

https://dspace.org/

The Activities Of The Urea Cycle Enzymes In Control, Regenerating And Neoplastic Rat Liver

Brebnor, Linda Denise

2015-01-12
http://hdl.handle.net/10539/16441

Downloaded from a DSpace repository. https://dspace.org/

THE ACTIVITIES OF THE UREA CYCLE ENZYMES IN CONTROL, REGENERATING AND NEOPLASTIC RAT LIVER

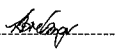
Linda Denise Brebner

A Dissertation Submitted to the Faculty of Science
University of the Witwatersrand, Johannesburg
for
the Degree of Doctor of Philosophy

Johannesburg 1977

DECLARATION

I declare that the thesis which is herewith submitted for the Degree of Doctor of Philosophy in the University of the Witwatersrand is entirely my own work and that it has not been previously submitted for a degree in any other university.



July 1977

ABSTRACT

Under conditions requiring rapid growth of liver cells such as occur in rat liver regenerating after partial hepatectomy and in hepatoma tissue, activities of urea cycle enzymes would be expected to drop for several reasons. Firstly, certain intermediates of urea synthesis are also required for polyamine and pyrimidine biosynthesis and secondly, little amino acid catalysis would be expected to occur since amino acids should be conserved for the synthesis of new proteins.

The effects of varying dietary protein content and hormone stimuli on the activities of urea cycle enzymes were tested in control and regenerating rat livers as well as in transplanted hepatomas and the livers of the transplant host animals.

A circadian rhythm experiment revealed a time-dependence for inducibility of urea cycle enzymes by glucagon. Hence all experimental animals were maintained on an '8-16' controlled lighting and feeding regime and sacrificed at 10:00 hours. The rats also showed an age-dependent inducibility; enzymes were inducible in 28- but not 35-day old animals.

A time-course study of changes of activities of urea cycle enzymes in regenerating livers shows that the levels of activity drop immediately after partial hepatectomy, reach a minimum at four hours and return to control values by 24 hours in animals maintained on a 22% protein diet. Actinomycin D and cordecepin, both inhibitors of transcription, prevent the initial drop in activity and produce an increase to levels above the controls at six and ten hours post-operatively.

In rats maintained on 0% or 10% protein diets there is an initial increase in activity after the operation with a drop becoming evident only at 15 hours and a minimum measured at 18 hours. The levels return to control values by 24 hours post-operatively.

After operation in rats maintained on a 75% protein diet, there is an initial decrease in activity and the activities return to control values by 12 hours.

In animals bearing transplanted hepatomas the activities of the urea cycle enzymes in the host liver are significantly less than those of nontumour-bearing controls in rats maintained on all diets. The activities in the hepatoma tissue were more dramatically decreased than those of the host livers. The urea cycle enzymes are responsive to dietary protein levels in all liver types with the hepatoma being the most responsive to diet.

Control livers of normal nontumour-bearing 35-day old animals are not responsive to hormonal stimulus, irrespective of the dietary protein content. The hormones tested included β-methasone, glucagon, β-methasone plus glucagon, and dibutyryl cyclic AMP. Surprisingly, both host livers and hepatomas from rats of the same age proved sensitive to all hormones tested. This inducibility was independent of dietary protein content. The fact that enzyme activity in the livers of animals bearing transplanted tumours is inducible whilst that of control animals is not, suggests the possible existence of a humoral substance responsible for urea cycle enzyme inducibility in tumour-bearing animals. This postulate was tested by injecting normal animals with plasma (or plasma plus glucagon) derived from tumour- or nontumour-bearing rats and comparing the inducibility in all groups of animals including both injected and noninjected controls. The animals injected intraperitoneally with plasma from tumour-bearing animals showed a decrease in urea cycle enzymes compared with controls, and plasma plus glucagon injected animals show inducibility above control values. This experiment would seem to support the hypothesis.

A semi-predictive, interpretive mathematical model is presented which describes the activities and behaviour of the urea cycle enzymes in normal, host and neoplastic rat liver.

TO MY FAMILY

ACKNOWLEDGEMENTS

I wish to thank Professor J.B. Balinsky and Dr A.R. Lee for their assistance during the course of this work. My appreciation is extended to my colleagues and to Professor N. van Schaik for their encouragement and enthusiasm and to Miss A. Hartley who assisted with the animals. I am grateful to Dr D. Bradu for his tremendous effort involved in the statistical analysis of the data presented.

My gratitude is extended to the C.S.I.R. and to the University of the Witwatersrand for bursaries awarded during the course of this research.

CONTENTS

		PAGE
1	*INTRODUCTION*	1
1.1	The Urea Cycle	1
1.2	The urea cycle enzymes	2
1.2.1	Carbamyl Phosphate Synthetase	2
1.2.2	Ornithine Transcarbamylase	3
1.2.3	Argininosuccinate Synthetase	4
1.2.4	Argininosuccinate Lyase	5
1.2.5	Arginase	6
1.3	Development and regulation of the capicity to synthesize urea.	6
1.3.1	Foetal and neonatal development of the urea cycle	6
1.3.2	Regulation of urea synthesis.	8
1.3.2.1	Dietary effects on the urea cycle enzymes	11
1.3.3	Circadian rhythms in enzyme activities ..	14
1.3.4	The rate of degradation of proteins ..	16
1.3.5	Hormone effects	16
1.3.5.1	Effects and mechanism of action of glucagon and cAMP	16
A.	Glucagon receptors	18
B.	Regulation of protein synthesis by cAMP	18
1.3.5.2	Mechanism of action of glucocorticoids	21
A.	Receptors	21
B.	Mechanism of action	24
C.	Effects of glucocorticoids ..	25
1.3.5.3	Hormonal effects on the urea cycle enzymes	26
1.4	Regeneration in rat liver after partial hepatectomy	28
1.4.1	General	28
1.4.2	Regulatory mechanisms operating in regenerating rat liver	31
1.4.2.1	Dietary effects on the proliferative response	31
1.4.2.2	Hormonal effects on the proliferative response	31
1.4.3	Relationship between polyamine and urea biosynthesis	32
1.5	Cancer	36
1.5.1	General	36
1.5.2	Ornithine imbalance in neoplastic growth ..	39
1.5.3	Urea cycle enzymes in neoplasia	39
1.5.4	Chemical carcinogenesis	40

PAGE

1.6 Aims of the project 41

2 MATERIAL AND METHODS 43

2.1 Materials 43

2.2 Animals 43

2.2.1 Rats for the study of hepatoma 44

2.3 Diets 44

2.3.1 Preparation of diets 47

2.4 Assay methods 47

2.4.1 Assays for urea cycle enzyme activities .. 47

2.4.1.1 Preparation of liver for quantitative assay 47

2.4.1.2 Spectrophotometric assay for CPS activity 48

2.4.1.3 Assay method for OTC 49

2.4.1.4 Assay method for ASS 50

2.4.1.5 Assay method for AL 50

2.4.1.6 Assay method for arginase .. 51

2.4.2 Colorometric Assays 51

2.4.2.1 Colorometric assay of citrulline .. 51

2.4.2.2 Colorometric determination of urea .. 52

2.4.2.3 Colorometric determination of proteins 52

2.5 Injections 58

2.6 Partial hepatectomy 58

2.6.1 Procedure for sham operations 59

2.7 Method for induction of hepatoma 59

2.7.1 Method of transplantation of hepatomas .. 60

2.8 Method of exsanguination of rats 60

3 RESULTS 62

3.1 Time-dependence of inducibility 62

3.2 Age-dependence of inducibility 70

3.3 Urea cycle enzymes in regenerating rat livers .. 75

3.4 Urea cycle enzymes in neoplasia 187

4 DISCUSSION 277

4.1 Assay systems 277

4.2 Time-dependence of urea cycle enzyme activities .. 279

4.3 Age-dependence of inducibility 281

4.4 Regeneration after partial hepatectomy 287

4.4.1 Effect of inhibitors of RNA synthesis on urea cycle enzyme activities in regenerating rat liver 290

4.4.2 Dietary effects on activities of urea cycle enzymes in regeneration 292

PAGE

4,5 Urea cycle enzyme activities in hepatoma .. 294
4.5.1 Statistical treatment of the data 302
4.5.1.1 Analysis of variance 302
4.5.2 'A mathematical model 302
4.6 Regulation of urea cycle enzyme activities .. 319
APPENDIX 321
BIBLIOGRAPHY 324

LIST OF TABLES

		PAGES
2.1	Composition of diets.	44
2.2	Steenbeck salt mixture.	45
2.3	Contents per Gevral vitamin capsule.	46
3.1a-3.1c	Circadian rhythm determination of urea cycle enzyme activities in rat liver and the determination of time-dependence of glucagon induction. Activities were expressed per gram wet weight of liver. ..	63
3.2a-3.2c	Circadian rhythm determination for urea cycle enzymes activities in rat liver and the determination of time-dependence of glucagon induction. Activities were expressed per mg protein.	66
3.3	A comparison of glucagon induction of urea cycle enzymes in 28 and 35-day old rats. Animals were maintained on a 10% protein diet.	71
3.4	A summary of glucagon induction of urea cycle enzymes in 28 and 35-day old animals maintained on a 10% protein diet.	72
3.5a-3.5c	CPS activities of control versus regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 0% protein diet.	77
3.6a-3.6c	ASS activities of control versus regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 0% protein diet.	81
3.7a-3.7c	AL activities of control versus regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 0% protein diet.	85
3.8a-3.8c	Arginase activities of control versus regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 0% protein diet.	89
3.9a-3.9c	CPS activities of control versus regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.	94
3.10a-3.10c	ASS activities of control versus regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.	98
3.11a-3.11c	AL activities of control versus regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.	102
3.12a-3.12c	Arginase activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.	106

		PAGE
3.13a-3.13e	CPS activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.	111
3.14a-3.14d	OTC activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.	117
3.15a-3.15e	ASS activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.	122
3.16a-3.16e	AL activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.	128
3.17a-3.17e	Arginase activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.	134
3.18a-3.18c	CPS activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.	141
3.19a-3.19c	ASS activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.	145
3.20a-3.20c	AL activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.	149
3.21a-3.21c	Arginase activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.	152
3.22a, 3.22b	A comparison of urea cycle enzyme activities in control vs. sham operated animals. The enzymes were assayed at four hours post-operatively.	158
3.23a, 3.23b	CPS activities from control and actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.	162
3.24a, 3.24b	ASS activities from control and actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.	165
3.25a, 3.25b	AL activities from control vs. actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.	168
3.26a, 3.26b	Arginase activities from control vs. actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.	171

PAGES

3.27a, 3.27b CPS activities of control vs. cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet. 175

3.28a, 3.28b ASS activities of control vs. cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet. 178

3.29a, 3.29b AL activities of control vs. cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet. 181

3.30a, 3.30b Arginase activities of control vs. cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet. 184

3.31a, 3.31b A comparison of CPS activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero % protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 189

3.32a, 3.32b A comparison of OTC activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero % protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 191

3.33a, 3.33b A comparison of ASS activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero % protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 193

3.34a, 3.34b A comparison of AL activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero % protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 195

3.35a, 3.35b A comparison of Arginase activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero % protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 197

3.36a, 3.36b A comparison of CPS activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 199

3.37a, 3.37b A comparison of OTC activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 201

PAGES

3.38a, 3.38b A comparison of ASS activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 203

3.39a, 3.39b A comparison of AL activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 205

3.40a, 3.40b A comparison of Arginase activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 207

3.41a, 3.41b A comparison of CPS activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 209

3.42a, 3.42b A comparison of OTC activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 211

3.43a, 3.43b A comparison of ASS activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 213

3.44a, 3.44b A comparison of AL activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 215

3.45a, 3.45b A comparison of Arginase activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 217

3.46a, 3.46b A comparison of CPS activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 219

3.47a, 3.47b A comparison of OTC activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet. Activities were expressed a) per gram liver, and b) as specific activity. 221

		PAGES
3.48a, 3.48b	A comparison of ASS activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet. Activities were expressed a) per gram liver, and b) as specific activity.	223
3.49a, 3.49b	A comparison of AL activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet. Activities were expressed a) per gram liver, and b) as specific activity.	225
3.50a, 3.50b	A comparison of Arginase activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet. Activities were expressed a) per gram liver, and b) as specific activity.	227
3.51	Activities of urea cycle enzymes from hormone-untreated control, host and hepatoma from rats maintained on 0, 10 and 75% protein diets expressed as a % of levels in rats maintained on a 22% protein diet. ..	257
3.52A	Activities of CPS of host and hepatoma expressed as a % of control levels.	258
3.52B	Host activities as % of control host. Hepatoma activities as % of control hepatoma.	58
3.53A	Activities of OTC of host and hepatoma expressed as a % of control levels.	259
3.53B	Host activities as % of control host. Hepatoma activities as % of control hepatoma.	259
3.54A	Activities of ASS of host and hepatoma expressed as a % of control levels.	260
3.54B	Host activities as % of control host. Hepatoma activities as % of control hepatoma...	260
3.55A	Activities of AL of host and hepatoma expressed as a % of control levels.	261
3.55B	Host activities as % of control host. Hepatoma activities as % of control hepatoma.	261
3.56A	Activities of Arginase of host and hepatoma expressed as a % of control levels.	262
3.56B	Host activities as % of control host. Hepatoma activities as % of control hepatoma.	262

PAGES

3.57a, 3.57b	Analysis of variance for results expressed a) per ... r, and b) as specific activity.	270
3.58a-3.58c	A comparison of activities of urea cycle enzymes for control vs. plasma and plasma+glucagon treatment in nontumourous animals. The plasma was obtained from both nontumourous and tumour-bearing animals. Rats were maintained on a 22% protein diet.	273
4A	Coefficient for activities as specific activity. ..	304
4B	Coefficient for activities per gram liver.	304
4.1	A comparison of observed data vs. predicted values for control livers. Activities are expressed per mg protein.	305
4.2	A comparison of observed data vs. predicted values for host livers. Activities are expressed per mg protein.	306
4.3	A comparison of observed data vs. predicted values for hepatoma. Activities are expressed per mg protein...	307
4.4	A comparison of computed averages for observed data vs. predicted values for control livers. Activities are expressed per mg protein.	308
4.5	A comparison of computed averages for observed data vs. predicted values for host liver. Activities are expressed per mg protein.	309
4.6	A comparison of computed averages for observed data vs. predicted values for hepatoma. Activities are expressed per mg protein.	310
4.7	A comparison of observed data vs. predicted values for control livers. Activities expressed per gram liver. ..	311
4.8	A comparison of observed data vs. predicted values for host liver. Activities expressed per gram liver. ..	312
4.9	A comparison of observed data vs. predicted values for hepatoma. Activities expressed per gram liver. ..	313
4.10	A comparison of computed averages for observed data vs. predicted values for control livers. Activities are expressed per gram liver.	314
4.11	A comparison of computed averages for observed data vs. predicted values for host. Activities are expressed per gram liver.	315
4.12	A comparison of computed averages for observed data vs. predicted values for hepatoma Activities are expressed per gram liver.	316

LIST OF FIGURES

		PAGE
1.1	The Urea Cycle	1a
2.1	Citrulline calibration curve	54
2.2	Urea calibration curve for arginase.	56
2.3	Urea calibration curve for AL.	55
2.4	Calibration curve for protein determination.	57
3.1	A demonstration of time-dependence of glucagon induction of urea cycle enzyme activities in the livers of 28-day old rats.	69
3.2	Activity/g liver of urea cycle enzymes from control and glucagon treated livers of 28 and 35-day old rats.	73
3.3	Specific activities of urea cycle enzymes from control and glucagon-treated livers of 28 and 35-day old rats. ..	74
3.4	CPS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 0% protein diet.	80
3.5	ASS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 0% protein diet.	84
3.6	AL activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 0% protein diet.	88
3.7	Arginase activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 0% protein diet.	92
3.8	CPS activities from regenerating rat livers exp[illegible] a % of zero-time controls. Rats were maintained on [illegible]tein diet.	97
3.9	ASS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 10% protein diet.	101
3.10	AL activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 10% protein diet.	105
3.11	Arginase activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 10% protein diet.	109
3.12	CPS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet.	116
3.13	OTC activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet	121

PAGE

3.14 ASS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. 127

3.15 AL activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. 133

3.16 Arginase activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22%protein diet. 139

3.17 CPS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 75% protein diet. 144

3.18 ASS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 75% protein diet. 148

3.19 AL activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 75% protein diet. 152

3.20 Arginase activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 75% protein diet. 156

3.21 Activities of CPS from 6 hour and 10 hour untreated regenerating and AMD - treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. 164

3.22 Activities of ASS from 6 hour and 10 hour untreated regenerating and AMD-treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. 167

3.23 Activities of AL from 6hour and 10 hour untreated regenerating and AMD-treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. 170

3.24 Activities of arginase from 6 hour and 10 hour untreated regenerating and AMD-treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. 173

3.25 Activities of CPS from 6 hour and 10 hour untreated and cordecepin-treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. .. 177

3.26 Activities of ASS from 6 hour and 10 hour untreated and cordecepin-treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. .. 180

3.27 Activities of AL from 6 hour and 10 hour untreated and cordecepin-treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. .. 183

3.28 Activities of arginase from 6 hour and 10 hour untreated and cordecepin-treated regenerating rat liver expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet. 186

PAGE

3.29 A comparison of urea cycle enzyme activities in control liver, host liver and hepatoma. Animals were maintained on a 22% protein diet. 230

3.30 Activities of CPS from hormone-untreated host livers and hepatomas expressed as a % of controls. Rats were maintained on various protein diets. 232

3.31 Activities of OTC from hormone-untreated host livers and hepatomas expressed as a % of controls. Rats were maintained on various protein diets. 233

3.32 Activities of ASS from hormone-untreated host livers and hepatomas expressed as a % of controls. Rats were maintained on various protein diets. 234

3.33 Activities of AL from hormone-untreated host livers and hepatomas expressed as a % of controls. Rats were maintained on various protein diets. 235

3.34 Activities of arginase from hormone-untreated host livers and hepatomas expressed as a % of controls. Rats were maintained on various protein diets. 236

3.35 Activity/g liver of CPS from hormone-treated host livers expressed as a % of control untreated host livers of rats maintained on various diets. 237

3.36 Specific activities of CPS from from hormone-treated host livers expressed as a % of control untreated host livers of rats maintained on various diets. 238

3.37, 3.38 Activity/g liver and specific activity of CPS from hormone-treated hepatomas expressed as a % of control untreated hepatomas of rats maintained on various diets. 239

3.39, 3.40 Activity/g liver and specific activity of OTC from hormone-treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets. 241

3.41, 3.42 Activity/g liver and specific activity of OTC from hormone-treated hepatomas expressed as a % of control untreated hepatomas of rats maintained on various protein diets. 243

3.43, 3.44 Activity/g liver and specific activity of ASS from hormone-treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets. 245

3.45, 3.46 Activity/g liver and specific activity of ASS from hormone-treated hepatomas expressed as a % of control untreated hepatomas of rats maintained on various protein diets. 247

3.47, 3.48 Activities/g liver and specific activities of AL from hormone-treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets. 249

3.49 3.50 Activity/g liver and specific activity of AL from hormone-treated hepatomas expressed as a % of control untreated hepatomas of rats maintained on various protein diets. 251

PAGE

3.51, 3.52 Activity/g liver and specific activity of arginase from hormone-treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets. 253

3.53, 3.54 Activity/g liver and specific activity of arginase from hormone-treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets. 255

3.55a, 3.55b CPS activities of control, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet. 264

3.56 OTC activities of control, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet. 266

3.57 ASS activities of control, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet. 267

3.58 AL activities of control, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in rats maintained on a 22% protein diet. 268

3.59 Arginase activities of control, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in rats maintained on a 22% protein diet. 269

3.60 A comparison of activities of urea cycle enzyme levels from control, plasma and plasma plus glucagon-treated rat livers. The plasma was obtained from tumour and nontumour-bearing animals. Rats were maintained on a 22% protein diet. 276

INTRODUCTION

1.1 THE UREA CYCLE

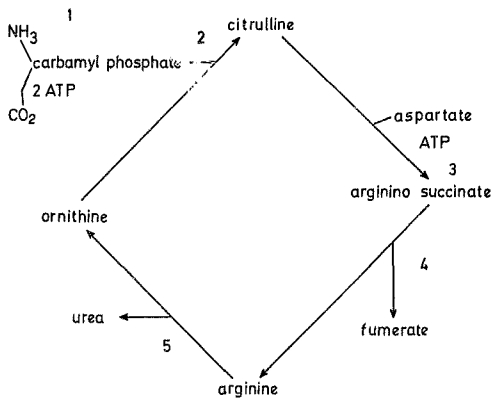
The major end product of nitrogen metabolism in man and other ureotelic vertebrates is urea (Cohen and Brown, 1960). Urea results from the detoxification of ammonia which arises from deamination reactions within the liver. Krebs and Henseleit (1932) demonstrated that the synthesis of urea occurs by a cyclic series of reactions and involves the sequential action of five enzymes (see fig. 1). The liver is the major site of urea synthesis although a small amount of activity of some urea cycle enzymes is found in the brain and kidneys, small intestine and pancreas (Yip and Knox, 1972; Szilagi, 1973) and in white blood cells (Reyero et al, 1974).

Ammonia is toxic to the central nervous system even in low concentrations. Land animals had to possess a mechanism for its detoxification early in their evolution, as soon as the supply of water became limited (Cohen and Brown, 1963).

Ornithine is an intermediate in urea synthesis, being the product of ornithine transcarbamylase (OTC) (E.C.2.1.2.2.) action (Grisolia and Cohen, 1951). It is also an intermediate in several other metabolic pathways which include polyamine synthesis via ornithine decarboxylase (ODC) (E.C.4.1.17) (Williams - Ashman et al, 1972) and transamination by ornithine amino transferase, (OAT) (E.C.2.6.1.13) to glutamate-γ - semi-aldehyde on the route to glutamate and proline biosynthesis (Sanada et al, 1970).

Carbamyl phosphate and aspartate, both intermediates in urea synthesis are also intermediates in the formation of pyrimidines (Ferdinandas et al, 1971). Therefore, alterations in urea cycle enzyme levels during conditions of rapid growth as exist in regenerating rat liver after partial hepatectomy, and in hepatomas could allow for the channelling of ornithine, carbamyl phosphate and aspartate to polyamine and pyrimidine biosynthesis to allow for cell proliferation. It is of interest therefore to study the possible alterations of urea cycle enzyme activities under these conditions. By varying dietary protein intake and hormonal stimuli it may be possible to determine the regulatory mechanism operating in the control of urea cycle enzyme activities and their possible relationship to proliferation processes.

FIG. 1.1 THE UREA CYCLE



1 Carbamyl Phosphate Synthetase
2 Ornithine Transcarbamylase
3 Argininosuccinate Synthetase
4 Argininosuccinate Lyase
5 Arginase

The present thesis describes experiments to study the effects of these variables.

1.2 THE UREA CYCLE ENZYMES

1.2.1 Carbamyl Phosphate Synthetase (E.C.2.7.2.5. ATP : carbamate phosphotransferase (dephosphorylating))

Mammalian tissue contains two distinct CPS isozymes, CPS I and CPS II (Tatibana and Shigesada, 1972). CPS I catalyses the formation of carbamyl phosphate from ammonia and bicarbonate. The overall reaction is irreversible (Metzenberg et al, 1957), requires two moles of ATP (ibid) and a cofactor N-acetylglutamate (Grisolia and Cohen, 1952 ; Metzenberg et al, 1957).

CPS I is found in the particulate fraction of the liver of vertebrates (Grisolia and Cohen, 1952), mostly in the mitochondria with some nuclear activity being exhibited (Mora et al, 1965). Acetylglutamate acts as an allosteric effector by altering the conformation and association of the enzymic subunits (Guthöhrlein and Knappe, 1968). The content of acetylglutamate varies with varying protein diets and is believed to be under a positive feedback regulation by arginine (Tatibana and Shigesada, 1972).

The major function of CPS I is believed to be for the provision of carbamylphosphate for urea biosynthesis (Jones, 1970), because it is limited to ureoteles which are unique in excreting urea as a breakdown product of protein and as it occurs in high levels in the livers of ureotelic vertebrates (ibid). CPS I is located in the mitochondria where the second enzyme of urea synthesis, ornithine transcarbamylase, is also found. Its activity varies with alterations in dietary protein content, and increases after birth at a time when urea is first synthesized (ibid).

The molecular weights of the two isozymes have been determined by sucrose density gradient centrifugation : that of CPS I was found to be 316 00 ± 42 000 daltons and that of CPS II was found to be 160 000 ± 10 000 daltons (Virden, 1972). Shephard (1975) determined the molecular weight of CPS I by gel filtration and found it to be 290 000 daltons. Immunological studies suggest that CPS I and CPS II are discrete proteins (Virden, 1972 ; Nakanishi, 1968) having different kinetic properties and different cofactor requirements.

PH optima were determined by Marshall et al. (1968). Kinetic studies and Michaelis Constants have been done by Kerson (1969) and Guthörhlein and Knappe (1969) and earlier studies were reviewed by Jones (1965). The Km values of ammonia and bicarbonate are 1,1 mM and 5,3 mM respectively.

The mechanism of the reaction is believed to proceed by three distinct steps depicted in the following equations (Anderson and Meister, 1965; Jones, 1965).

$$E + HCO_3^- + ATP \longrightarrow E\text{-carboxyphosphate} + ADP \quad (1\,a)$$

$$E\text{-carboxyphosphate} + NH_4 \longrightarrow E\text{-carbamate} + Pi \quad (1\,b)$$

$$E\text{-carbamate} + ATP \rightleftharpoons E + \text{carbamyl phosphate} + ADP \quad (1\,c)$$

Later studies by Chabas et al. (1972) led to the suggestion that five consecutive steps are involved with acetylglutamate phosphate, a possible intermediate as follows:

E + acetylglutamate $\rightleftharpoons$ E-acglu

$\downarrow\uparrow$ + ATP 2a

E.2-acetamido-glutaric anhydride + ADP + Pi

$\downarrow\uparrow$ HCO_3^-

E. or -N^3-acetylglutamate mixed carboxylic-carbonic anhydride

$\downarrow\uparrow$ + NH_4^+

E. acetylglutamate . carbamate

$\downarrow\uparrow$ + ATP

ADP + E.acetylglutamate + carbamyl phosphate

1.2.2 Ornithine Transcarbamylase
[EC 2.1.3.3 carbamylphosphate-L-ornithine carbamyl transferase] (OTC)

This enzyme catalyzes the second step in the ornithine cycle. It is found in the particulate fraction of liver cells of all ureotelic vertebrates (Brown and Cohen, 1959). It was first purified by Reichard (1957) as well as by Burnett and Cohen (1957). The pH optimum of bovine OTC was determined by Marshall and Cohen (1972) and found to occur at pH 8,5. Snodgrass (1968) found that the apparent

Km for ornithine decreases with increasing pH in the range 6,2 - 7,9. Snodgrass (1968) suggested that the ionic species with net zero charge is the active species or substrate and probably also the inhibitor. Marshall and Cohan (1972) have confirmed these observations. The zwitterion or active species constitutes only 10% of the species with net zero charge (ibid). OTC was found to be a trimer of three identical subunits, the molecular weight of the multimer being 108 000 daltons (Reichard, 1957).

Studies on substrate activation and inhibition revealed that OTC is stimulated by ornithine and carbamyl phosphate, while OAT is inhibited by these substances. OTC is also activated by α-ketoglutarate while OAT is inhibited. It is suggested that α-ketoglutarate may be an allosteric ligand for OTC (Shimmoyashi, 1972).

Cytochemical (Mizutani, 1968) and electronmicroscopic studies (Mercker, 1969) have demonstrated that OTC is located in the mitochondrial matrix of rat and mouse liver and kidney cells.

1.2.3 Argininosuccinate Synthetase [EC 6.3.4.5. L-Citrulline : L-aspartate lyase (AMP)] (ASS)

ASS is the cytosolic enzyme involved in the conversion of citrulline to argininosuccinate by the addition of aspartate. The kinetics of the reaction were determined by Rochovansky and Ratner (1967) and this work together with earlier studies by the same authors (1961) led them to postulate a two step reaction as follows

$$E + \text{citrulline} + MgATP^{2-} \rightleftharpoons E\begin{matrix}\diagup \text{AMP-citrulline} \\ \diagdown MgPPi\end{matrix} \qquad (2a)$$

$$E\begin{matrix}\diagup \text{AMP-citrulline} \\ \diagdown MgPPi\end{matrix} + \text{aspartate} \rightleftharpoons E + \text{argininosuccinate} + MgPPi + AMP^{2-} \qquad (2b)$$

The rate of the reaction is greatly dependent on pH : the optimum pH in the forward direction is 8.7 and in the reverse direction lies at pH 6.0 (Petreck and Ratner, 1956).

ASS has a molecular weight of 175 000 daltons, being a tetramer of four identical subunits, each of 45 000 daltons with two pairs of dimers being crosslinked by disulphide bonds (Ratner, 1973). The enzyme shows an absolute requirement for Mg^{2+} since $Mg\,ATP^{2-}$ is the

active form of ATP (Ratner, 1973). The enzyme is highly specific with only the natural isomers of aspartate and citrulline being active (Rochovansky and Ratner, 1962;1967).

ASS is believed to catalyze the rate limiting step in the synthesis of urea, its activity being 300 fold less than that of arginase (McLean and Gurney, 1963). In general the affinity of the enzyme for its substrates is not affected by changes in ATP concentrations, but a reduction in concentration of either citrulline or aspartate increases the affinity of the enzyme for the second substrate when present in low concentrations.

1.2.4 Argininosuccinate Lyase (E.C. 4.3.2.1 L-argininosuccinate arginine lyase) (AL)

AL has been shown to catalyze the reversible conversion of argininosuccinate to arginine and fumarate (Ratner and Petrack, 1951 ; 1953). The number of binding sites for argininosuccinate as determined by equilibrium dialysis indicate that at high argininosuccinate concentrations there are four binding sites and that at low concentrations there are two (Ratner, 1973). Arginine shows similar binding patterns (ibid). The maximum of four binding sites agrees with a tetrameric structure with each monomeric subunit having its own catalytic site.

The enzyme has been found to be a tetramer of four identical subunits, the molecular weight of the monomers being 50 000 daltons and that of the tetramer being 202 000 daltons (Lusty and Ratner, 1972). Studies on subunit association and equilibria were carried out by Schultze et al (1970). The data agrees with the arrangement of monomers with their centres of mass at the virtices of a tetrahedron (Lusty and Ratner, 1972). The kidney and liver enzymes have identical molecular weights and oligomeric structures suggesting that they are the products of one gene (Schulze et al, 1970).

1.2.5 Arginase (EC 3.5.3.1 L-arginine ureahydrolase)

Arginase is the most abundant of the urea cycle enzymes and has the greatest turnover rate of the five. Gasiorowska et al (1970) demonstrated that arginase consists of two separate isozymes one with a pH optimum at pH 9,5 and one with a pH optimum at 7,5. Only the former is found in the liver but both are found in the brain and kidney. Later studies by Hartzfeld and Raper (1976) have identified three isozymes by polyacrylamide gel electrophoresis. Isozyme I is found in all extrahepatic tissues with the exception of the

submaxillary glands, isozyme II is found in the liver and the submaxillaries and isozyme III is found in the kidney, intestine and pancreas.

Glass and Knox (1973) showed by molecular weight determinations that the two isozymes found in the mammary gland differ chemically. Liver arginase is homogeneous with respect to molecular weight, being a tetramer of identical subunits (MW 30 000 daltons) (Hirch et al, 1968) whereas mammary gland arginase contains two components of MW 94 000 and 42 000 daltons.

Mora et al. (1960) reported differences between arginase from ureotelic and uricotelic species. For chicken and lizard arginase, a molecular weight of 276 - 278 x 10^3 daltons was reported (ibid.). Hirch - Kolb and Greenberg (1968) calculated the molecular weight of rat liver arginase to be 118 000 daltons. SDS-Polyacrylamide gel electrophoresis gave a simple protein band suggesting that the subunits are identical.

Inactivation of rat liver arginase occurs at low pH at 0^oC (Hosoyama et al, 1971). Reactivation occurs in neutral buffer in the presence of Mn^{2+}. Acid pH results in dissociation of the multimer into subunits of MW 64 000 daltons and 32 000 daltons at pH 4,0 and 2,0 respectively. Four moles of Mn^{2+} are required per mole of arginase for maximal activity (Kolb et al, 1971) and activation occurs by a conformational change in the multimer structure.

1.3 DEVELOPMENT AND REGULATION OF THE CAPACITY TO SYNTHESIZE UREA

1.3.1 Foetal and neonatal development of the urea cycle

The capacity to synthesize urea depends on the activity of the rate limiting enzyme argininosuccinate synthetase which is detectable only on the 14th day of gestation (Räihä and Schwartz, 1973). Much work has been done on the development of the urea cycle enzymes in general (Kenney and Cohen, 1959; Räihä and Schwartz, 1973; Miller and Chu, 1970) and of arginase and OTC in particular (Schuit and Dickie, 1973; Illenerova, 1966; Greengard et al, 1970; Bradley, 1973).

In rats, urea cycle enzyme activities are measurable only four days before birth at the same time that the metanephric kidney becomes functional (Cohen, Räihä and Schwartz, 1973; Kennan and Cohen, 1959).

There is a rapid rise one day before birth and the activities remain at this level until the third postnatal week when on about day 16, there is a very sharp increase in urea cycle enzyme activities reaching the adult levels by day 28. The second period of rise coincides with weaning (Greengard et al, 1970) and is probably correllated with an increased protein consumption. On day 14 postnatally, the eyes open and the rats begin to eat solid food for the first time and are thus exposed to a greater variety of protein.

Argininosuccinate synthetase (ASS) and argininosuccinate lyase (AL) activity have been detected by day 18 of gestation (Kennan and Cohen, 1959). Arginase activity has been detected by the eighth day of gestation and is distributed throughout the foetal embryo. By the 12th day the levels in the liver increase and on the 16th day the activity increases in the foetal kidney (Bradley, 1973).

It is thought that the thyroid and glucocorticoid hormones successively promote the developmental formation of urea cycle enzymes in the late foetal and late sucklin, periods respectively since the thyroid gland begins to operate on day 16 of gestation coinciding with the first appearance of urea cycle enzymes, whilst the pituitary and adreno-cortical activity increase by the 14th postnatal day (Levine and Mullins, 1966) at about the same time as the second rise of urea cycle enzyme activity occurs.

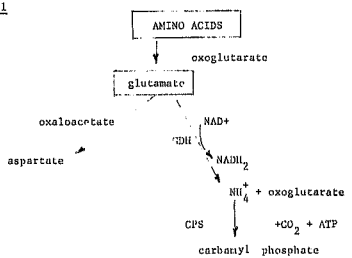
Adrenalectomy on the 12th postnatal day prevents the accumulation of arginase, (Greengard et al, 1970) reinforcing the belief that this rise is influenced by glucocorticoids. An intraperitoneal injection of hydrocortisone (25mg / 10 ml) (but not thyroxine) between days 5-8 postnatally results in the rapid increase in urea cycle enzyme activities within 24 hours. A single injection of thyroxine (3mg / foetus) (but not hydrocortisone) enhances the prenatal development (Greengard et al, 1970). After the 28th postnatal day the urea cycle enzymes are not inducible by a single injection of hydrocortisone; rather, they require at least 3 days of injections to obtain a significant induction (McLean and Gurney, 1963).

1.3.2 Regulation of urea synthesis

Alanine is metabolized more readily than any other amino acid in the liver. The main step in metabolism is the transamination with oxoglutarate, the carbon skeleton forming either glucose or lactose and the amine nitrogen going to urea or ammonia.

In the ornithine cycle, half of the ure. nitrogen must be supplied as aspartate, the other half as carbamyl phosphate (Krebs et al, 1973) according to scheme 1.

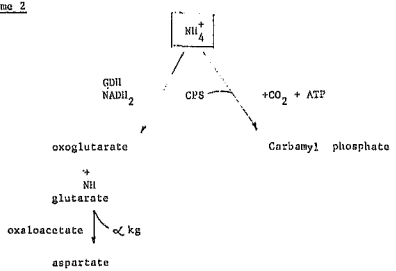
Scheme 1



The fact that supplies of aspartate and carbamyl phosphate are co-ordinated requires some kind of feedback control regulating whether glutamate undergoes transamination to aspartate or dehydrogenation to form ammonia, and whether ammonia forms carbamyl phosphate or glutamine.

If free ammonia is the nitrogen source, then carbamyl phosphate and aspartate are derived from it according to Sch-- 2.

Scheme 2



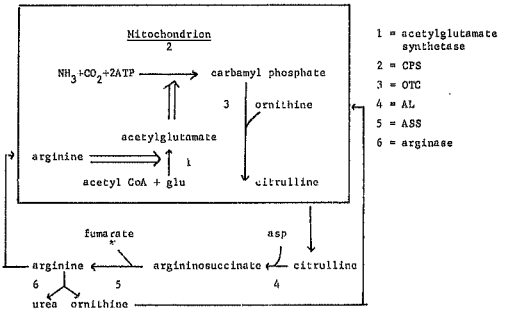
The free ammonia could be derived from glutamine or asparagine, the amino groups of adenine, guanine and their derivatives and the degradation products of all α -keto amino acids. Additional free ammonia can arise by the decomposition of urea by bacteria; this can account for the metabolism of up to 20% of the urea formed (Krebs et al, 1973).

The fact that alanine can be metabolized to glucose by deamination followed by gluconeogenesis or to urea via Scheme 1 above and that glucose and urea are formed in equal amounts as the products of metabolism of alanine requires that there are equivalent rates of flux of malate and aspartate from the mitochondria and equal rates of flux of these metabolites through mitochondrial glutamate dehydrogenase (GDH) and αketoglutarate dehydrogenase (αKGDH).

Since glutamate can be formed from alanine and since NADH and ammonia can inhibit deamination of glutamate by GDH and stimulate its transamination by aspartate aminotransferase, the regulation of urea and glucose production from alanine via glutamate in the mitochondria depends on the mitochondrial ratio of $NADH/NAD^+$ due to reactions involving both GDH and α KGDH. Interactions involving these enzymes regulate the flux of metabolites across the mitochondrial membrane by regulating the steady state metabolite levels (Williamson et al, 1974).

Tatibana et al. (1972a) assume that acetylglutamate levels serve as a regulator of carbamyl phosphate synthesis and that the role of arginine is that of positive feedback regulation. This is shown in Scheme 3. Acetylglutamate levels approximately double in livers of rats maintained on high protein diets, parallel with increases in CPS activities and urea synthesized (Sangduk et al., 1972).

Scheme 3



Urea synthesis is initiated in mitochondria by the action of CPS in forming carbamyl phosphate. Carbamyl phosphate is relatively unstable (Shigisada and Tatibana, 1971a; 1971b) with a half life of 40-50 mins at 37°C and this necessitates its rapid conversion to citrulline which is ensured by high concentrations of both ornithine and the transcarbamylase, OTC.

Arginine can increase the maximum acetylglutamate synthetase activity six fold and can stimulate its synthesis ten fold in the mitochondria (Shigesada and Tatibana, 1971a). At sub-optimal concentrations of ornithine, CPS is not fully active. An increase of ornithine concentrations in a cell causes activation of CPS by raising in the first instance the steady state concentration of

arginine which in turn increases the concentration of acetyl glutamate thereby activating CPS.

Control of the amount of carbamyl phosphate synthesized is very important since ammonia is also converted to pyrimidines and glutamate and via glutamate to all essential amino acids, for protein synthesis. Ammonia is also important in the maintenance of mitochondrial redox systems since it is a key component of these systems (Williamson, et al, 1967). Therefore it is important that certain ammonia reserves are always available for maintenance of mitochondrial redox systems. A further control mechanism ensuring the coordinated synthesis of carbamyl phosphate is that of feedback inhibition of CPS by carbamyl phosphate and aspartate and closely related substances such as glutamate (Krebs et al, 1973).

1.3.2.1 Dietary effects on urea cycle enzymes

Schimke (1962) was the first to show the concerted regulation of an entire pathway, namely the response of urea cycle enzymes to increased protein intake. All the urea cycle enzymes were measured and it was found that activities of all five of the enzymes increased in a coordinated manner directly proportional to the protein intake and to the amount of urea excreted. The increases and decreases in enzyme activity were due directly to changes in absolute amount of enzyme protein.

It was found by Doesthale and Tulpule (1969) that the activity of urea cycle enzymes depended both on quality and quantity of protein intake. Vegetable protein is of a lower quality generally than animal protein and animals fed on a vegetable protein diet have lower urea cycle enzyme activities than those fed on an equivalent diet of animal protein.

Starvation results in the catabolism of less essential metabolites and the preservation of essential metabolic components for the maintenance of life. Generally, starved animals show increased gluconeogenesis (Nordlie et al, 1965; Gaswami and Chatagner, 1966), decreased levels of RNA and protein (Schimke, 1962) with ribosomal RNA and proteins decreasing in parallel since they have similar half lives. There is a drop in blood glucose (Freedland and Szepesi, 1971) with a drop in insulin. The latter allows gluconeogenesis to proceed since insulin is antagonistic to glucagon which stimulates

gluconeogenesis. In starvation there is an increase in glucagon for glycogenolysis and glucocorticoids for gluconeogenesis (Olsen, 1975). The Embden-Meyerhof pathway, gluconeogenesis and the tricarboxylic acid cycle are maintained preferentially for the production of energy and formation of glucose.

During starvation the activities of urea cycle enzymes increase although there is a 30% drop in foetal liver enzymes and urea excretion increases. During fasting for four days, the urea cycle enzyme activities increase two-fold, and after 7 days of fasting they have risen approximately four-fold (Schimke, 1962).

Purification of arginase and OTC, followed by immunological assay showed that increased activities are due directly to increased content of specific proteins (Schimke, 1962a; Schimke, 1964).

Under conditions of protein deprivation, there is a shift in enzyme activities for the conservation of the amino nitrogen and essential amino acids. Energy requirements are derived from dietary carbohydrate. It has been shown that there is a drop in the amount of urea excreted with a concomitant drop in urea cycle enzyme activities directly proportional to a decrease in the absolute amount of enzyme proteins (Schimke, 1962b). Arginase activity has been shown to decrease on a zero % protein diet resulting in the conservation of arginine for protein synthesis (Waterlow and Stephen, 1968). The amino acids released by protein catabolism are more efficiently reutilised for protein synthesis in the vital tissues such as the liver at the expense of less important tissues such as the muscles. Amino acids entering the liver are incorporated into new proteins rather than deaminated to produce urea (Deonthale and Tulpule, 1969).

When rats are maintained on a high protein diet, urea excretion increases as do the levels of the urea cycle enzymes themselves (Schimke, 1962a). There is no necessity to save the amino nitrogen so for most amino acids catabolized, the ammonia released will be excreted as urea. Ashida and Harper (1961) also showed that with an increase in protein intake there is a concomitant increase in arginase activity.

Experiments in forced feeding of different amino acids to rats have shown that tryptophan is the most effective in increasing urea cycle enzyme activities without increasing the amount of urea in the plasma or the urine (Muramatsu and Nakagawa, 1971). Phenylalanine, leucine, threonine and methionine also increase the urea cycle enzyme activities but to a lesser extent than tryptophan while histidine and

lysine cause a drop in the activity of arginase. The different enzymes of the urea cycle respond differently to these amino acids with OTC responding least. Wannemacher *et al.* (1975) postulated that the availability of amino acids in rats maintained on different protein diets regulates protein synthesis by affecting the number of ribosomes available. On low protein diets there would be an inhibition in the synthesis of new ribosomes. Tryptophan may regulate protein synthesis by regulating the number of polysomes aggregated.

Schimke (1964) and Szepesi and Freedland (1969) have shown that the diet effects occur through altered rates of synthesis and degradation of the enzyme protein. Using incorporation experiments, they found that in starvation and changes from high (70%) to low (8%) dietary protein content there was an increased rate of degradation together with a decreased rate of synthesis resulting in an overall drop in the absolute amount of arginase. The half life of the enzyme changed from five to three days thus reaching the new steady state in a shorter time.

When rats were changed from low (8%) to a high (70%) protein diet there was a two-fold increase in the synthesis of arginase enzyme protein with little change in the rate of degradation. When rats were changed from a 90% protein free to a casein diet the half life of arginase changed to one day. In all cases the half-life returned to five days once the new steady state was attained.

Das and Waterlow (1974) found that when rats were changed from a high (154 g / kg) to a low (40 g /kg) casein diet all five of the urea cycle enzyme activities dropped reaching the new steady state within 30 hours. They calculated the half life of the enzymes to be in the order of seven hours which is shorter than the values found by other workers. The main difference between the work of these authors and those previously mentioned is that Das and Waterlow conducted their experiments such that their animals were trained to a fixed feeding and lighting regime rather than ad lib feeding. This might in some way alter the measurements of half-life values.

1.3.3 Circadian rhythms in enzyme activities

Wurtman (1969) has reported that ad lib. feeding of rats maintained on a 12-12 lighting regime (12 hours dark and 12 hours light) results in large daily oscillations in tyrosine aminotransferase activities (TAT). In vivo free tryptophan concentrations increase by 50% several hours before the increase in TAT is observed. Omission of tryptophan from the diet results in the complete extinction of TAT periodicity. Tryptophan concentrations in the plasma vary with the time of day, with feeding patterns and dietary protein intake. The rate of uptake of amino acids is influenced by steroids and insulin both of which show circadian rhythms and so may generate rhythms in amino acid concentrations. Glucocorticoid levels peak two hours before the onset of darkness (Wurtman and Axelrod, 1967) and TAT peaks at about five hours after the onset of darkness, showing a four-fold increase in activity compared with levels measured during the light period. When rats are maintained on controlled feeding regimes, the amount of light they are subjected to each day is controlled and may vary from one regime to another. On these regimes they are fed for a fixed period of time, the time at which they are fed being constant from day to day. So for example, a '12-12' regime represents twelve hours dark and twelve hours light, the animals being fed for the 12 hours of darkness. In an '8-16' regime the animals are maintained again on 12 hours of light and 12 hours of darkness being fed for the first eight hours after the onset of darkness and then starved for 16 hours, four of which are during the dark period and 12 hours during the light period. More dietary tryptophan would be available for uptake by the liver shortly after feeding at a time when glucocorticoid levels have already increased. It may therefore be possible that TAT messenger ribonucleic acid (mRNA) is already present but is only translated into TAT enzyme when tryptophan concentrations increase after feeding. Tryptophan concentrations have a special significance in the control of hepatic protein synthesis, both in the formation and availability of ribosomes and the disaggregation of polysomes (Wunner et al, 1966; Freminet et al, 1968). Omission of tryptophan from the diet results in the reversible disaggregation of polysomes.

In studying the regulation of the level of an enzyme it is of great importance to determine whether there is a time dependenc

variation in enzyme activity since a several fold increase in enzyme activity may be attributed to a particular treatment before it is recognised that an identical rise occurs at the same time of day in untreated animals.

As tryptophan also plays a role in the induction of urea cycle enzyme levels (Muramatsu and Nakagawa, 1971), ad lib. feeding may result in a great spread in the induction of enzymes in different animals depending on when they are taking food. Since in the experiments of Das and Waterlow (1971) the rats were maintained on controlled feeding regimes, the induction of urea cycle enzyme levels may be more synchronized and so appear to occur within a shorter time span. If this were so it would not be inconsistant with the fact that the half-lives of the urea cycle enzymes appear to be shorter in these experiments compared to those of other workers (Schimke, 1964; Szepesi and Freedland, 1969) where the experimental animals were not maintained on controlled feeding or lighting regimes.

A number of different types of rhythms are seen in biological systems, those with high frequencies (from half and hour to two to five days) and those with low frequencies (greater than five days) (Vanden Driessche, 1975). Circadian rhythms have great adaptive advantage, for since the rhythm is initiated in advance, the organisms will have reached the optimal performance state before external conditions are attained to which the rhythms are adaptive. For example, in rats which are nocturnal feeders, the levels of glucocorticoids increase at about two hours before the onset of darkness and hence of feeding. This allows for the more efficient uptake of amino acids at the time when they become available, after feeding and digestion has been initiated.

The potential for circadian rhythms must be hereditary since the natural periods of oscillation are specific. They show a dependence on transcription since the rhythms are impaired if RNA synthesis is inhibited but are independant of protein synthesis (Vanden Driessche, 1975).

Several models for the generation of these rhythms have been proposed depending on interactions of light, temperature, geophysical factors and metabolite availability and are reviewed by Vanden Driessche (1975). Biological rhythmicity influencing hormonally inducible events such as DNA synthesis, RNA synthesis mitotic index and liver enzyme activities have been reviewed by Glaser (1975).

1.3.4 The Rate of degradation of proteins

Schimke and Doyle (1970) have postulated that degradation may play a role in regulating enzyme activities. All protein molecules are available to degradation by proteases which are present in excess at all times. The shifting of cofactor and substrate concentrations as might occur under different physiological and hormonal conditions may lead to a variety of effects on specific enzymes either to stabilize or to labilize them thereby altering their rates of degradation.

Dice et al (1973) have suggested that there is a correlation between the size of proteins and their relative rates of degradation and generally the greater the size the greater the target is for protease attack. This is a general characteristic of eukaryotic soluble proteins. According to this theory it is the size of the subunit rather than the size of the multimer that is important and in heteromultimers the larger subunit is degraded more rapidly than the smaller. This theory of degradation is however, still in need of confirmation.

1.3.5 Hormone Effects

1.3.5.1 Effects and mechanism of action of glucagon and cAMP

Cyclic AMP has been recognised as the second messenger for a number of different hormones such as adrenocorticotropic hormones, thyroid stimulating hormone, vasopressin, luteinizing hormone, parathyroid hormone and glucagon (Strada and Robinson, 1974).

Rall and Sutherland (1958) found that glucagon administration causes a large increase in hepatic cAMP levels. It now seems that many of the metabolic effects of glucagon are mediated by cAMP (Kenney, 1970).

Glucagon interacts with adenylcyclase in the membrane which in turn catalyzes the formation of cyclic AMP from ATP.

A. Glucagon Receptors

The receptor of glucagon, namely the adenylcyclase system is found in the plasma membrane and sarcoplasmic reticulum of muscle in all eukaryotes (Strada and Robinson, 1974). The receptor is a two-component system embedded in the lipid matrix of the cell membrane. It consists of a regulatory subunit in contact with the extracellular space with receptors for one or more hormones and a

catalytic subunit in contact with ATP in the cytoplasm. Interaction of hormone with the receptor results in a conformational change which activates the system and converts ATP to cAMP.

Rodbell et al (1971) showed that discrete regions of the glucagon molecule, which is a polypeptide of 29 amino acids are important for receptor binding. The entire molecule is the active species but histidine at the NH_2 terminal end is unimportant for binding. At physiological concentrations (10^{-11}M) glucagon exists as a monomer and the glucagon receptor complex is stabilized by hydrophobic interactions (Sasaki et al,1975). The flexibility of the structure of glucagon allows rapid degradation by proteolytic enzymes thus giving sophistication in control.

Hormone activation of adenylcyclase requires ATP or GTP which bind reversibly to allosteric sites on the enzymes molecule (Rodbell, 1973). These nucleotides increase the rate of binding and dissociation of glucagon from the regulatory component.

The regulatory component exists in two states which are in equilibrium and these bind glucagon with different rates of association and dissociation. Only one of the states is competent in activating adenylcyclase. GTP or ATP act at topographically distinct sites which results in the shifting of activity to the active form. The competent form has the appropriate association and dissociation rate constant for glucagon to account for rapid onset and cessation of hormone action (Rodbell, 1973).

In rats, the adenylcyclase system is present and responsive to glucagon and isoproterenol four days prior to birth (Butcher and Potter, 1972). They demonstrated a close parallelism between the age-dependent effect of glucagon on increasing cAMP levels and TAT induction. Adenylcyclase is present and responds to glucagon during gestation prior to TAT induction by glucagon.

cAMP concentration is controlled not only by its rate of synthesis by adenylcyclase, and its rate of release into the extracellular fluid but also by its breakdown by the enzyme phosphodiesterase (Strada and Robison, 1974; Schmidtke et al, 1976). There is evidence that this enzyme exists in multiple molecular forms and that it is sensitive to different hormones. It is believed that insulin

activates phosphodiesterase thereby preventing the accumulation of cAMP (Strada and Robison, 1974). Glucocorticoids and other steroids inhibit phosphodiesterase activity and it has been speculated that many "*permissive effects*" *of glucocorticoids on* cAMP mediated responses reflect the direct interaction of these hormones with phosphodiesterase.

B. Regulation of protein synthesis by cAMP

(i) Effects of cAMP

Most work in this field has been done on the regulation of synthesis of tyrosine aminotransferase (TAT) and is extensively reviewed by Wicks (1974). It was found that glucagon and cAMP act selectively in the liver in contrast to other tissues. They stimulate hepatic protein catabolism. Cyclic AMP does not mediate the action of glucocorticoids; rather, different mechanisms operate (Kenney, 1970). TAT is not activated by these hormones but synthesis of new enzyme molecules occurs (Wicks, 1968a, b; 1969), as verified by immunological assay (Wicks, 1971).

TAT is only feebly induced by cAMP and the action of this substance is greatly enhanced by theophylline, an inhibitor of phosphodiesterase activity (Wicks 1968a, b). TAT is also inducible by glucagon and insulin whose effects are mediated by cAMP. Dibutyryl cAMP (dBcAMP) induces TAT much more strongly than either glucagon or isoproterenol and its effect is not enhanced by theophylline (ibid). One possible action of cAMP may be the release of nascent polypeptide chains from polysomes although this view is not widely held. It has been shown to cause the release of TAT protein molecules from washed polysomes of neonatal rat liver (Chuah and Oliver, 1971).

The microsomal factor responsible for the release of polypeptide chain by cAMP in vitro has been purified and is found to be a protein of molecular weight 40 000, capable of binding cAMP and possessing cAMP-dependent protein kinase activity (Donovan and Oliver, 1972).

Although these experiments are provocative and do provide direct evidence for an effect of cAMP at the translational level, there are several features of the system which deserve comment.

Firstly, the release of TAT from the washed polysomes required very high concentrations of cAMP. The release of phosphoenol-pyruvate carboxykinase is not detectable in the neonatal microsome-polysome system in spite of the fact that characteristics of its regulation by cAMP are essentially identical to that of TAT (Wicks, 1969). Finally, no direct evidence has been provided that the TAT released is truly nascent polysome-bound TAT. Even with these objections it is difficult to escape from the suggestion that the release of TAT in neonatal liver may present a unique developmental phenomenon that is unrelated to induction of TAT in mature animals. The fact that the cAMP-dependent release factor is present in adult liver but that release of polypeptide does not occur in adult microsomes suggests that some change occurs in the polysomes during development.

Studies using inhibitors of RNA synthesis support the suggestion that cAMP acts at the level of translation (Holt and Oliver, 1969; Wicks et al, 1973; Butcher et al, 1972).

Some examples of cAMP and glucagon acting at the transcriptional level have been found and are reviewed by Wicks (1974). Glutamate dehydrogenase has been found to be inducible by glucagon and dBcAMP in an actinomycin D-sensitive process (Yaroni and Halinsky, personal communications, unpublished). Ornithine decarboxylase is also inducible by an actinomycin D-sensitive process (Beck et al, 1972) as is serine dehydratase (Peraino and Pitot, 1964). Dibutyryl cAMP may be a more potent inducer than cAMP since it is resistant to hydrolysis by phosphodiesterase (Moore et al, 1968)

(ii) Mode of action

a) Translational control

Where mechanisms of physiologically relevant effects of cAMP have been elucidated in mammalian cells they are found to involve protein phosphorylation catalyzed by the cAMP-dependent protein kinase resulting in the phosphorylation of nuclear acid proteins and nuclear histone proteins (Eil and Wool, 1973; Strada and Robison, 1974). Phosphorylation of ribosome - associated proteins

results in a slight shift in the affinity of subunits for each other (Walton and Gill, 1973) with a slight increase in synthesis of polyphenylalanine in vitro (Eil and Wool, 1973; Eil and Wool,1973b) although this change is probably not significant.

It is conceivable that growing nascent polypeptide chains of certain inducible enzymes are substrates for ribosome-bound-cAMP-dependent protein kinases. This may facilitate elongation, or termination by favourably altering the conformation of the complex in the vicinity of the polypeptide growing point. Upon the release of the completed enzyme, specific phosphatases may remove the phosphate moiety and the native enzyme in the cytosol would no longer be the substrate for protein kinases as is true for TAT and phosphoenol-pyruvate carboxykinase (Wicks et al, 1972).

The possibility exists that the messenger RNA's (mRNA) of all inducible enzymes may have a common 5' sequence allowing for discrimination in competition for ribosomal subunits in forming complexes when a common ribosome-associated protein is phosphorylated. Some evidence exists as to discrimination in the translation of certain mRNA's in eukaryotic cells (Rourke and Heywood, 1972; Nudel et al, 1973).

b) Transcriptional control

Transcriptional induction has been observed in isolated nuclei, liver slices and whole animals. cAMP-dependent phosphorylation of nuclear non-histone proteins may play a role in the induction of specific species of mRNA since the addition of phosphorylated nuclear nonhistones to rat DNA results in an increased synthesis of RNA, (Watson and Langan, 1973). It remains to be established that the synthesis of specific mRNA's is promoted in this process.

The possibility must be considered that cAMP-dependent phosphorylation of nonhistone nuclear proteins plays a role in enzyme induction (Johnson and Allfrey, 1972) in view of the report that addition of phosphorylated nonhistone chromatin proteins from rat liver stimulates RNA synthesis in vitro when naked rat liver DNA was used as the template (Shea and Kleinsmith, 1973).

1.3.5.2 Mechanism of action of glucocorticoids

Steroid hormones act via a complex coordinated set of reactions which alter the cellular phenotype through alterations in gene expression. Many examples are known showing changes in specific enzymes in the target cells. Steroids are very small molecules (MW 300) and are therefore capable of passing through the plasma membrane into the cytoplasm where they bind with receptors specific to the target tissue (Rousseau, 1975). Specific receptors are found in the rat thymus, liver, kidney, brain, skeletal heart muscle, testis, lung, stomach, spleen and several transformed cultures (O'Malley et al, 1970; Golder et al, 1972)

Steroids are transported in the blood by the corticosteroid binding protein (CBG), transcortin, which is a specific α-globulin, and to a lesser extent by albumin. Less than 10% of the foetal blood steroid (10^{-9} M/l) is unbound and it is the unbound steroid which is believed to have physiological consequence in interaction with the target tissue. Transcortin may mediate the kinetics of inactivation of cortisol by the liver. Dexamethasone and β-methasone, synthetic steroids, do not bind to transcortin and this may account for their greater biological potency.

A. Receptors

Much work has been done on the identification and purification of the cytosolic receptors of glucorticoids and other steroid hormones (Higgins et al, 1974; Litwack et al, 1973; Ribarac-Stepić et al, 1973; Rousseau et al, 1973; Litwack and Rosenfield 1975; Acs et al, 1975); O'Malley and Schrader (1976) have reviewed the literature for information on these receptors and their mechanism of action.

(i) Glucocorticoid receptors

Several workers have demonstrated the existence of cortisol-binding proteins in the liver. Beato et al. (1969) showed that

H^3-cortisol binds specifically to soluble proteins in rat liver. Later studies by these authors (1970) and by Rousseau et al. (1973) suggest that the function of these receptors is to facilitate the movement of the glucocorticoid to the nucleus in a temperature-independent transfer process. The binding of the glucocorticoid to the cytosolic receptor is temperature-dependent (Kalimi et al., 1973). Once the steroid-receptor is transferred to the nucleus, from 4 000 to 8 000 nuclear binding sites become evident. The possibility exists that only a small fraction of receptor-glucocorticoid complexes are involved in meaningful interactions with the genome and it is possible that the complex interacts with nonstructural repeated sequences of DNA corresponding to evolutionary-related glucocorticoid responsive genes of different target tissues. Short et al. (1970) found at least three different receptors of glucocorticoid in the liver with varying affinities for the steroids. Wira and Munck (1970) and Mosher et al. (1971) have demonstrated the existence of glucocorticoid-specific receptors in thymus cells and these appear to be localized in the nucleus.

Tsai and Hnilica (1971) found that a small amount of cortisol binds to a trypsin-resistant fraction of the arginine-rich histones and they therefore suggest that cortisol probably binds to a non-histone protein associated with the arginine-rich histones. It is however not known whether this interaction turns on the transcription of specific structural genes or turns off the transcription of specific "repressor" genes (Wicks, 1974). Since glucocorticoids interact with receptors and are then translocated to the nucleus, as are the sex steroids, it is not inconceivable that their mechanism of action is very similar to that of the sex steroids. Since a great deal of work has been done on the sex steroids they will be discussed in some detail. The chemical structure of all steroids is similar and therefore the possibility exists that the glucocorticoid-receptors are similar and interact in a similar way to that of other steroids and, in particular, to the sex steroids.

(ii) Sex steroid receptors

Although much work has been done on glucocorticoid receptors, the best understood steroid receptor system is that of the sex steroids.

Since the chemical structure of all steroids is so similar the possibility exists that the glucocorticoid receptors are similar to and interact in a similar way to that of the other steroids and in particular to the sex steroids.

It was found that in female rats, radioactive oestrogen was retained longer in the uterus than anywhere else (O'Malley and Schrader, 1976) and it was found that in target cells, radioactive steroid accumulates in the nucleus and that movement into the nucleus precedes all other changes.

It was found that oestrogen bound selectively to a protein MW 200 000 which is present only in target cells and bound very tightly to oestrogen and it was found that the receptors not only sequestrated the hormone molecules in the target cells but also concentrated them in the nucleus (O'Malley and Schrader, 1976).

O'Malley and Means (1974) found that the hormone-receptor complex binds directly to the chromatin and that there were about 5 000 acceptor sites per nucleus for this hormone-receptor complex. They also found that the hormone-receptor complex binds directly to isolated chromatin, progesterone-receptor complex binds preferentially to chromatin from oviduct nuclei compared with other tissues and that the receptor alone does not bind chromatin. At least five different steroid binders have been recognised with different molecular weights and different affinities for steroids, these being Binders I and III (Morey and Litwack, 1969), Binders II and IV (Litwack et al, 1973) Binder IIB (Litwack and Rosenfield, 1975).

The steroid binding protein is located in the AP_3 fraction of nonhistone chromosomal proteins (O'Malley and Schrader, 1976). Chromatin stripped of histone bound the complex as tightly as with histone. The receptor-hormone complex interacts with all DNA irrespective of origin when stripped of protein, therefore the DNA is not responsible for specificity (O'Malley and Schrader, 1976).

The progesterone receptor has been purified and found to be a dimer of two nonidentical subunits of MW greater than 100 000. The dimer is cigar shaped with the A and B subunits lying side by side and each containing a simple hormone receptor site. The dimeric receptors have no affinity for naked DNA. Subunit A does not bind to intact chromatin but binds very strongly to naked DNA. The B subunit has no affinity for naked DNA but binds to intact chromatin (ibid).

B. Mechanism of action of steroids

O'Malley and Schrader (1976) have proposed the following model for the mechanism of action of sex steroids. The steroid interacts with the cytoplasmic receptor which exists as two conformers one active and one inactive. Only a small proportion of these are in the active conformation and these are responsible for the basal enzyme levels (Rousseau, 1975). Once the hormone binds to the receptor there is an activation followed by a temperature sensitive translocation of the complex to the nucleus (Ishii et al, 1971; Beato et al.,1973). In the nucleus the complex binds via the B subunit to particular nonhistone proteins of the AP_3 fraction. The dimer may then disaggregate or separate and the A subunit hormone complex may interact directly with the DNA. It is unknown whether A recognises specific nucleotide sequences or whether it binds to positions whose specificity is defined by the B subunit binding specificity.

Using rifampcin, an antibiotic which inactivates all RNA polymerase bound to initiation sites, it was found that the number of initiation sites is directly proportional to the number of hormone-receptor complexes present (O'Malley and Schrader, 1976).

in vivo hybridization techniques using copy DNA (cDNA) of chick ovalbumin mRNA showed that the number of copies of this specific messenger rose from zero to 10 000 molecules per cell within a matter of hours of hormone treatment. In *in vitro* experiments using chromatin in cell free extracts it was found that new ovalbumin mRNA is synthesized only in the presence of the hormone-receptor complex, not in the presence of hormone only and only if the receptor was present as the dimer.

Kalimi et al. (1973) have proposed a model implicating the receptor-hormone complex wherein the complex acts as a positive regulator of gene activity, similar to the regulation of the lac-operon in *E. coli* by the cAMP binding protein. Final expression depends on additional negative controls which may or may not be under hormonal regulation .

C. Effects of glucocorticoids

One of the primary actions of glucocorticoids is cell growth inhibition and concomitant expression of specific enzymes (Rousseau, 1975). In the thymus of rats and mice there is a decrease in activity of RNA polymerase after glucocorticoid treatment. The DNA template is much more active after cortisol treatment (Homoki et al, 1968) and this is attributed to increased nucleolar ribosomal RNA polymerase rather than an increased availibility of the DNA template in the chromatin.(Yu and Feigelson, 1971).

In young rats cortisone treatment results in the rapid decrease in DNA synthesis due to a drop in the activity of nuclear DNA polymerase (Henderson et al, 1971) without an effect on mitochondrial DNA polymerase (Kimberg and Loeb, 1971). This effect is seen in most tissues including hepatomas (Henderson et al,1971). RNA and protein synthesis increase after cortisol treatment but with prolonged treatment there is a drop in the induction of these molecules implying that continued DNA synthesis is required for the stimulation of transcription. Inhibition of DNA synthesis is common in tissues where glucocorticoids are stimulatory, namely in liver and malignant cells in culture. There is an inhibition of DNA synthesis and growth of livers in partially hepatectomized rats treated with glucocorticoids (Jones and Logan, 1972).

Cortisol specifically induces particular enzyme proteins by an actinomycin D-sensitive process (Raina and Rosen, 1968; Peraino et al, 1966). Schwartz (1972) found that glucocorticoid action on embryonic chick retina results in the biosynthesis of glutamine synthetase itself through altered genomic expression.

Several of the permissive effects of glucocorticoids for cAMP-mediated responses are thought to occur by inhibition of phosphodiesterase activity (Nanganiello and Vaughn,1972).

Evidence suggests that the earliest manifestation of glucocorticoid action is the initial inhibition of protein synthesis (Yim and Kim, 1975).

Shelton-Karp (1974) found that glucocorticoids play a role in the normal diurnal variation of chromatin availability in the liver since adrenalectomy greatly reduces the nocturnal rise and an intraperitoneal

injection of hydrocortisone (50 µg/100g) eight hours prior to killing adrenalectomized animals restores the nocturnal rise in chromatin availability.

1.3.5.3 Hormonal effects on the urea cycle enzymes

The polypeptide hormones of the pancreas, insulin and glucagon, have been implicated in the regulation of a large variety of hepatic enzymes (Kenney, 1970). In their action on the urea cycle enzymes they are antagonistic to each other. McLean and Novello (1965) found that glucagon increased the level of urea cycle enzymes, but this increase is probably not caused merely by increased amino acid catabolism with the release of free ammonia for detoxification; rather, the effect appears to be more specific, since in the in vitro experiments of Snodgrass and Lin (1976) where the amino acid supply is not limiting, inductions in the levels of urea cycle enzymes were still observed. Insulin is antagonistic to glucagon since it causes nitrogen retention (ibid) and these results are in agreement with those of Mortimore (1972).

In prenatal rats the arginine synthetase system, namely argininosuccinate synthetase (ASS) and argininosuccinate lyase (AL) are inducible by dBcAMP, glucagon and triamcinolone. The effects of dBcAMP and glucagon are nonadditive suggesting that their mechanism of action is the same but, the effects of dBcAMP and triamcinolone are additive. The latter suggests different mechanisms of action for cAMP and the steroids in the induction of this system (Schwartz, 1972). The effect of glucagon on the arginine synthetase system was confirmed by Räihä and Schwarts (1973) who found that a single intraperitoneal injection of glucagon (100µg/animal) induced ASS and AL in five day old rats but not in foetal or new born rats. In vitro, glucagon and dBcAMP double the activity of the arginine synthetase systems from 19,5 day foetal explants. Noxley et al. (1974) have demonstrated a 54% increase in urea synthesis in rats treated with glucagon, a 24% increase with epinephrine and a 29% increase with parathyroid hormone. All of these act by activating adenylcyclase thereby increasing intracellular levels of cyclic AMP.

Corticosteroids have been shown to increase tissue breakdown and there is a co-ordinated increase in the activities of urea cycle enzyme activities (Schimke, 1963). Adrenalectomized rats show a

concerted drop in the activities of the cycle, the cytoplasmic enzymes dropping more than the mitochondrial ones, with arginase showing the greatest drop (Schimke, 1963; McLean and Gurney, 1963). Cortisone acetate-treated adrenalectomized rats show a recovery in the activities of arginase, AL and ASS, with arginase responding soonest.

Adrenalectomy performed on the thirteenth postnatal day prevents the normally observed developmental rise in arginase and OTC activities. The observed increase in these activities following a 48 hour starvation period is prevented by adrenalectomy (Illnerova, 1966).

In vivo administration of corticosteroids to adult rats results in a low increase in the levels of urea cycle enzymes observable only after several days of repeated treatment (McLean and Gurney, 1963; Schimke, 1963). In fact in animals aged 29 days and more only daily injections for three days resulted in a significant increase in arginase activity (McLean and Gurney, 1963; Christowitz, 1976)

Krečák and Palatý (1967) have proposed that several critical periods in ontogenic development exist. These involve different target tissues becoming sensitive to the appropriate hormone at specific times as well as different isozymic patters becoming apparent at different stages of ontogenic development. The first of these critical periods occurs during the first postnatal week and a second one falls between the second and fourth week during the period of weaning. Since day 28 of postnatal development represents the last day of weaning, this could account for the fact that on day 29 and beyond the regulatory mechanism operating in rats are different as the second critical period of development has been completed. These periods are so critical that by altering the environmental conditions of young rats it is possible to permanently alter the development of the adults (Krečák and Palatý, 1967). Premature weaning of the rats results in altered regulation of the oestral cycle, altered fertility and altered activity of the adrenal glands.

Schimke (1963) found that intact adrenals were not necessary for the response of urea cycle enzymes to a high protein diet and from this concluded that cortisol is not involved in the regulatory response of the enzyme to dietary protein level.

1.4 REGENERATION OF RAT LIVER AFTER PARTIAL HEPATECTOMY

1.4.1 General

When rats are subjected to partial hepatectomy wherein 70 percent of the liver is removed, the remaining cells begin to grow and divide in order to replace the cells that have been lost. The metabolism and ultrastructures of the remaining liver cells begin to alter very soon after the operation and within three weeks the volume of the remaining liver lobes has increased to that of the liver prior to the operation (Higgins and Anderson, 1931). Electron microscopy studies by Hradil et al. (1965) and Virogh (1966) showed that on the first day after partial hepatectomy the liver undergoes disorganization. On the second day "dark cells" rich in organelles and clear cells containing few organelles are distinguishable. In the early stages of regeneration the Golgi apparatus appears hypertrophied and the bile canaliculi increase in number and become dilated. These phenomena may reflect the increased load imposed by the secretion of bile upon the reduced liver parenchyma. Ultrastructural appearances of hepatocytes are normal by 168 hours following partial hepatectomy (Kamura and Asushi, 1969).

Intracellular communication was examined in partially hepatectomized rats and found to be as good as in normal livers in contrast to the lack of communication in tissues with cancerous growth (Lowestein and Penn, 1967).

Electrophoresis of blood serum from partially hepatectomized rats revealed abnormal fractions in the α_2-globulins and albumin similar to the abnormal fractions previously called the metabolic parameter of cancer which is unique to neoplastic processes (Laperda et al., 1972).

Total liver protein, RNA and DNA has returned to normal by ten days after partial hepatectomy. A maximum in the labelling and mitotic index occurs between 12-48 hours after the operation (Gerhard, 1975). The first two days of regeneration are compensatory and have one round of replication and mitosis. The ploidy is 3.6c per cell and is equal in all cells. In normal nondividing cells the average ploidy is 2c. Days three to fourteen are reconstructive and there is an increased number of cells with a slight decrease in ploidy.

The total number of hepatocytes increases 2,6 times by the end of regeneration and by the end of the first week 85% of the total cell number is regained (ibid).

Pyrimidine and DNA synthetic enzymes increase while the catabolic enzymes decrease during the post-operative period. The changes include an increased capacity to synthesize DNA (the polymerase peaks at 48 hours) and pyrimidines, and a decreased potential for catabolism and recycling these metabolites. This confers a selective advantage to regenerating liver cells and provides an increased replicative potential (Holtzer and Denken, 1975). DNA polymerase II increases 6-10 fold, 18-30 hours after partial hepatectomy (Baril et al, 1973).

The RNA synthetic capacity of regenerating liver nuclei greatly exceeds that of normal liver nuclei, this being largely due to an increase in the number of DNA sites available for transcription in the DNA-dependent-RNA polymerase reaction (Pogo et al, 1969). Berg et al. (1967), using radioisotopic techniques, found that an increased rate of synthesis of nuclear RNA occurs twelve hours after partial hepatectomy and cytoplasmic labelling occurs between fifteen and eighteen hours.

Fausto et al. (1968) found three cytoplasmic RNA peaks, a 4S, an 18S and a 28S peak; 4S was labelled first, 18S specific activity peaked at 12 hours and that of 28S at 24 hours post operatively.

Kalf et al. (1971) showed that DNA synthesis in vitro is stimulated by factors present in post microsomal fractions of regenerating, neoplastic and foetal liver but absent from normal tissue. La Breque et al. (1973) have isolated an hepatic stimulator substance of hepatic regeneration and found it to be soluble, heat stable, sensitive to TCA treatment and to have a molecular weight greater than 10 000. Schumm et al. (1973) have isolated nondialyzable compounds from the cytosol that act post transcriptionally to regulate the processing and/or transport of mRNA and found that differences exist in those factors in normal, regenerating and neoplastic liver.

Within 18 hours of partial hepatectomy, the ratio of histone to DNA drops by 10 to 15%. The kinetics of the change correlate with increased template activity (Garrard and Bonner, 1973). Within 12 hours a 20% turnover in histone I is detectable. Within one and

a half hours histone-bound proteases increase two-fold. These may be involved in the displacement of histone from the chromatin leading to gene derepression (ibid.).

By 30 hours of regeneration the in vivo rate of protein synthesis has increased by 60% compared with control rates. There are approximately 10% more ribosomes bound as polysomes in regenerating liver compared with normal liver; the rate of translation is constant, therefore the rate of initiation must be greater in ribosomes of regenerating versus normal liver cells (Scornik, 1974).

Walker and Potter (1972) have studied the effect of partial hepatectomy on altering isozyme patterns of hexokinase, aldolase and pyruvate kinase. Changes in these isozymes following partial hepatectomy suggest that when hepatocytes need to undergo cell division they cease to produce some of the isozymes characteristic of the fully differentiated state and revert to the production of isozymes characteristic of the foetal stage of ontogeny. For example, within 23 hours of partial hepatectomy there is a large increase in type-III pyruvate kinase with a concomitant decrease in type-I pyruvate kinase (Tanaka et al, 1967; Walker and Potter, 1972) and glucokinase (Lea et al, 1970; Walker and Potter, 1972). There are no changes in either aldolase or hexokinase isozyme patterns (Walker and Potter, 1972).

Hopkins et al (1973) found that in rats maintained on an 8-16 feeding regime thymidine kinase activity and thymidine incorporation into liver DNA exhibited marked daily oscillations during liver regeneration. Yanagi and Potter (1977) found that thymidine kinase (TK), TAT and ODC activities were stimulated sequentially by partial hepatectomy in the livers of rats maintained on controlled feeding and lighting schedules. The earliest response was for ODC at 2 hours, reaching the maximal level by 4 hours, while the activity of TK peaked only at 48 hours postoperatively.

The same authors observed a significant decrease in the activity of ornithine amino transferase by 24 hours and although a drop was seen in the activity of serine dehydratase, the drop appeared to be unrelated to the regenerative process since the lowered level of enzyme activity was also found in sham operated animals (ibid).

1.4.2 Regulatory mechanisms operating in regenerating rat liver

1.4.2.1 Dietary effects on the proliferative response

Starved rats (48 hours) show a five hour delay in the proliferative response which is probably due to a deficiency of RNA and protein required for proliferation or perhaps the energy requirements are not met (Stirling et al, 1973). Starvation may activate stress pathways resulting in cortisol release which may then inhibit the mitotic index. Administration of fairly large doses of cortisol for three days prior to partial hepatectomy results in the inhibition of the mitotic index. Protein deprivation does not alter the flow of ribosomal RNA from the nucleus into the cytoplasm but does slow down the entry of cells into mitosis (ibid).

When partially hepatectomized rats were maintained on controlled feeding and lighting regimes they displayed two peaks in DNA synthesis, the first appears at 23 hours and is independent of the time of the day, the second appears between 5 and 72 hours and is dependent on and follows the stimulus of the controlled feeding schedule (Barbirroli and Potter, 1971).

Schulte-Herman (1975) has stressed the importance of the feeding regime in the appearance of DNA synthetic rhythms in proliferating cells. He found that in rats maintained on different regimes with ad lib. versus controlled feeding, the rhythmic variations in feeding were responsible for the synchronization of proliferation.

If partially hepatectomized rats were fed at the end of the light phase, DNA synthesis peaked at 20 and 37 hours after the operation. Rats fed at the end of the dark phase showed peaks at 23 and 47 hours. Hence the first peak in synthesis is responding directly to partial hepatectomy whilst the second peak is dependent on the feeding regime.

Rats maintained for three weeks in the light and fed ad lib. show no diurnal fluctuations in DNA synthesis (ibid).

1.4.2.2 Hormonal effects on the proliferative response

The effect of glucocorticoids as inhibitors of DNA synthesis is well known (Kim and Kim, 1970) and hormone treatment of animals

for three days results in an 80% drop in DNA polymerase activity (Henderson and Loeb, 1970). DNA synthesis and liver growth is inhibited by glucocorticoids in partially hepatectomized rats (Thomson and Lippman, 1974). There is also an inhibition of thymidine and thymidylate synthesis in corticosteroid-treated partially hepatectomized rats (Sakuma and Terayama, 1967).

Cyclic-AMP concentration displays two waves of increase following partial hepatectomy, one at three hours and one at 12 hours. The second wave of cyclic-AMP accumulation appears to be associated with the initiation of DNA synthesis (MacManus et al., 1973).

Other workers have shown, however, that high concentrations of cAMP inhibit cell growth (Bombik et al., 1973; Holtzer and Denken, 1975). Low levels of cAMP are found in malignant cells and in liver 24 hours after partial hepatectomy and in cells involved in mitosis, whereas cells in the stationary phase display high levels of cyclic AMP.

Phosphodiesterase activities are very much greater in rapidly dividing tissues than in nondividing cells (146% greater after partial hepatectomy, 433% greater in rapidly growing tumours) and so it appears that low levels of cyclic AMP allow proliferation.

The discrepancy in these results may be explained by the fact that MacManus et al. (1973) used β-andrenergic blockers to inhibit the second wave of cAMP accumulation and found a parallel inhibition in DNA replication. These inhibitors may have secondary effects on proliferation, independent of the cAMP response.

Morley et al. (1975) measured serum hormone levels after partial hepatectomy and found that glucagon had increased by 6 hours, insulin remained unchanged for 72 hours, growth hormone dropped between 6 and 48 hours and thyroxine dropped between 24 and 48 hours.

1.4.3 Relationship between polyamine and urea biosynthesis

The polyamines putrescine, spermidine and spermine occur in considerable concentration in all plants and animals studied. These substances have been implicated in transcriptional and translational controls, in cell division and in protein and RNA synthesis (Fausto et al., 1975). Polyamines permit more complex folding of RNA and in this way may stabilize the primary structure. They could act by removing newly transcribed RNA from the DNA-enzyme complex thereby making more enzyme available for RNA synthesis. It has been suggested that transcriptional regulation is achieved by the polyamines

interacting with the nucleic acids with neutralization of the negative phosphate groups and stabilization of the structure. Polyamines have also been implicated in polynucleotide chain initiation, elongation and chain selection.

Translational controls are thought to involve ribosomal subunit association, methylation of tRNA species, aminoacylation of tRNA and aminoacyl-tRNA binding to the 30S ribosomal subunits (ibid).

In rapidly dividing tissue such as regenerating rat liver the concentration of spermidine increases in parallel with RNA content for the period 24-96 hours after partial hepatectomy (Russel and Lambardini, 1971).

One of the earliest responses measurable after partial hepatectomy is the rapid increase in activity of ornithine decarboxylase (ODC) (Hölta and Jänne, 1972), the enzyme catalyzing the rate limiting step in polyamine biosynthesis (Tabor and Tabor, 1972).

Increases in ODC activity occur immediately after partial hepatectomy reaching a maximum 4 hours after the operation when levels are 17 fold greater than that of controls (Yanagi and Potter, 1977). The increase in ODC activity is blocked by an injection of puromycin or cycloheximide anytime within the first 24 hours after partial hepatectomy (Fausto, 1968), and by actinomycin D within the first hour. These results suggest the de novo synthesis of new ODC protein. The half life of ODC is measured as 11 minutes.

Ornithine levels increase within the first two hours and by 18 hours are four to five times greater than in sham operated controls (Fausto et al, 1975). The balance of ornithine metabolism depends on the relative activities of ODC, OTC, and OAT. The activity of OTC is very much greater than that of ODC in normal livers but in neoplasia there is a great increase in ODC activity with a corresponding drop in OTC (Weber et al, 1972; Williams-Ashman et al, 1972).

Bhide (1971), Weber et al (1972), Fausto (1975) Jänne and Hölta (1975) have been unable to measure a corresponding drop in OTC activity in the early hours of regeneration following partial hepatectomy but Bhide (1971) has shown a 40% drop in OTC at 72 hours and Weber (1972) has shown a 17% drop at 24 hours after the operation.

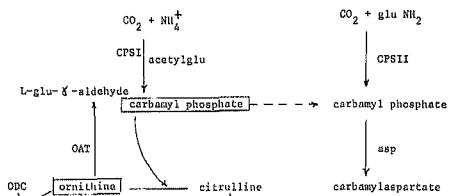
Carbamyl phosphate is required for the synthesis of both urea and pyrimidines. It is possible that excess carbamyl phosphate may be channelled to pyrimidines by CPS I, the urea cycle isozyme. Such a cross over has been seen in certain inborn errors of metabolism (Bourget et al, 1971) and has been demonstrated experimentally in liver slices. This crossover requires that carbamyl phosphate be transported across the mitochondrial membrane.

Excess ammonia may be channelled through glutamate to orotate biosynthesis (Jones, 1972). The relationship between urea, polyamine and pyrimidine biosynthesis is shown in figure 1.2 (see p.35).

Both L-arginine-glycine aminotransferase (E.C.2.1.4.1.) which catalyzes the reverse formation of L-ornithine and guanidoacetic acid from glycine and arginine, the key step in creatine biosynthesis, and ornithine aminotransferase (OAT), which catalyzes the formation of glutamic 2-semialdehyde from L-ornithine and ketoglutarate, utilise ornithine (Weber et al, 1972)

The relative amounts of these enzymes and the choice of the pathway to which ornithine is channelled plays an important role in the amount of urea synthesized and hence the activities of urea cycle enzymes. Adaptation of the urea cycle enzymes to the metabolic load imposed on the liver remnant after partial hepatectomy might provide precursors for both polyamine and pyrimidine synthesis during liver regeneration.

Figure 1.2


$CO_2 + NH_4^+$
CPSI
acetylglu
CO_2 + glu NH_2
CPSII
L-glu-γ-aldehyde
carbamyl phosphate
carbamyl phosphate
OAT
asp
ODC
ornithine
citrulline
OTC
carbamylaspartate
urea
argininosuccinate
dihydroorotate
orotate
putrescine
spermidine
spermine (Polyamines)
RNA
UTP
UMP
orotidylate

1.5 CANCER

1.5.1. General

Cancer is a term that encompasses a large group of diseases, all of which are characterized by the loss of control of cell division. Neoplastic transformation introduces into the body a new race of cells with a common ontogeny but differing biologically, structurally and chemically from their ancestors.

A distinct pattern is emerging from studies on minimal deviation liver tumours and that is that in most cases, the enzymes that characterize adult liver occur in these hepatomas at levels that resemble foetal or newborn rat livers during the period of transition. The question therefore arises as to whether each strain of minimal deviation hepatoma is descended from an immature parenchyma liver cell whose further maturation was somehow blocked (Potter, 1969).

Potter et al. (1972) have reviewed the ideas on "oncogeny as blocked ontogeny" where ontogeny is the unfolding of environmental-response *systems for modulating gene expression according to a programmed time* schedule and oncogeny is the accumulation of locked in gene configurations of availability that were scheduled to be unlocked at different times in normal ontogeny.

Much work has been done on the morphological and biochemical differentiation of hepatomas in relation to studies on foetal and neonatal rat liver. In general slow-growing, highly differentiated hepatomas contain many biochemical characteristics of the adult liver type with some properties of foetal tissue. The fast-growing, undifferentiated type hepatomas more closely resemble foetal liver cells and retain fewer of the adult-type characteristics (Walker and Potter, 1972).

Aldolase has three isozymic forms in the adult, Aldolase A being present in muscle tissue, Aldolase B in liver and Aldolase C in the brain (Rutter et al, 1963; Baron et al, 1969). In the foetus Aldolase A predominates in the liver and this is found to be true in precancerous livers of rats fed 3-me-4-dimethylaminobenzene (DAB) (Potter et al. (1972).

Four isozymes of hexokinase have been described (McLean and Brown, 1966). The isozyme with a very high Km for glucose, glucokinase, is found exclusively in the adult liver (Sapag-Hagar et al,

1972). Sugimura et al (1972) have shown that reversion to non malignancy i.e. decarcinogenesis is accompanied by loss of transplantability, changes in membrane properties and a concomitant alteration from foetal type hexokinase isozyme patterns to those patterns seen in normal adult livers.

George Weber (1966) proposed the molecular correlation concept of the ordered pattern of gene expression in neoplasia. This concept postulates the operation of meaningful, ordered and correlated expression of morphological, biological and biochemical behaviour in neoplastic tissue linked with the expression of malignancy and growth rate. This theory takes into account that critical changes must be heritable and that progression in neoplasia must be reflected in progressive etabolic imbalance.

This theory has been reinforced by many biochemical studies such as those on gluconeogenesis in normal versus hepatoma tissue where it has been found that there is a progressive imbalance in gene expression with increased tumour growth. In the hepatoma spectrum, with increased tumour growth there is a parallel decrease in gluconeogenic enzymes and an increase in glycolytic enzymes as is the pattern in foetal livers (Weber, 1972).

A similar correlated series of changes in enzyme activity is seen in the pathways involved in pyrimidine and nucleic acid metabolism. With differentiation there is a decrease in DNA polymerase activity and the overall synthetic pathways with a gradual increase in catabolic activities. In hepatoma, however, there are increases in the activities of the anabolic pathways with correlated decreases in the catabolic ones (ibid). Regenerating rat livers following partial hepatectomy show similar patterns in the alteration of expression of genes involved in DNA metabolism. There is a sharp rise in the incorporation of thymidine into DNA and a decrease in the catabolic utilization of this precursor until 24 to 36 hours post-operatively. This is interpreted to mean that changes result in the repression of the derepressed pathway of thymidine catabolism and in the derepression of the repressed pathway for utilization of thymidine for the synthesis of DNA (Weber et al, 1971).

Utsunomiya and Takeshi (1966) found that all hepatomas had a slightly greater percentage of RNA in a polysome fraction than normal liver and also had a higher proportion of monomer and dimer ribosomes than normal liver.

In 1951 Mider pointed out the pronounced avidity of cancer cells for amino acids which reflects their remarkable capacity for protein synthesis. Burke (1962) studied the development of hepatomas in rats fed DAB and found that during precancerous growth periods there was an increased synthesis of liver and plasma proteins and decreased catabolism of amino acids.

Intracellular communications have been studied using intracellular electrical techniques in primary and transplantable hepatomas (Lowenstein and Kanno, 1967). Normal liver cells communicate with each other through junction membranes which are freely permeable to small ions and probably to larger molecules and ions as in other connected cell systems. Hepatoma cells show no such communication, their surface membrane being a strong barrier to diffusion all around the cell. Modjanova and Malenkov (1973) have studied alterations in surface contact during progression of hepatomas and have found that adhesion properties weaken with progression of the tumour with an increase in the number of isolated cell islands.

Experiments on the determination of protein turnover in Morris hepatomas indicate that there are random defects in the control of degradation of membrane proteins of hepatomas and that the membrane-bound proteins turn over independently of the total particulate membrane (Moyer and Pitot, 1973).

Electrophoresis of the nuclear proteins in normal cells and Novikoff ascite tumour cells indicate both qualitative and quantitative differences (Yeoman et al, 1973a; 1973b). These may involve differences in control proteins, differences in the modification of the same proteins or specific increases in the nuclear metabolism in tumours.

Ouellette and Taylor (1973) have shown that in a specific Morris hepatoma (5123D) there is a greater proportion of transfer RNA molecules capable of accepting amino acids per mg tRNA than in normal liver. This may represent differential rates of tRNA processing and maturation in these tissues.

1.4.2. Ornithine imbalance in neoplastic growth

Weber (1966) showed that tumour cells can synthesize protein and nucleic acids at increased rates whilst amino acid and protein catabolism decreased as compared with that of normal liver. Hepatoma cells while undergoing selection for rapid division and growth have at the same time been selected for a streamlined metabolism. This requires imbalance in key enzymes of various metabolic pathways resulting in imbalance in the opposing and competitive metabolic pathways in the tumour.

The interrelationship of polyamine, urea and pyrimidine biosynthesis is not as important in tumour growth as it is in regenerating liver. Williams-Ashman et al. (1972) showed that ODC activity increases in parallel with an increase in tumour growth rate. In a spectrum of hepatomas studied there was a drop in OTC activity paralleling the increase in tumour growth rate (Weber et al., 1972). Several other studies have also revealed a drop in OTC activity in Morris hepatomas (Jones et al., 1961; Weber, 1973). In some hepatomas OTC activities drop to less than 1% of that of control values (Weber, 1973).

The gradual drop in OTC activity with increasing tumour growth rate may have an increased sparing effect on the availability of carbamyl phosphate and aspartate for nucleic acid synthesis.

With the increasing amounts of precursors for DNA and RNA synthesis there is a concurrent increase in the first two enzymes of orotate utilization viz. aspartate transcarbamylase and dihydroorotase (Weber, 1972). These two enzymes increase in activity with an increase in growth rate of the tumour.

There is an inverse relationship between the activity of OAT and tumour growth rate, therefore it may be sparing ornithine for polyamine biosynthesis in tumour growth (Williams-Ashman, 1972). Since it catalyzes the reversible formation of glutamic-semialdehyde from L-ornithine and α-ketoglutarate, it may catalyze the formation of L-ornithine from glutarate or proline thereby increasing the availability of ornithine for polyamine biosynthesis.

1.5.3. Urea cycle enzymes in neoplasia

Burke and Miller (1956) found that rats fed on DAB showed marked decreases in the ability to synthesize urea. McLean (1964) measured all five urea cycle enzymes in precancerous and tumourous livers induced by DAB and found their activities were greatly reduced compared to that of normal livers.

There have been many reports on the activity of hepatic arginase in experimental animals with transplantable hepatomas and in all cases hepatic arginase activity is depressed (Fujiwara, 1929; Weil, 1935; Greenstein, 1941).

Wu et al. (1967) measured arginase, ASS and AL in various Morris hepatomas and Novikoff ascite tumours and in all cases found the activities lower than in controls. They found that ASS activity was inducible by cortisol in two of the Morris hepatomas studied.

Morris et al. (1964) and Potter et al. (1969) studied the inducibility of urea cycle enzymes in different hepatomas and found no significant changes. Wu et al. (1971) found however that arginase and ASS activities were inducible by cortisol in fourteen different Morris hepatomas studied. They found also that arginase activity in the hepatomas was responsive to cortisol, but not arginase in the host liver. The lack of inducibility of urea cycle enzymes in certain hepatomas may depend on the type of tumour being studied and the rate of growth.

Examination of various transplantable tumours revealed that CPS II activities were correlated with increased tumour growth rate (Hagar and Jones, 1967; Yip and Knox, 1970) whilst CPS I shows an inverse relationship with growth rate i.e. is present in slow growing highly differentiated Morris hepatomas but is absent from fast growing undifferentiated tumours (Lawson et al, 1975). These later studies found that although CPS II was present in normal liver and all Morris hepatomas studied, its activity in the hepatomas was lower than that of the CPS I isozyme.

Ono et al. (1963) and Bhide (1971) found that OTC levels were considerably decreased in hepatomas.

1.5.4. Chemical carcinogenesis

Carcinogenic stimuli can be conveniently divided into two major groups, those agents containing information translatable as such by the cell e.g. viruses and those not containing such translatable information e.g. chemicals and radiation.

The chemical carcinogen after appropriate activation and conversion to a reactive species reacts with several macromolecules including RNA, DNA and protein. Meyer and Barber (1973) have presented evidence to show that DAB interacts with microsomal membranes to alter their conformations which could account for the loss of activity of certain microsomal enzymes.

Within a short time, after treatment, some liver cells divide,

the new population having acquired the ability to grow in vivo under conditions where normal cells would not. Treatment with 3-me-DAB results in alterations in the DNA structure. One of the early consequences of 3-me-DAB ingestion is the repair synthesis of DNA but presumably in the proliferating cells the DNA has been replicated before full repair of the altered DNA was possible (Potter et al, 1972)

Once initiated the ' nching and distortion of DNA could become increasingly grea :r as the cells continue to proliferate. This could result in the progression of the hyperplastic nodule until the point when a malignant tumour forms.

During the early stages of carcinogenesis by DAB there is an increased synthesis of nucleolar RNA both in vivo and in vitro (Godwin et al, 1967)and this appears to be due to increased activity of nucleolar RNA polymerase isozyme.

The activities of all urea cycle enzymes are greatly reduced in primary liver tumours induced by 3-me-DAB (Mclean, 1964).

The transplantable hepatomas used in this study were induced by feeding 3-Me-DAB in the diet. In these transplantable hepatomas, the nucleus to cytoplasm ratio is increased as is the nucleulus to nucleus ratio (Albrecht and Liebenberg, 1972). The nucleolus is usually found associated with the nuclear envelope, most polysomes are free in the cytoplasm and little rough endoplasmic reticulum is visible. Relatively few mitochondria are seen and so appear to be degenerating. The plasma membrane forms projections resembling microcilli and open spaces between the cells are joined by desmosomas (ibid).

From the above ultrastructural changes it was concluded that this hepatoma is typical of dedifferentiated liver cells.

1.6. AIMS OF THE PROJECT

The molecular correlation theory predicts that the activities of the urea cycle enzymes should decrease (a) during the regeneration which follows partial hepatectomy, and (b) in hepatoma. The predicted drop in the activities of these enzymes is in accordance with the theory that urea cycle intermediates become important metabolites in anabolic processes which are necessary for cell proliferation.

The aims of this project were to determine:

(i) whether urea cycle enzyme activities in regenerating rat liver and hepatoma follow the pattern predicted by the molecular correlation theory;

(ii) the effect of dietary protein on the activities of these enzymes involved in catabolic nitrogen detoxification in such rapidly proliferating systems;

(iii) the effects of certain hormones on the activities of urea cycle enzymes in regenerating liver and hepatoma, and whether hormone inducibility is age-dependent;

(iv) whether there is interaction between transplanted hepatoma and host liver, in the same animal and, if so, whether the interaction is regulated by host liver or hepatoma;

(v) whether urea cycle enzymes are controlled in a concerted fashion;

(vi) whether there is a time-dependent variation in urea cycle activities.

In the regenerating rat liver and hepatoma tissue, both of which are rapidly dividing systems, a drop in the activites of the urea cycle enzymes and hence the amount of urea excreted would be consistent with decreased amino acid catabolism. Arginine, an intermediate of the urea cycle could then be spared for the synthesis of new essential proteins, and the other intermediates carbamyl phosphate and aspartate could be spared and rerouted to pyrimidine biosynthesis for the synthesis of nucleic acid which is essential for growth. Ornithine, another urea cycle intermediate, is also an intermediate in the synthesis of polyamines via ODC. Therefore, a decrease in activity of urea cycle enzymes would be consistent with the fact that ODC activities are known to increase both in hepatomas and in regenerating rat livers after partial hepatectomies.

In this study we set out to determine the activities of the urea cycle enzymes in normal, regenerating and neoplastic rat liver under varying conditions of dietary intake and hormonal stimulation.

METHODS AND MATERIALS

2.1 MATERIALS

The British Drug House Ltd., Poole, England supplied the dipotassium hydrogen phosphate, potassium-dihydrogen phosphate, ammonium bicarbonate, magnesium sulphate, L-ornithine HCl, L-aspartate, potassium sulphate, copper sulphate (hydrated), sulphuric acid, potassium chloride, calcium lactate, iron citrate and nigrosine.

E. Merck Company, Darmstadt, Germany supplied the iron III chloride, sodium chloride, manganese sulphate, N-glycyl glycine, sodium hydroxide, tris (hydroxymethyl) aminomethane, phosphoric acid, diacetyl monoxine, absolute ethanol, urea, perchloric acid, ether, glucose, disodium hydrogen phosphate, calcium phosphate, potassium iodide and the 1-phenyl-1, 2-propanedione-2-oxime (PPDO).

The Sigma Chemical Company, St. Louis, Missouri, U.S.A., supplied the ATP, N-acetyl glutamate, casein (light white vitamin free), dilithium carbamyl phosphate, L-ornithine, urease, argininosuccinate, L-arginine HCl, bovine serum albumin (BSA), sodium potassium tartrate and cornstarch.

Anchor, Industria, S.A., supplied the food yeast. β-methasone was obtained as Betasolan soluble injection (2mg/ml) from Glaxo-Allenbury, Johannesburg, S.A. Glucagon and N^6-2'-O-dibuteryl adenosine-3'-5'-monophosphate cyclic (dibuteryl c-AMP) were obtained from Sigma Chemical Company. Thalamonal, Lethidrone and Libitane were obtained from Goldfields Veterinary Supplies, Johannesburg, S.A. Gevral Vitamin-Mineral Nutritional Supplement was supplied by Lederle Laboratories, Isando, S.A.

2.2 ANIMALS

Male and female albino Sprague-Dawley rats were bred and supplied by the University animal facility for this investigation. Animals were supplied at 21 days of age and then sacrificed at 28 or 35 days of age depending on the experiment.

All animals were maintained on the '8-16' schedule of Potter et al. (1968). Under this regime the daylight was reversed. The animals were housed in a room sealed form external light, at a constant room temperature of 22°C and artificial illimination was switched on from 20:00 hours to 08:00 hours. Food was supplied at

08:00 hours and removed at 16:00 hours with water being available continuously. Apart from these times animals were only disturbed for hormonal injections or anaesthetic prior to operations or removal for sacrifice. Rats were fed on diets containing 0%, 10%, 22% or 75% protein (2.3). They were weaned at 20 days on to one of these diets and adapted to the appropriate diet and the '8-16' regime for at least one week prior to sacrifice.

2.2.1 Rats for the study of hepatoma

Albino Wistar strain rats bearing transplantable 3-MeDAB induced hepatomas were supplied by Dr C. Albrecht, C.S.I.R. These were seven week old males.

2.3 DIETS

The 22% protein diet was supplied in the form of normal laboratory rat pellets from Epol. They contained 56% carbohydrate, 4,2% fat, 8% fibre, 22% protein, 1% calcium, 1,69% phosphate and 0,45% salt. Metabolisable energy was found to be 2490 Kcal/Kg and the total digestable nutrients comprised 71% of the total. The 0%, 10% and 75% protein diets were made up as shown in table 2.1, the cornstarch was hydrolyzed by boiling in water, it was then redried in a drying oven and pulverized in a Waring blender. The brewer's yeast contributed 5% toward the total protein content of the diet.

Table 2.1 Composition of diets

Ingredient	0%	10%	75%
Casein	-	5%	70%
Sugar	27%	10%	-
Cornstarch	70%	62%	7%
Brewer's Yeast	-	10%	10%
Steenbock salt mixture*	3%	3%	3%
Vitamin supplement×	5 capsule/Kg		

* See table 2.2

× " " 2.3

Table 2.2 Steenbeck Salt Mixture

Salt	Quantity (g)
Sodium chloride	22,36
Magnesium sulphate	24,60
di-Sodium hydrogen orthophosphate	35,80
Calcium tetrahydrogen di-orthophosphate	68,80
Calcium lactate	15,40
Potassium Iodide	0,15
Ferric Citrate	5,98
di-Potassium hydrogen orthophosphate	63,60

Table 2.3 Contents per Cevral Capsule

Ingredient	Quantity
Vitamin A acetate	5000 U.S.P. units
Vitamin D	500 U.S.P. units
Thiamine mononitrate (B_1)	5 mg
Riboflavin (B_2)	5 mg
Pyridoxine HCl (B_6)	0,5 mg
Vitamin B_{12}	1 µg
Ascorbic Acid	50 mg
d- α -Tocopheryl Acetate	10 I.U.
Niacinamide	15 mg
Calcium Pantothenate	5 mg
Calcium (as $CaHPO_4$)	145 mg
Phosphorus (as $CaHPO_4$)	100 mg
Elemental Iron (as Ferrous Fumerate)	10 mg
Magnesium as MgO	1 mg
Potassium as K_2SO_4	5 mg
Iodine as KI	0,1 mg
Copper as CuO	1 mg
Manganese as (MnO_2)	1 mg
Zinc (as ZnO)	0,5 mg
L-Lysine HCl	25 mg
Choline Bitartrate	50 mg
Inositol	50 mg

2.3.1 Preparation of Diets

The ingredients were added to the melted lard, mixed well and allowed to cool before adding the vitamin supplement. Diets were stored at -20°C and mixed with water to a thick consistency just before feeding.

2.4 ASSAY METHODS

2.4.1 Assays for urea cycle enzyme activities

2.4.1.1 Preparation of liver for quantitative assay

The rats were sacrificed by cervical dislocation, the livers were removed, chilled, weighed and homogenized for 30 seconds in 24 volumes (w/v) ice cold 10mM phosphate buffer, pH 7,4, using an Ultra Turrax homogenizer. The homogenate was then divided into three parts.

One part was diluted with two volumes of the phosphate buffer, and stored on ice to be used for the assay of ASS. The final dilution was 1 in 50 (w/v).

A second portion was diluted to a final dilution of 1 : 100 (w/v) with 10 mM manganese sulphate buffer. This was used for the assay of argininosuccinate lyase. A small volume of this extract was then further diluted to 1 : 300 (w/v) with 10 mM manganese sulphate for the assay of arginase. The diluted homogenate for AL was stored on ice until required for assay, while that for arginase was kept at room temperature for one hour prior to assay for the maximal activation of arginase by manganese ions.

A volume of the original homogenate was frozen in a mixture of dry ice and acetone and allowed to thaw at room temperature. This was then diluted ten times in 10 mM buffer, pH 7.4, and assayed for CPS activity. The one in 250 homogenate was diluted a further twenty times in the 10 mM phosphate buffer, pH 7.4, and this final dilute extract used for the assay of OTC.

The protein concentration of the homogenate was determined in the 1 : 5000 (w/v) dilution by the method of Lowry et al. (1951) as described under Protein determination (2.4.2.3).

The enzyme levels in the hepatoma tissue were greatly reduced and so the homogenate of this tissue was diluted to a lesser extent than normal liver or host liver. The original hepatoma tissue was diluted with nine volumes (w/v) of phosphate buffer (pH 7,4). One volume of the 1 : 10 homogenate was removed and diluted with one volume of manganese sulphate buffer and this dilution was used for the assay of AL and arginase.

The original homogenate was diluted twenty times and the 1 : 200 dilution was used for the assay of OTC as well as to determine the protein content of the hepatoma tissue.

The original 1 : 10 extract was used for the assay of both CPS and ASS activities in the hepatoma.

Activity was expressed in international units, one unit being that amount of enzyme per gram wet weight of liver required to catalyze the production (or depletion) of 1 µmole of product or substrate per minute under assay conditions.

Specific Activity was defined as activity per mg of liver protein with activity defined as above.

2.4.1.2 Spectrophotometric assay for CPS activity (Metzenberg et al., 1958; Brown and Cohen, 1959)

The reaction followed is:

(i) Ammonia + bicarbonate + 2ATP $\xrightarrow[\text{CPS}]{\text{N-acglu}}$ carbamyl phosphate + 2ADP + Pi

(ii) *Carbamyl phosphate + ornithine* $\xrightarrow[\text{OTC}]{}$ *citrulline* + Pi

The beef liver OTC was prepared as follows:

100g of fresh beef liver was homogenized on ice and strained through cheesecloth. The homogenate was frozen in a mixture of dry ice and acetone, thawed and centrifuged at 23 000g to remove cell debris. The supernatant was heated at 60°C for 20 minutes and again centrifuged to remove denatured protein. The preparation was stored at -18°C and diluted 1 : 10 (w/v) with distilled water before use.

The final concentration of reagents in the assay volume was as follows:

50 µM ammonium bicarbonate; 10 µM magnesium sulphate; 5 µM L-ornithine HCl; 5 µMoles n-acetyl glutamate (pH 7,0); 5 µM ATP. *Equal volumes of the first four reagents* were combined and bubbled with carbon dioxide for about 30 minutes to a pH of 6,8. The assay tubes were placed in a water bath at 37°C, 5 ml of 1M $HClO_4$ pipetted into

the blank tubes. The ATP was added to the gassed mixture with the OTC extracts in a ratio of 1 : 2 : 1 (v/v) of ATP: gassed mixture: OTC; 0,5 ml of this mixture was pipetted into each tube and equilibrated at 37°C for two minutes. The reaction was initiated by addition of 0,5ml of the CPS extract and stopped after fifteen minutes incubation by the addition of 1M $HClO_4$. The precipitated proteins were centrifuged down at 1 000g for ten minutes using an MSE bench centrifuge prior to citrulline determination (2.4.2.). All assays are done in duplicate.

2.4.1.3 Assay method for ornithine transcarbamylase (Brown and Cohen, 1959)

The reaction followed is :-

$$\text{Carbamyl phosphate} + \text{ornithine} \xrightarrow{\text{OTC}} \text{citrulline} + \text{Pi}$$

The assay system contained 0,5 ml each of L-ornithine HCl, pH 8,0, to a final concentration of 10 µMoles and N-glycylglycine, pH 8,3, to a final concentration of 45 µMoles, and 10 µMoles of freshly prepared carbamyl phosphate. The assay tubes containing the reaction mixture are placed in the water bath at 37°C. A reagent blank blank is made up by the addition of 0,5 ml of phosphate buffer and 2 ml $HClO_4$ at zero time to test for the amount of citrulline present in the reaction mixture at zero time.

A reagent blank is made up by the addition of 0,5 ml of phosphate buffer instead of enzyme and is stopped together with the sample tubes with 2ml $HClO_4$. These test for the amount of citrulline formed in the reaction mixture during the incubation period.

The reaction in the sample tubes is initiated by the addition of the OTC homogenate (1:5000 dilution) to a final volume of 2 ml after a two minute equilibration time of the tubes at 37°C. The reaction is allowed to proceed for ten minutes and is terminated by the addition of 2 ml of $HClO_4$ (1M).

The reagent blank blank corrects for citrulline present at zero time in the reagent blank and the corrected reagent blank is then subtracted from sample readings to correct for the effect of incubation on the reaction mixture. Citrulline determinations (2.4.2.1) are then carried out in the supernatant after precipitated proteins have been centrifuged down at 1 000g for ten minutes in an MSE bench centrifuge. All assays are done in duplicate.

2.4.1.4 Assay method for Argininosuccinate Synthetase (Ratner 1955; Modified by Wixom et al., 1972)

The reaction measured is the depletion of citrulline from the reaction mixture as follows:

$$\text{citrulline} + \text{aspartate} \xrightarrow[\text{ATP}]{\text{ASS}} \text{argininosuccinate}$$

The assay system consisted of 1µmole of L-citrulline (pH 7,5), 5 µmoles L-aspartate (pH 7,5), 5 µmoles of magnesium sulphate, 5µmoles of ATP, 100µmoles of tris (hydroxymethyl) aminomethane (pH 7,5), approximately 1,0 unit of urease plus enzyme extract to a final volume of 1,0ml.

The reaction was initiated by the addition of citrulline to the sample tube which had previously been equilibrated to 37°C for two minutes. The reaction was terminated after 20 minutes incubation by the addition of 4,0 ml perchloric acid (0,5M). Zero time controls are obtained by adding citrulline after twenty minutes when $HClO_4$ has been added. This allows for urease to breakdown any urea which may be present in the enzyme extract without citrulline being used up in the reaction. Both samples and blanks were assayed in duplicate and citrulline determinations (2.4.2.1) done on the supernatant after precipitated protein had been removed by centrifugation at 1 000g for twenty minutes in an MSE bench centrifuge.

2.4.1.5 The assay method for Argininosuccinate Lyase

The reaction followed is:

$$\text{argininosuccinate} \xrightarrow{\text{AL}} \text{arginine} + \text{fumarate}$$

$$\text{arginine} \xrightarrow[\text{arginase}]{} \text{urea} + \text{ornithine}$$

Argininosuccinate is stored as the barium salt and before use potassium sulphate must be added to precipitate the barium. The precipitated barium as barium sulphate is removed by centrifugation at 1 000g for ten minutes in an MSE bench centrifuge.

The assay system contains 2µmoles of argininosuccinate and 50 µmoles of phosphate buffer, pH 7,4, excess arginase and enzyme extract in a final volume of 1,0 ml. The sample tubes were placed in the water bath at 37°C for two minutes to equilibrate the temperature.

Zero time blanks were set up by dispensing 2,0 ml of $HClO_4$ (1M) before adding enzyme extract. The reaction was initiated by addition of extract to the incubating mixture and allowed to proceed for 30 minutes prior to termination by 2,0 ml of $HClO_4$ (1M). Precipitated denatured protein was spun down at 1 000g for 10 minutes in an MSE bench centrifuge. Arginine produced by the AL reaction is converted to urea by the excess arginase present in the homogenate. Samples were then taken for urea determination as specified in 2.4.2.2.

2.4.1.6 The Assay method for Arginase
(Brown and Cohen, 1959; modified by Balinsky and Baldwin, 1962)

The reaction followed is:-

$$\text{arginine} \xrightarrow{\text{arginase}} \text{ornithine} + \text{urea}$$

The reaction mixture contained 25µmoles of L-arginine HCl (pH 9,5) and the arginase extract made up to a final volume of 2,5 ml.

Sample tubes containing arginine were equilibrated at 37°C for two minutes. Control tubes were made by adding 5,0 ml of $HClO_4$ (0,5M) to sample tubes at zero time, prior to the addition of enzyme extract. The reaction was initiated by the addition of enzyme extract to the incubating mixture and allowed to proceed for ten minutes prior to termination by the addition of 2,5 ml of $HClO_4$ (0,5M). The precipitated denatured protein was removed by centrifugation at 1 000 g for ten minutes in an MSE bench centrifuge. All assays were done in duplicate. Samples were taken from the supernatant to determine the amount of urea present. This method is described in 2.4.2.2.

2.4.2 Colorimetric Assays

2.4.2.1 Colorimetric determination of citrulline
(Archibald, 1944 modified by Ratner, 1955)

Reagents

1) Acid mixture :- 3 parts syrupy orthophosphoric acid : 1 part sulphuric acid : 6 parts water containing iron III chloride (0,5mM).

2) 0,75% aqueous solution of diacetyl monoxime.

3) 1mM solution of citrulline in 0,8N orthophosphoric acid.

Dilutions were made with 1M $HClO_4$.

To 2 ml of sample, 2 ml of acid mix were added. In the dark room 1 ml of diacetyl monoxime was added, the tubes were stoppered and shaken vigorously and placed in rapidly boiling water in the dark for 15 minutes. The tubes were immersed in a nigrosine dye-containing ice bath to cool and for protection against light.

The absorbance was read at 490 nm in an Hitachi Model 101 spectrophotometer. The amount of citrulline produced in each assay was determined from a calibration curve (fig. 2.1). The unit of enzyme activity was defined as that activity which produced 1 µmole of citrulline per minute. The calibration curve was plotted using a computer program (see appendix) and all absorbance values were read off this curve and activities computed automatically.

2.4.2.2 Colorimetric determination of urea

Two ml of the AL assay sample or 0,5 ml of the arginase assay sample were added to 5,0 ml of the acid mix described above. To this was added 5 ml of 1-phenyl-1, 2-propanedione-2-oxime solution in absolute alcohol (3% w/v) in the dark, stoppered, shaken vigorously and emersed in a boiling waterbath for one hour. The tubes were removed and placed in a nigrosine dye-containing ice bath to cool them and protect the solutions from the light.

The absorbance was then read at 540 nm in an Hitachi Model 101 spectrophotometer. Water was used as a blank. Two standard curves, for urea produced by AL (fig 2.2.) and arginase (fig 2.3) were constructed. These were plotted using a computer program (see appendix) absorbance values were read off and activities were computed automatically.

2.4.2.3 Colorimetric determination of proteins (Lowry et al., 1951 ; Layne, 1957)

(i) Reagent A (i) 2,7% potassium sodium tartrate
(ii) 1,0% copper sulphate
(iii) 2,0% sodium carbonate in 0,1 N sodium hydroxide.

Reagents (i) and (ii) were first mixed and then reagent (iii) was added in a ratio of 1 : 1 : 48 (w/v).

(2) Reagent B:- Folin Ciocalteau reagent was diluted 1 : 2 with water.

(3) Standard protein dilutions were prepared from a stock solution of 0,5% Bovine Serum Albumin (BSA).

To 1 ml of sample, 5 ml of reagent A was added. The tubes were shaken and allowed to stand for 20 minutes. After this time 0,5 ml of reagent B was added, the solutions mixed immediately and the tubes were left for 30 minutes before reading the absorbance at 750 nm in an Hitachi Model 101 spectrophotometer. The amount of protein present was determined from a calibration curve (fig. 2.4.).

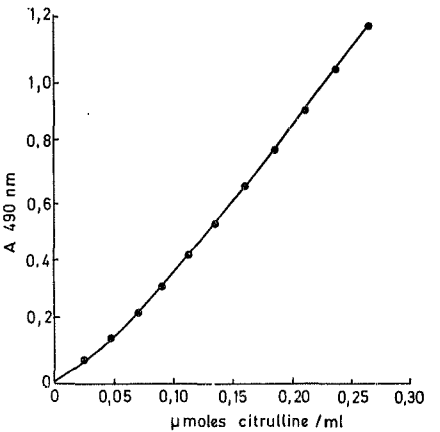


Fig. 2.1 Citrulline calibration curve.

1 ml of standard solution was heated in a boiling water bath for 15 minutes in the presence of 0,5 ml of diacetyl monoxime and 2 ml acid mixture. The absorbance was read at 490 nm.

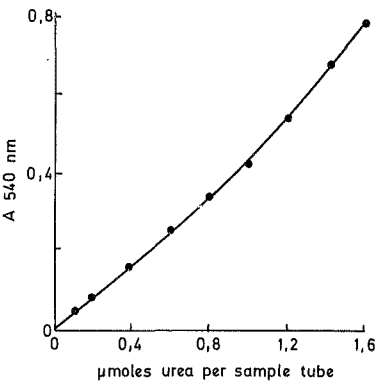


Fig. 2.2 Calibration curve for urea in Al assay.

2 ml of sample was boiled for 1 hour in the presence of 5 ml acid mixture and 0,5 ml PPDO solution in the dark. The absorbance was read at 540 nm.

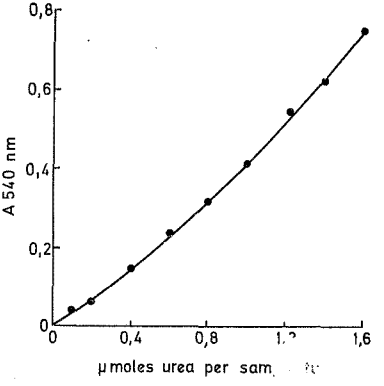


Fig. 2.3 Calibration curve for urea in arginase assay.

0,5 ml sample was boiled for 1 hour in the presence of 5 ml acid mixture and 0,5% alcoholic PPDO solution. The absorbance was read at 540 nm.

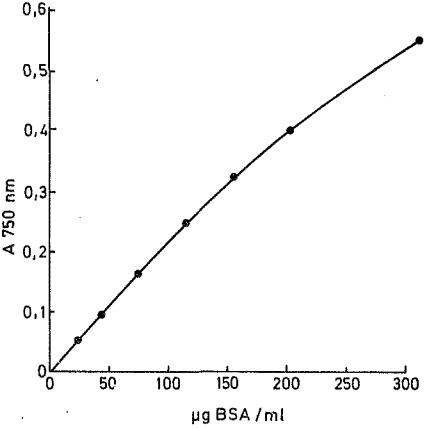


Fig. 2.4 Protein calibration curve.

5 ml of reagent A was added to 1 ml of sample. After 20 minutes 0,5 ml Folin Ciocalteau reagent was added. The colour was developed for 30 minutes and the absorbance read at 750 nm.

2.5 INJECTIONS

Animals were killed 24 hours after an injection unless otherwise stated.

Glucagon was dissolved in dilute HCl and brought to pH 7.4 with sodium hydroxide. Rats were given a single intraperitoneal injection of 250 µg / 50 g.

β-methasone was obtained as Betsolan, the veterinary preparation containing 2 mg/ml of B-methasone phosphate B.P.I. A single intraperitoneal injection of 100 µg / 50 g was given.

Dibuteryl cAMP was dissolved in distilled water and rats injected intraperitoneally with 250 µg / 50 g.

The anaesthetic, thalamonal (0,05 mg / ml fentanyl + 2,5 mg / ml droperidal) was injected intramuscularly (0,15 ml / 100 g) about five minutes before operating and the rats were brought around immediately after the operation with a single intramuscular injection of lethidrone (Nalorphrine hydrobromide 20 mgs /ml), an antidote to the anaesthetic.

A single intraperitoneal injection of cordecepin (1 mg/ 100g) was given at zero time in partially hepatectomized rats, the same being true for actinomycin-D treatment (AMD) (50 µg/50g). The cordecepin and AMD-treated animals were sacrificed at 6 and 10 hours postoperatively.

2.6 PARTIAL HEPATECTOMY (Higgins and Anderson, 1931)

The liver of the rat is composed of four main lobes. The median lobe is cleft by a longitudinal fissure which divides it into a right and left central lobe. The right central lobe is flanked by the right lateral lobe, the latter being transversely divided by a fissure into two smaller lobes, the posterior one capping the anterior pole of the right kidney. The left lateral lobe is large and lies immediately behind the left central lobe. The caudate lobe is also divided transversely into two smaller lobes which lie within the curvature of the stomach.

The operation was carried out under thalamonal anaesthesia (0,15 ml/100 g). Careful asepsis was maintained at all times. The ventral surface of the rat was wiped with hibitane and all surface hair removed by shaving from the top of the rib-cage to about 6 cm posteriorly from the xiphoid process of the stomach. Through a mid-line incision reaching three to four centimetres posteriorly from the xiphoid process, the large median lobe was easily delivered, securely ligated by vetifil, a synthetic surgical suture, and then excised. In this was portions of the liver parenchyma ranging from 65% to 75% of total liver excised, leaving within the peritoneum the right lateral and small caudate lobes which could then regenerate. The excised liver was immediately stored on ice.

The abdomen was closed in two separate layers; the peritoneum and abdominal wall being closed first and then the integument. The animals were immediately brought around with lethidrone (0,1 ml / animal), the antidote to the anaesthetic.

No special postoperative care was excercised except that a 1% saline solution was given in place of water. The animals were fed according to the '8-16' regime.

2.6.1 Procedure for sham operations

The animals were anaesthetised and shaved as above and an incision of the same size and in the same position was made as for partially hepatectomized rats. The abdomen was closed in two layers, the first closing the peritoneum and abdominal muscles, the second the integument. They were brought around immediately by a single intramuscular injection of lethidrone (0,1 ml) The animals were sacrificed by cervical dislocation four hours postoperatively, the livers were removed and assayed for urea cycle enzyme activity (2.4.1.).

2.7 METHOD FOR INDUCTION OF HEPATOMA (Albrecht, 1972)

In order to study the fundamental differences between normal and cancerous tissue it is essential to have a constant supply of tumours which are similar in terms of biological and morphological characteristics.

The liver tumours were originally induced by feeding male Wistar rats a diet containing 1,63 g of 3'-Me-4- dimethylamino-benzene (DAB) in 24 ml of olive oil/kg stock food for five months, after which the rats were fed stock diets until sacrifice six weeks later.

2.7.1 Method of transplant of hepatoma

The hepatoma derived from the Wistar strain was not directly transplantable into Sprague Dawley rats. A cell culture of the hepatoma was started in this laboratory and after the cells had been in culture for about four weeks it was found that the hepatoma line was now transplantable into Sprague Dawley rats. Once the hepatoma was growing in Sprague Dawley it was then transplantable into other Sprague Dawley animals.

The hepatoma of freshly sacrificed animals is dissected out, care being taken not to puncture the haematoma feeding the hepatoma. Only growing hepatoma tissue is used, care being taken to avoid necrotic tissue. The neoplastic tissue is covered with 0,9% saline, cut very finely with a sharp pair of scissors and then approximately 1 - 2 mls of tissue is sucked up into a clean tuberculin syringe fitted with either a 15 x g or a 16 x g needle.

The rats into which the hepatoma is to be transplanted are lightly anaesthetised with ether. The neoplastic tissue is injected into the muscle on the inside of the hind leg. The tumour is allowed to grow for two weeks after which the animals must be sacrificed and the tumours transplanted into new animals.

For studies on hepatomas, both the hepatoma and the host liver are dissected out, weighed, diluted and homogenized (2.4.1.1.) for enzyme assay.

2.8 METHOD OF EXSANGUINATION OF RATS

Both normal nontumour-bearing and tumour-bearing animals were fatally anaesthetised by injecting intraperitoneally pentabarbitone sodium (15 mg/animal). This was obtained as Sagatal, a commercial preparation of pentabarbitone sodium containing 60 mg/ml, and was obtained from Maybaker, South Africa (Pty) Ltd.

When the animals were deeply anaesthetised, the thoracic cavity was opened on the left side and the lungs reflected to expose the posterior vena cava. A 21 gauge needle was inserted into the vessel and 5 ml of blood was withdrawn.

The blood was dispensed immediately into EDTA treated centrifuge tubes to prevent clotting. To clean centrifuge tubes was added 1 ml of a 10% solution of EDTA and the excess water was removed by drying in a hot oven. EDTA is useful as an anticoagulant as it has excellent preserving power for the preservation of cellular elements. The tubes containing the blood were immediately covered and the contents mixed. The blood cells were removed by centrifugation at 2 000 rpm for ten minutes in an MSE bench centrifuge. The remaining plasma (supernatant) was then injected intraperitoneally into normal 35-day old nontumour-bearing animals (2ml/animal).

SECTION 3 - RESULTS

3.1 Time-dependence of inducibility

An experiment was conducted to determine whether the levels of urea cycle enzyme activities vary with the time of day. All animals were trained to a reversed daylight, controlled feeding schedule of twelve hours dark and twelve hours light, being fed a 22% protein diet for the first eight hours after the onset of darkness. They were trained to this regime for seven days prior to sacrifice. Two groups of 28-day old animals were assayed for hepatic urea cycle enzyme activities, the first group being controlanimals, the second group being assayed after a single 24 hours intraperitoneal injection of glucagon (250µg/5[illegible] g rat). The animals were sacrificed at three hourly intervals for 24 hours and the activities of ASS, AL, and arginase determined immediately. The results expressed as activity per gram liver are shown in tables 3.1a - 3.1c and those for specific activities are shown in tables 3.2a - 3.2c. They are represented graphically in figure 3.1. As can be seen from these tables and the graph, the activities of the three enzymes being studied do not alter significantly with the time of day, but there is a time dependence in the inducibility of the enzymes. They show a maximum in inducibility at 10.00 a.m. which is equivalent to two hours after the onset of darkness, since the lights are automatically switched off at 08:00 hours and then switched on again at 20:00 hours. ASS and AL respond in a similar way to glucagon treatment both being induced by about 1,6 times compared with the control levels. Arginase responds less markedly than the other enzymes measured, the induced level being approximately 1,3 times greater than the control.

Table 3.1a Circadian Rhythm determination for urea cycle enzyme activities in rat livers and the determination of time dependence of glucagon induction.

	Activity/g Liver					
	ASS		AL		ARG	
Time	Control	Glucagon	Control	Glucagon	Control	Glucagon
07:00	0,944	0,563	1,200	1,600	895,501	776,250
	0,859	0,625	1,295	1,050	1320,002	652,522
	0,509	0,791	0,700	0,800	870,022	716,255
	0,588	0,567	0,955	0,855	892,500	723,750
Mean	0,725	0,654	1,037	1,076	994,506	717,180
SE	0,077	0,003	0,133	0,182	108,647	25,360
10:00	0,857	0,963	0,750	1,550	746,251	913,002
	0,813	0,781	0,750	1,300	851,252	995,097
	0,581	1,016	1,125	1,701	922,505	969,991
	1,106	0,819	1,485	1,175	840,009	819,905
Mean	0,839	0,923	1,028	1,431	840,004	924,749
SE	0,091	0,059	0,176	0,118	36,197	38,864
13:00	0,563	0,538	1,210	0,725	954,001	885,007
	0,581	0,625	0,710	1,100	649,691	673,750
	0,547	0,428	0,752	1,052	855,125	951,875
	0,666	0,625	1,101	1,025	747,022	745,070
Mean	0,589	0,554	0,943	0,975	801,460	863,925
SE	0,026	0,047	0,063	0,849	65,920	43,208

Table 3.1b Circadian Rhythm determination for urea cycle enzyme activities in rat livers and the determination of time dependence of glucagon induction.

Activity/g Liver

	ASS		AL		ARG	
Time	Control	Glucagon	Control	Glucagon	Control	Glucagon
16:00	0,544	0,469	0,715	0,750	975,002	783,750
	0,975	0,897	1,101	0,825	802,551	697,502
	0,469	0,467	1,012	0,825	1035,000	712,500
	0,430	0,561	1,025	1,215	667,525	813,753
Mean	0,604	0,598	0,963	0,903	870,001	751,875
SE	0,125	0,101	0,085	0,105	83,570	27,920
19:00	0,428	0,406	1,104	0,850	645,001	888,175
	0,375	0,547	0,700	0,902	840,022	756,250
	0,491	0,741	0,950	0,925	723,750	849,375
	0,797	0,640	0,905	0,829	866,250	776,250
Mean	0,523	0,583	0,915	0,876	786,750	817,513
SE	0,094	0,071	0,083	0,022	51,580	30,909
22:00	0,563		1,075		960,000	
	0,500		1,013		8.5,050	
	0,688		0,863		435,025	
	0,500		0,900		555,017	
Mean	0,562		0,963		708,750	
SE	0,044		0,049		63,335	

Table 3.1c Circadian Rhythm determination for urea cycle enzyme activities in rat livers and the determination of time dependence of glucagon induction.

	Activity/g Liver		
	ASS	AL	ARG
Time	Control	Control	Control
01:00	0,563	1,001	1068,751
	0,430	0,720	687,001
	0,520	0,810	945,025
	0,530	1,150	559,503
Mean	0,510	0,920	815,060
SE	0,028	0,096	58,250
04:00	0,565	0,750	922,500
	0,494	0,951	943,750
	0,634	1,113	937,500
	0,548	0,940	604,500
Mean	0,565	0,938	805,063
SE	0,033	0,074	82,64

Table 3.2a Circadian Rhythm determination for urea cycle enzyme activities in rat livers and the determination of time dependence of glucagon induction.

Specific Activity

	ASS		AL		ARG	
Time	Control	Glucagon	Control	Glucagon	Control	Glucagon
07:00	0,0054	0,0040	0,0091	0,0053	5,114	5,008
	0,0034	0,0032	0,0069	0,0103	5,228	4,340
	0,0033	0,0043	0,0045	0,0070	5,613	4,093
	0,0033	0,0036	0,0053	0,0056	4,958	4,825
Mean	0,0039	0,0038	0,0065	0,0066	5,228	4,667
SE	*0,001*	*0,0002*	*0,001*	*0,001*	*0,139*	*0,211*
10:00	0,0038	0,0064	0,0054	0,0105	5,330	6,087
	0,0025	0,0043	0,0064	0,0072	4,864	5,528
	0,0021	0,0088	0,0096	0,0148	5,052	6,363
	0,0043	0,0047	0,0058	0,0067	5,442	4,686
Mean	0,0032	0,0061	0,0068	0,0098	5,397	5,656
SE	0.001	0,001	0,001	0,002	0,223	0,378
13:00	0,0036	0,0047	0,0058	0,0105	6,154	5,770
	0,0027	0,0034	0,0071	0,0075	4,641	4,679
	0,0038	0,0043	0,0045	0,0078	4,966	5,197
	0,0045	0,0035	0,0065	0,0050	5,054	5,549
Mean	0,0036	0,0040	0,0060	0,0077	5,203	5,299
SE	0,0003	0,0003	0,0005	0,0011	0,215	0,238

Table 3.2b Circadian Rhythm determination for urea cycle enzyme activities in rat livers and the determination of time dependence of glucagon induction.

Specific Activity

	ASS		AL		ARG	
Time	Control	Glucagon	Control	Glucagon	Control	Glucagon
16:00	0,0023	0,0035	0,0097	0,0052	4,063	5,405
	0,0065	0,0062	0,0044	0,0059	3,379	4,982
	0,0035	0,0033	0,0097	0,0061	3,981	4,453
	0,0033	0,0033	0,0076	0,0063	3,745	5,260
Mean	0,0039	0,0041	0,0062	0,0059	3,792	5,025
SE	0,0009	0,0007	0,0010	0,0002	0,153	0,382
19:00	0,0033	0,0029	0,0091	0,0063	4,071	5,663
	0,0013	0,0028	0,0063	0,0064	4,000	4,232
	0,0038	0,0049	0,0058	0,0067	4,047	4,781
	0,0066	0,0048	0,0053	0,0062	4,303	4,929
Mean	0,0038	0,0039	0,0066	0,0064	4,105	4,885
SE	0,0007	0,0050	0,0008	0,0001	0,068	0,297
22:00	0,0050		0,0073		4,726	
	0,0021		0,0057		4,933	
	0,0043		0,0061		4,270	
	0,0047		0,0053		3,065	
Mean	0,0039		0,0061		4,289	
SE	0,0010		0,0004		0,418	

Table 3.2c Circadian Rhythm determination for urea cycle enzyme activities in rat livers and the determination of time dependence of glucagon induction.

	Specific Activity		
	ASS	AL	ARG
Time	Control	Control	Control
01:00	0,0053	0,0060	5,150
	0,0023	0,0064	3,710
	0,0022	0,0063	4,022
	0,0044	0,0062	4,025
Mean	0,0036	0,0063	4,227
SE	0,0007	0,0001	0,317
04:00	0,0042	0,0058	3,550
	0,0027	0,0067	3,950
	0,0024	0,0081	3,382
	0,0038	0,0071	5,425
Mean	0,0033	0,0069	4,076
SE	0,0004	0,0005	0,310

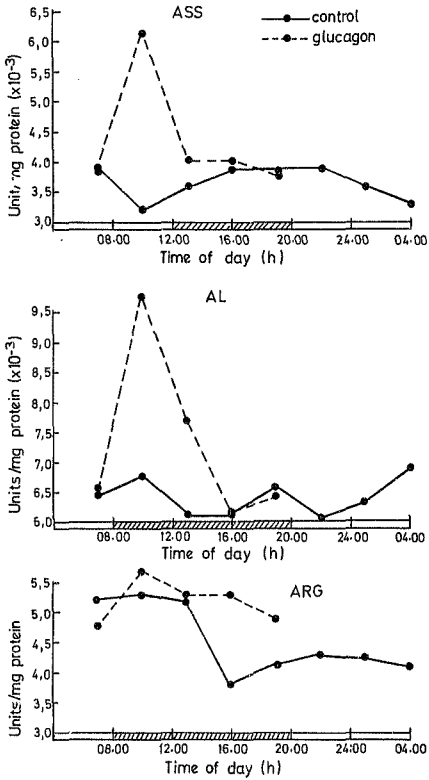


Fig. 3.1 A demonstration of the time-dependence of glucagon induction of urea cycle enzyme activities in the livers of 28 day old rats.

3.2 Age-dependence of inducibility by glucagon

A comparison of induction by glucagon (250ug/50g) was carried out on 28- and 35-day old animals which were maintained on a 10% protein diet. All animals were maintained on a reversed 8-16 feeding and lighting regime and were sacrificed at 10:00 hours, or two hours after the onset of feeding and darkness. The results are shown in tables 3.3 and 3.4 and figures 3.2 and 3.3.

From these results it can be seen that the urea cycle enzymes do not respond i.e. are not inducible by glucagon in 35-day old animals, but they are significantly induced in 28-day old rats, all of the enzymes studied showing a concerted change. When considering changes in specific activities, with glucagon treatment it can be seen that OTC and AL are the most responsive, being 1,5 fold greater than nonhormone-treated controls; CPS and ASS show a 1,3 fold increase with arginase being the least responsive.

Table 3.3 A comparison of Glucagon induction of urea cycle enzymes in 28 and 35 day old rats. Animals were maintained on a 10% protein diet.

	CPS		ASS		AL		ARG	
	A/g	S.A.	A/g	S.A.	A/g	S.A.	A/g	S.A.
28 day	1,798	0,0150	0,632	0,0037	1,098	0,0083	875,427	5,836
Control	1,839	0,0123	0,602	0,0039	1,460	0,0094	880,730	5,181
	1,681	0,0099	0,523	0,0026	1,210	0,0069	875,000	6,035
	1,574	0,0109	0,712	0,0040	1,085	0,0060	745,325	5,411
Mean	1,723	0,0120	0,617	0,0036	1,213	0,0077	844,121	5,616
SE	0,060	0,0001	0,039	0,0001	0,860	0,0008	32,957	0,194
Glucagon	*2,538*	*0,0139*	*0,752*	*0,0051*	*1,810*	*0,0095*	*921,445*	*6,143*
	2,441	0,0163	0,675	0,0042	1,811	0,0113	902,020	5,638
	2,541	0,0172	0,653	0,0043	1,925	0,0130	851,207	5,833
	2,512	0,0141	0,793	0,0045	1,967	0,0122	979,073	6,615
Mean	2,508	0,0158	0,718	0,0045	1,878	0,0113	913,439	6,057
SE	0,023	0,0006	0,033	0,0002	0,040	0,0007	26,416	0,213
35 day	1,827	0,0138	0,512	0,0032	1,182	0,0090	766,610	5,790
Control	1,677	0,0116	0,628	0,0033	1,702	0,0118	659,802	4,551
	1,713	0,0095	0,704	0,0030	0,820	0,0091	917,825	5,101
	1,822	0,0114	0,567	0,0028	0,864	0,0067	910,302	5,693
Mean	1,759	0,0116	0,602	0,0031	1,142	0,0091	813,634	5,274
SE	0,038	0,008	0,041	0,0001	0,203	0,0001	61,965	0,283
Glucagon	1,532	0,0085	0,613	0,0026	1,494	0,0084	900,610	5,125
	1,790	0,0102	0,643	0,0030	1,016	0,0078	622,321	6,207
	1,812	0,0104	0,566	0,0036	1,052	0,006	914,600	5,232
	1,624	0,0102	0,518	0,0032	1,164	0,0072	792,110	4,959
Mean	1,689	0,0098	0,583	0,0031	1,181	0,0073	807,410	5,354
SE	0,067	0,0004	0,003	0,0002	0,108	0,0005	67,496	0,268

A/g = Activity/g Liver
S.A. = Specific Activity

Table 3.4 Summary of Glucagon induction of urea cycle enzymes in 28 and 35 day old rats maintained on a 10% protein diet.

	CPS		ASS		AL	
	28day	35day	28day	35day	28day	35day
Activity/g Control	100± 3,1	100±2,1	100± 6,3	100± 6,8	100± 7,8	100± 17,7
Glucagon	145,46± 1,2	96,02± 4,0	116,36± 5,3	96,84± 6,1	155,82± 3,2	103,41± 9,5
Specific Activity Control	100± 6,3	100± 6,8	100± 2,8	100± 3,2	100± 10,3	100± 1.1
Glucagon	131,61± 1,3	84,4±± 3,4	125,00± 5,5	100± 6,5	146,75± 9,1	80,20± 5,6

	ARG		OTC	
	28day	35day	28day	35day
Activity/g Control	100± 3,9	100± 7,6	100± 6	100± 6,0
Glucagon	108,21± 3,1	99,23± 7,8	144,1± 9,2	77,50± 10,5
Specific Activity Control	100± 3,5	100± 5,4	100± 6,0	00± 8,0
Glucagon	107,85± 3,7	101,52± 5,1	157,2± 10,5	85,0± 7,0

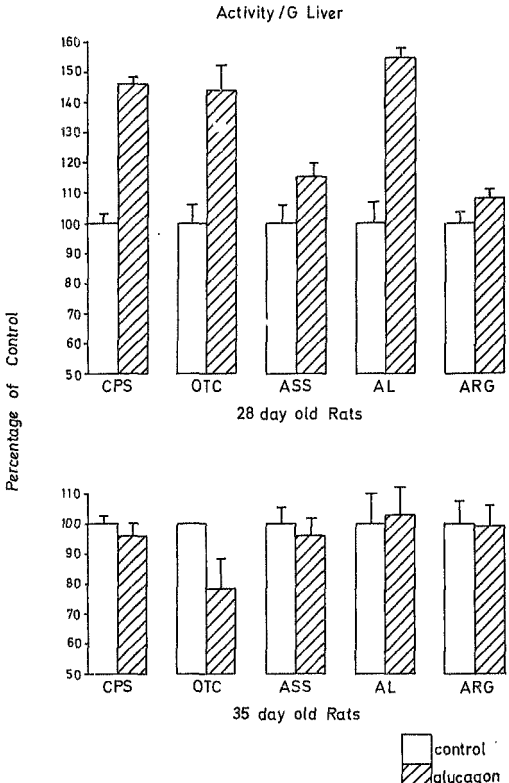


Fig. 3.2 Activity/g liver of urea cycle enzymes from control and glucagon treated livers of 28- and 35-day old animals.

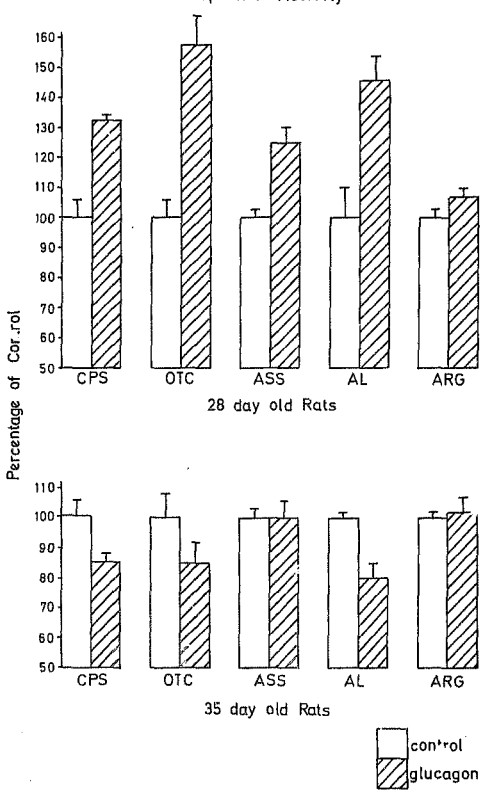


Fig. 3.3 Specific activities of urea cycle enzymes from control and glucagon treated livers of 28- and 35-day old rats.

3.3 Urea cycle enzymes in regenerating rat liver after partial hepatectomy

A series of experiments was conducted, first, to determine the time course of change in urea cycle enzyme activities in regenerating rat liver after partial hepatectomy, and secondly to determine the effect of diet on these changes. Hepatic CPS, ASS, AL and arginase was measured in partially hepatectomized rats during the first 24 hours of regeneration in rats maintained on a zero %, 10% or 75% protein diet and all five of the urea cycle enzymes were measured in regenerating livers of rats maintained on a 22% protein diet. Thirty five day old rats were maintained on an 8-16 controlled lighting and feeding regime, being fed for the first eight hours after the onset of darkness. The animals were trained to this regime and to the particular diet being studied for one week prior to sacrifice. The enzymes were assayed at zero time in the excised liver lobes at the time of operation and in the regenerated liver at the time of sacrifice. All animals were sacrificed at 10:00 hours, therefore for the four hour point, the rats were operated on at 06:00 hours and zero time controls measured at that time; for the eight hour point, the liver lobes were excised and assayed at 02:00 hours. All operated animals were sacrificed at 10:00 hours and the urea cycle enzymes they assayed in the regenerating liver at that time.

For each experimental animal studied, the liver lobes removed and assayed at zero time were used as control values for the regenerated liver from the same animal. All results are expressed both as activity / gram liver and as specific activity and since the results are similar when expressed in either way, this suggests that it is less likely that effects are due to changes in liver components such as total protein content, water content or carbohydrate content.

The results are also expressed as a % of zero time control and these values are represented graphically. The significance of the differences in activities between control and regenerating livers was determined using the paired t-test, the t and probability (P) values are shown in the tables of results. Probabilities of less than 5% are considered to show significant differences.

Rats were maintained on a protein deficient (zero %) diet for one week prior to sacrifice. They were operated on at various times and changes in hepatic CPS, ASS, AL and arginase determined after 4, 8, 12, 18 and 24 hours of regeneration following partial hepatectomy.

Results are shown in tables 3.5a to 3.8c and figures 3.4 to 3.7.

The enzymes studied behave in a concerted, coordinated way, the pattern of change being qualitatively but not quantitatively the same. The results are similar when expressed either as activity/g liver or specific activity and this argues against a nonspecific effect on the urea cycle enzymes.

The enzymes show an initial increase in activity, the increase being significant at 4 hours of regeneration and continues until 12 hours of regeneration. By 18 hours there is a dramatic and significant decrease in activity which has increased again above control values by 24 hours of regeneration. CPS and ASS show a very similar pattern of change, (figures 3.4 and 3.5), the activities increasing 1,3 fold between 4 and 12 hours of regenerat' and dropping to between 0,55 and 0,8 fold of control values. AL and arginase also show similar patterns of cha..ge (see figures 3.6 and 3.7). The magnitude of change being greater than that of CPS and ASS. Between 4 and 12 hours of regeneration there is a 1,6 fold increase in enzyme activity and at 18 hours they drop to between 0,5 to 0,65 times the control activities.

Table 3.5a CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	1,436	2,469	171,64	122,38±	0,0093	0,0190	205,08	127,97±
	1,734	1,760	101,50	8,5	0,0120	0,0121	101,42	8,91
	1,708	2,224	130,21		0,0131	0,0171	130,14	
	1,273	1,800	141,40		0,0082	0,0144	175,40	
	1,113	1,202	108,01		0,0086	0,0096	111,92	
	1,123	1,216	108,31		0,0086	0,0111	128,07	
	1,111	1,164	104,74		0,0081	0,0106	131,43	
	1,025	1,1609	113,24		0,0076	0,0089	117,50	
Mean	1,315	1,624			0,0094	0,0128		
SE	0,099	0,184			0,001	0,001		
t	-2,460				-3,050			
P	0,043				0,018			
8 hour	1,328	1,486	119,00	131,66±	0,0099	0,0099	100,61	123,37±
	1,540	2,224	144,42	9,64	0,0123	0,0171	138,88	4,70
	1,854	2,028	109,39		0,0145	0,0176	121,59	
	1,640	3,183	194,09		0,0099	0,0205	206,85	
	1,035	1,333	129,38		0,0079	0,0092	116,33	
	1,037	1,216	117,26		0,0083	0,0101	122,05	
	1,199	1,433	119,57		0,0083	0,0102	123,70	
	1,048	1,259	120,14		0,0070	0,0093	133,48	
Mean	1,334	1,770			0,0098	0,0130		
SE	0,111	0,240			0,001	0,001		
t	-2,570				-2,810			
P	0,037				0,026			

Table 3.5b CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	1,012	1,407	139,03	118,08±	0,0075	0,0108	144,00	122,55±
	1,114	1,204	108,84	5,14	0,0106	0,0115	108,49	7,0
	8,137	9,741	119,71		0,0603	0,0779	129,19	
	7,798	8,191	105,04		0,0624	0,0780	125,00	
	5,912	6,558	110,93		0,0678	0,0656	96,76	
	5,912	7,388	124,97		0,0537	0,0708	131,84	
Mean	4,918	5,748			0,0437	0,0524		
SE	1,295	1,469			0,011	0,013		
t	-3,010				-2,810			
P	0,030				0,026			
18 hour	3,480	3,114	89,48	81,18±	0,0199	0,0133	66,83	76,57±
	4,067	2,412	59,31	6,30	0,0173	0,0127	73,41	5,40
	4,405	4,225	95,91		0,0238	0,0228	95,93	
	3,756	2,867	76,33		0,0230	0,0154	66,96	
	4,225	3,585	84,85		0,0197	0,0157	79,70	
Mean	3,987	3,241			0,0207	0,0160		
SE	0,166	0,311			0,002	0,002		
t	2,900				0,750			
P	0,044							

Table 3.5c CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	2,788	3,617	131,17	129,65±	0,0151	0,0176	116,56	145,64±
	4,937	5,070	102,69	8,6	0,0091	0,0175	192,31	13,5
	3,634	4,359	119,29		0,0197	0,0242	122,84	
	3,620	5,367	148,26		0,0177	0,0252	142,37	
	4,448	6,534	146,90		0,0207	0,0319	154,11	
Mean	3,889	4,989			0,0165	0,0233		
SE	0,428	0,491			0,002	0,003		
t	-3,820				-4,490			
P	0,019				0,011			

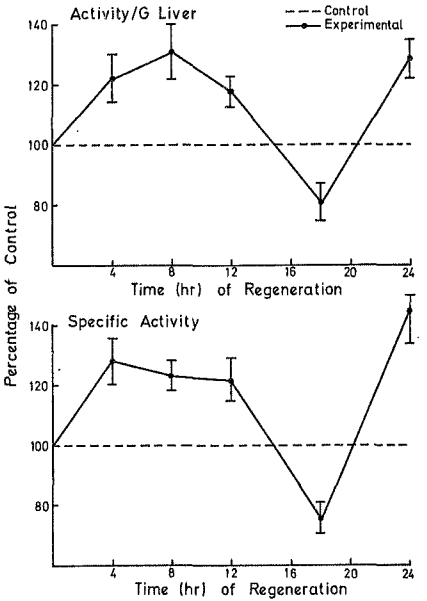


Fig. 3.4 GPS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a zero% protein diet.

Table 3.6a ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	1,196	1,258	105,18	119,09± 6,1	0,0081	0,0092	113,58	128,49± 6,8
	1,311	1,488	113,50		0,0090	0,0103	114,44	
	1,059	2,092	197,54		0,0080	0,0161	201,25	
	1,541	1,746	113,30		0,0113	0,0123	108,85	
	1,010	1,338	132,48		0,0078	0,0099	126,92	
	1,148	1,390	121,08		0,0088	0,0132	150,00	
	1,381	1,400	100,65		0,0103	0,0136	132,00	
	0,934	1,376	147,42		0,0069	0,0106	153,62	
Mean	1,199	1,511			0,0088	0,0119		
SE	0,072	0,098			0,0001	0,0010		
t	2,566				3,085			
P	0,002				0,003			
8 hour	2,208	2,065	102,74	127,40± 11,36	0,0134	0,0140	104,48	126,22± 11,8
	1,570	2,205	139,73		0,0126	0,0170	134,92	
	1,027	1,898	184,81		0,0086	0,0165	191,86	
	0,816	1,154	141,42		0,0063	0,0080	126,98	
	1,308	1,382	105,66		0,0105	0,0115	109,52	
	1,482	1,626	109,72		0,0102	0,0116	113,73	
	1,082	1,166	107,76		0,0078	0,0080	102,56	
Mean	1,329	1,642			0,0099	0,0123		
SE	0,151	0,162			0,001	0,001		
t	1,413				1,697			
P	NS				0,040			

Table 3.6b ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	1,104	1,510	136,78	127,9 ± 7,3	0,0081	0,0116	143,21	129,86 ± 6,4
	1,548	1,671	107,94		0,0135	0,0152	112,59	
	1,472	1,839	124,93		0,0148	0,0167	112,84	
	1,241	1,808	145,69		0,0118	0,0134	113,56	
	1,194	1,934	161,98		0,0096	0,0143	148,96	
	1,337	1,786	133,58		0,0107	0,0170	158,88	
	1,726	1,757	101,80		0,0144	0,0176	122,22	
	1,131	1,250	110,52		0,0094	0,0119	126,60	
Mean	1,349	1,694			0,0115	0,0147		
SE	0,080	0,077			0,0008	0,0008		
t	3,120				2,829			
P	0,002				0,007			
18 hour	2,689	1,552	57,72	47,49 ± 2,85	0,0131	0,0089	67,94	58,84 ± 3,9
	2,158	1,217	56,39		0,0105	0,0072	68,57	
	3,033	1,251	41,25		0,0135	0,0074	54,81	
	2,821	1,050	37,22		0,0128	0,0058	45,31	
	2,464	1,190	48,30		0,0130	0,0070	53,84	
	3,042	1,452	47,73		0,0119	0,0085	71,42	
	3,103	1,360	43,83		0,0148	0,0074	50,00	
Mean	2,784	1,296			0,0128	0,0075		
SE	0,132	0,064			0,001	0,0003		
t	0,143				5,077			
P	0,001				0,001			

Table 3.6c ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	1,233	1,449	117,52	98,70±	0,0065	0,0083	127,69	117,68±
	1,670	1,242	74,37	17,21	0,0088	0,0071	80,68	9,8
	1,261	1,678	133,07		0,0063	0,0093	147,62	
	2,238	1,885	84,23		0,0112	0,0105	93,75	
	1,149	1,084	94,34		0,0057	0,0057	100,00	
	1,273	1,638	128,67		0,0071	0,0094	132,39	
	1,015	1,567	154,38		0,0060	0,0085	141,67	
Mean	1,406	1,506			0,0074	0,0084		
SE	0,158	0,102			0,0007	0,0006		
t	0,592				1,685			
P	NS				NS			

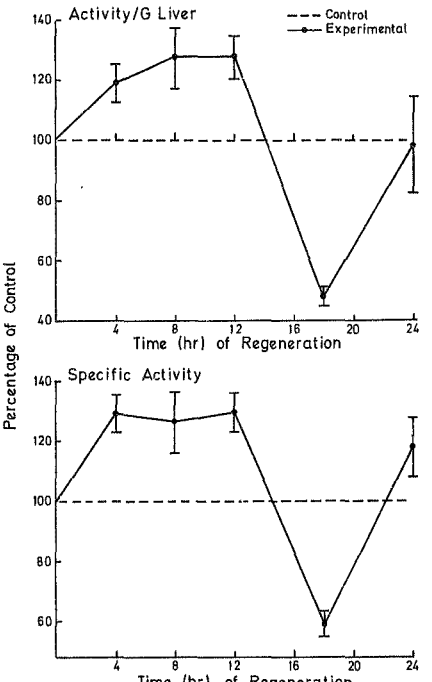


Fig. 3.5 ASS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a zero protein diet.

Table 3.7a

AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	0,2240	0,2800	125,00	149,94±	0,0020	0,0036	180,00	148,29±
	0,7080	0,8170	123,08	18,8	0,0049	0,0060	122,45	15,7
	0,3200	0,5830	182,19		0,0025	0,0035	140,00	
	0,2320	0,2870	123,71		0,0019	0,0027	142,11	
	0,7760	0,9990	128,74		0,0060	0,0071	118,33	
	0,4440	0,6870	154,73		0,0053	0,0091	171,70	
	1,1460	1,3370	116,67		0,0085	0,0122	143,53	
	1,0060	1,1340	112,72		0,0075	0,0087	116,00	
Mean	0,6070	0,7655			0,0048	0,0068		
SE	0,126	0,135			0,001	0,001		
t	-5,42				-4,10			
P	0,001				0,005			
8 hour	0,5540	0,5890	106,32	119,83±	0,0037	0,0038	102,70	-23,04±
	0,8320	0,9330	112,00	5,15	0,0067	0,0072	107,47	6,3
	0,7260	0,7860	108,86		0,0061	0,0068	111,48	
	0,7580	0,8370	110,42		0,0046	0,0054	117,39	
	1,0130	1,1990	118,36		0,0070	0,0083	118,57	
	0,6740	0,8740	129,67		0,0054	0,0073	135,29	
	1,0130	1,5220	150,25		0,0070	0,0109	155,71	
	0,6250	0,7670	122,72		0,0042	0,0057	135,71	
Mean	0,7744	0,9384			0,0056	0,0069		
SE	0,060	0,103			0,0001	0,001		
t	-3,07				-3,19			
P	0,018				0,015			

Table 3.7b *AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.*

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	0,3170	0,6080	191,80	160,73±	0,0025	0,0047	188,00	158,09±
	0,5830	0,9110	156,26	13,9	0,0051	0,0067	131,37	12,5
	0,3640	0,6200	170,30		0,0036	0,0058	161,11	
	0,4140	0,8130	196,38		0,0039	0,0063	158,97	
	0,6390	0,9310	146,83		0,0047	0,0076	161,70	
	0,5990	1,0170	169,78		0,0048	0,0057	118,70	
	0,3070	0,3420	111,40		0,0026	0,0034	130,77	
	1,0480	1,0830	103,34		0,0087	0,0090	103,45	
Mean	0,5340	0,7931			0,0045	0,0061		
SE	0,0870	0,0089			0,001	0,001		
t	-4,95				-5,15			
P	0,002				0,001			
18 hour	0,3750	0,2250	60,00	64,20±	0,0092	0,0051	57,29	70,92±
	0,4150	0,2470	59,52	6,14	0,0020	0,0015	71,78	5,9
	0,2560	0,0980	38,28		0,0013	0,0006	46,40	
	0,4280	0,2760	64,49		0,0021	0,0015	73,21	
	0,3710	0,2170	58,49		0,0019	0,0014	76,88	
	0,3660	0,2940	80,33		0,0018	0,0014	74,86	
	0,3240	0,2860	88,27		0,0018	0,0017	96,00	
	0,5050	0,4660	92,28		0,0025	0,0025	99,60	
Mean	0,3800	0,2636			0,0028	0,0020		
SE	0,0260	0,036			0,001	0,000		
t	5,83				5,020			
P	0,001				0,001			

Table 3.7c AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	0,4340	0,3520	81,11	81,91±	0,0023	0,0020	87,39	89,98±
	0,8800	0,7760	88,18	6,0	0,0046	0,0044	95,68	6,5
	0,8730	0,5230	59,91		0,0044	0,0029	66,59	
	1,4810	1,2160	82,11		0,0074	0,0068	91,35	
	1,0320	0,6700	64,92		0,0050	0,0035	70,18	
	0,4120	0,3740	90,78		0,0021	0,0020	93,93	
	0,6980	0,6560	93,98		0,0038	0,0034	89,12	
Mean	0,8300	0,6520			0,0042	0,0036		
SE	0,140	0,111			0,001	0,001		
t	3,27				3,00			
P	0,017				0,024			

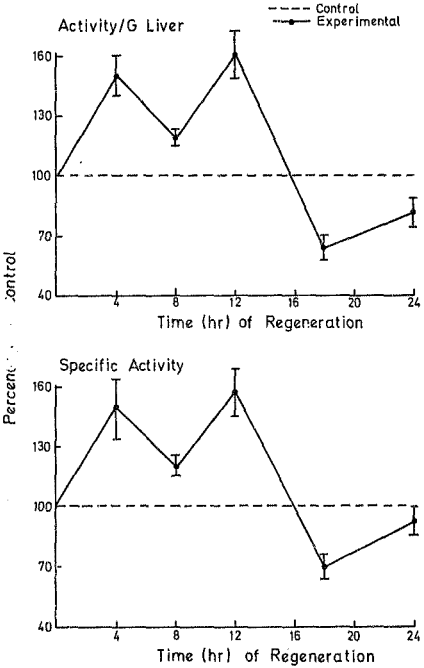


Fig. 3.6 AL activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a zero % protein diet.

Table 3.8a ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	855,411	942,00	110,12	124,91±	5,5188	7,2462	131,30	122,15±
	942,593	1000,50	106,14	7,0	6,6006	6,9000	104,50	5,0
	841,807	892,851	106,06		6,4754	6,8681	106,06	
	700,450	1060,420	151,13		5,6036	6,8414	122,09	
	603,753	764,11	126,56		4,6443	5,3193	114,53	
	567,252	835,004	144,90		4,4327	6,4231	144,90	
	765,33	827,504	146,38		5,1386	6,1297	119,29	
	32,503	689,94	129,57		3,9445	5,3067	134,53	
Mean	701,136	876,541			5,2145	6,3804		
SE	56,[illegible]	43,368			0,334	0,261		
t	-4,				-5,02			
P	0,003				0,002			
8 hour	662,987	807,972	132,85	120,48±	5,0999	5,5722	109,29	117,00±
	615,033	710,687	115,55	4,2	4,9203	5,9224	120,37	5,50
	815,735	884,334	108,41		5,8257	6,0984	104,67	
	678,788	893,108	131,57		4,5253	6,6156	136,25	
	787,100	880,649	111,39		6,2988	6,5233	103,56	
	550,003	674,197	122,58		4,5834	5,8626	127,91	
Mean	684,941	808,491			5,2089	6,0990		
SE	41,294	39,037			0,2899	0,1644		
t	-5,85				-3,02			
P	0,002				0,029			

Table 3.8b ARG Activities of contr. vr. Regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	603,229	920,072	152,50	163,76±	4,4684	7,0844	158,54	145,53±
	477,421	870,972	182,43	15,5	4,1515	6,4516	157,55	7,5
	438,712	911,295	207,72		4,3871	5,6956	127,55	
	433,873	880,649	199,40		4,1321	6,5233	157,87	
	667,024	755,398	113,25		5,3362	7,1942	134,82	
	477,334	887,543	185,94		3,9778	6,6754	167,81	
	689,239	724,102	105,06		5,7437	6,5825	114,60	
Mean	540,977	850,004			4,6000	6,6010		
SE	109,366	77,328			0,2545	0,1851		
t	-4,63				-7,54			
P	0,004				0,000			
18 hour	913,842	310,737	34,00	48,78±	4,1538	2,5158	60,57	54,09±
	743,285	643,561	99,96	8,9	4,3329	3,6271	83,90	7,9
	1175,171	691,781	58,02		6,9128	3,2578	47,13	
	796,750	472,806	59,30		4,4264	2,3064	52,11	
	1080,450	664,834	61,30		5,0259	3,4991	59,62	
	1075,530	638,988	59,41		5,0038	3,1170	62,30	
	1410,380	632,401	44,84		6,5599	3,5133	53,56	
	1270,320	998,115	78,57		6,8666	4,9906	72,68	
Mean	1058,291	631,653			5,4103	3,3534		
SE	81,450	69,104			0,417	0,289		
t	5,84				6,35			
P	0,001				0,0001			

Table 3.8c ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a zero% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	230,860	511,351	221,45	185,88±	1,2151	1,9214	157,36	151,84±
	465,479	614,720	132,06	16,13	2,7170	3,2353	119,07	12,40
	383,530	982,715	252,28		1,9477	2,9136	149,49	
	471,579	998,543	211,74		2,3579	2,9927	126,92	
	336,925	569,645	169,07		1,6425	2,9981	182,53	
	390,093	405,747	104,01		1,9026	2,3186	121,86	
	371,340	724,860	195,20		1,9053	3,9182	205,65	
Mean	379,429	686,783			1 9554	2,8997		
SE	33,861	86,392			0,1821	0,2421		
t	-4,00				-4,41			
P	0,007				0,005			

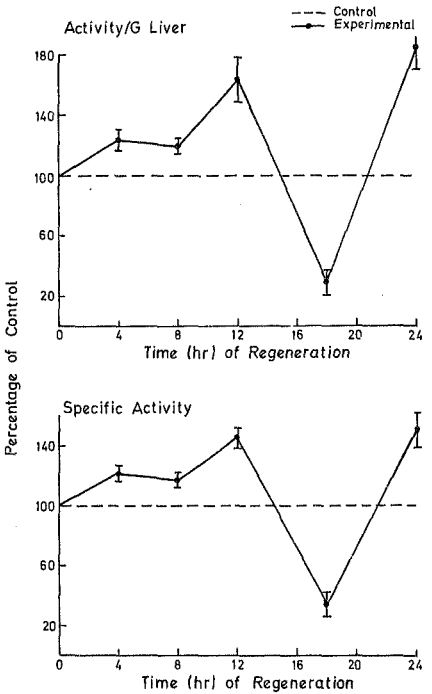


Fig 3.7 Arginase activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a zero % protein diet.

The next experiment was conducted to determine the effect of a low or 10% protein diet on the pattern of change in the activities of hepatic CPS, ASS, AL and arginase at 4, 8, 12, 18 and 24 hours of regeneration after partial hepatectomy. All conditions were as described previously.

From tables 3.9a to 3.12c and figures 3.8 to 3.11 it can be seen that the enzymes studied behave in a concerted way. The results expressed either as activity/g or specific activity are similar arguing against some nonspecific effect. The enzymes show an initial significant increase in activity between 4 and 12 hours of regeneration. At 15 hours activities begin to decrease, reaching a nadir by 18 hours and begin to increase again by 21 hours of regeneration. CPS shows an initial increase in activity reaching 1,3 fold the value of the controls. By 18 hours it has dropped to 0,8 times the control value and by 24 hours is still below the activity of the zero time control (see figure 3.8). ASS shows an initial 1,4 fold increase in activity, begins to drop by 15 hours, reaching a nadir at 18 hours where the activity is 1,82 times that of controls. By 21 hours the activities again begin to increase and by 24 hours are 1,4 fold greater than the control values (see figure 3.9). Between 4 and 12 hours AL shows a 1,35 fold increase in activity above control values, drops to reach a nadir by 18 hours and has not returned to control levels by 24 hours (see figure 3.10). Arginase shows an initial 1,3 fold increase above control activities between 4 and 12 hours after the operation, by 15 hours the activity begins to drop, reaching a minimum at 18 hours when the activity is 0,75 times control values. At 21 hours the activity begins to increase and by 24 hours the activity has returned to control activities (see figure 3.11).

Table 3.9a CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	2,493	2,565	102,89	125,92±	0,0238	0,0205	86,13	121,91±
	2,734	3,752	136,24	9,6	0,0297	0,0300	101,00	8,93
	2,403	2,467	102,66		0,0200	0,0206	103,00	
	1,453	2,048	140,95		0,0116	0,0164	141,38	
	2,241	4,033	179,96		0,0140	0,0218	155,71	
	2,665	2,795	104,88		0,0162	0,0169	104,89	
	1,575	2,071	131,49		0,0105	0,0126	119,52	
	1,753	1,898	108,27		0,0130	0,0146	112,39	
Mean	2,167	2,704			0,0173	0,0192		
SE	0,176	0,281			0,002	0,002		
t	-2,51				-2,57			
P	0,04				0,037			
8 hour	2,393	2,651	110,78	137,08±	0,0218	0,0241	110,55	131,48±
	2,891	4,275	151,68	7,43	0,0256	0,0353	151,56	8,87
	3,371	4,154	123,23		0,0270	0,0322	122,96	
	2,853	4,163	145,92		0,0272	0,0347	127,57	
	1,613	2,685	166.46		0,0108	0,0179	166,51	
	1,577	2,099	133,10		0,0111	0,0113	101,90	
	1,316	2,040	155,02		0,0088	0,0141	160,43	
	1,183	1,306	110,40		0,0076	0,0087	114,15	
Mean	2,150	2,921			0,0175	0,0223		
SE	0,294	0,402			0,003	0,004		
t	-4,70				-4,05			
P	0,002				0,005			

Table 3.9b CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	2,273	3,297	120,99	125,46± 12,1	0,02730	0,03300	120,88	125,03± 2,8
	1,357	3,093	131,23		0,00890	0,01220	141,91	
	2,077	2,627	126,28		0,00890	0,01220	126,51	
	1,115	1,400	126,00		0,01442	0,01787	137,08	
	1,374	2,023	123,96		0,01690	0,01885	123,93	
	1,417	1,450	102,30		0,01742	0,02296	111,54	
	2,493	3,215	128,96		0,01360	0,01930	131,80	
Mean	1,821	2,486			0,0153	0,01950		
SE	0,195	0,303			0,0020	0,003		
t	-3,240				-7,290			
P	0,019				0,0001			
18 hour	3,623	2,529	69,80	79,63± 6,07	0,0181	0,0137	75,69	77,10± 8,5
	4,785	3,227	67,47		0,0245	0,0161	65,71	
	4,725	2,364	50,03		0,0242	0,0118	48,96	
	2,765	2,760	99,82		0,0178	0,0153	85,96	
	2,829	2,697	95,33		0,0218	0,0174	79,82	
	5,156	3,821	74,11		0,0344	0,0255	74,73	
	2,694	2,500	86,39		0,0233	0,0185	84,96	
	2,364	2,226	94,16		0,0135	0,0135	97,12	
Mean	3,642	2,676			0,0222	0,0165		
SE	0,387	0,185			0,002	0,002		
t	2,930				4,220			
P	0,022				0,004			

Table 3.9c CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	3,111	2,259	72,61	76,64±	0,01900	0,01189	61,29	87,47±
	2,641	2,147	81,29	5,8	0,01820	0,01652	90,77	9,2
	3,211	1,320	41,11		0,01647	0,00978	59,38	
	4,246	2,641	62,22		0,02573	0,01601	62,22	
	4,837	3,531	73,00		0,02615	0,02435	93,12	
	3,008	2,953	98,17		0,01823	0,01514	83,05	
	3,838	2,642	68,84		0,02020	0,01960	97,46	
	3,557	1,564	44,04		0,01732	0,01203	69,46	
Mean	3.556	2,382			0,02020	0,01570		
SE	0,255	0,255			0,001	0,002		
t	4,912				3,950			
P	0,002				0,006			

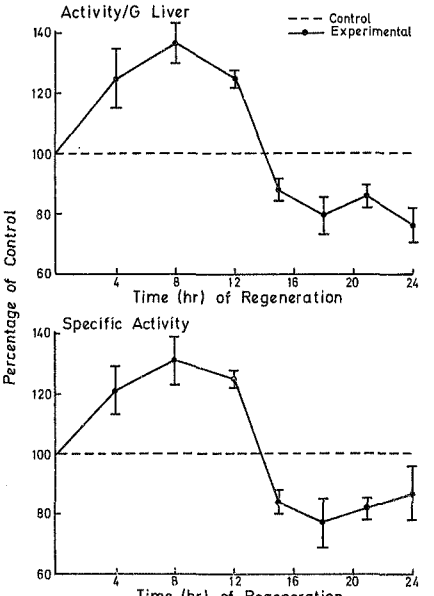


Fig. 3.8 CPS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 10% protein diet.

Table 3.10a ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	2,665	2,749	103,15	110,65±	0,0213	0,0196	92,02	114,16±
	1,623	2,111	130,07	7,1	0,0135	0,0176	130,37	6,5
	1,206	1,408	116,75		0,0096	0,0133	138,54	
	2,822	2,100	74,42		0,0176	0,0168	95,45	
	2,554	3,044	119,19		0,0154	0,0184	119,48	
	2,305	2,907	126,12		0,0154	0,0176	114,29	
	2,585	2,703	104,86		0,0191	0,0208	108,90	
Mean	2,251	2,431			0,0160	0,0177		
SE	0,228	0,220			0,001	0,001		
t	-1,073				-3,590			
P	NS				0,012			
8 hour	1,609	1,806	112,31	136,05±	0,0152	0,0164	107,89	123,75±
	2,070	2,428	117,29	8,4	0,0189	0,0194	102,64	7,5
	2,089	2,925	140,02		0,0166	0,0234	140,96	
	1,850	2,085	112,70		0,0154	0,0198	128,57	
	1,620	2,522	155,68		0,0116	0,0133	114,66	
	1,377	1,801	130,79		0,0089	0,0119	133,71	
	1,526	2,247	147,25		0,0098	0,0145	147,96	
Mean	1,734	2,259			0,0137	0,0170		
SE	0,103	0,153			-3,590			
t	-4,770				0,012			
P	0,003				0,			

Table 3.10b ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Contr.	Mean ± SE
12 hour	1,649	1,128	68,49	139,38±	0,0164	0,0113	68,90	142,11±
	1,742	1,442	82,78	10,03	0,0109	0,0144	132,11	7,7
	1,132	1,882	166,25		0,0091	0,0149	163,74	
	1,732	2,608	150,58		0,0138	0,0214	155,07	
	1,862	2,124	114,07		0,0143	0,0163	113,99	
	1,214	2,043	168,_9		0,0101	0,0157	155,45	
	1,716	2,648	154,31		0,0143	0,0189	132,17	
Mean	1,578	1,982			0,C127	0,0161		
SE	0,108	0,212			0,001	0,001		
t	-1,78				-3,390			
P	NS				0,012			
18 hour	1,439	1,182	82,14	82,80±	0,0072	0,0064	88,89	84,23±
	1,869	1,469	78,60	3,7	0,0096	0,0072	75,00	5,0
	1,693	1,029	60,78		0,0092	0,0051	55,43	
	1,771	1,726	97,46		0,0114	0,0096	84,21	
	1,194	1,074	89,95		0,0085	0,0083	97,65	
	1,742	1,500	86,11		0,0116	0,0100	86,21	
	1,697	1,422	83,79		0,0126	0,0109	86,51	
	1,414	1,182	83,59		0,C088	0,0088	100,00	
Mean	1,602	1,323			0,0099	0,0083		
SE	0,081	0,086			0,001	0,001		
t	4,210				3,390			
P	0,004				0,012			

Table 3.10c ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	0,890	0,990	105,00	140,78± 10,1	0,0059	0,0062	105,08	135,34± 10,1
	0,890	1,279	143,71		0,0061	0,0098	160,66	
	1,035	1,793	173,24		0,0080	0,0133	166,26	
	1,346	1,658	123,18		0,0082	0,0100	121,95	
	1,211	1,989	156,73		0,0081	0,0137	169,14	
	1,374	1,410	102,62		0,0102	0,0104	101,96	
	1,077	1,537	142,71		0,0083	0,0114	137,35	
	0,990	1,769	178,69		0,0076	0,0118	151,28	
Mean	1,108	1,553			0,0078	0,0108		
SE	0,065	0,113			0,000	0,001		
t	-4,290				-4,100			
P	0,005				0,005			

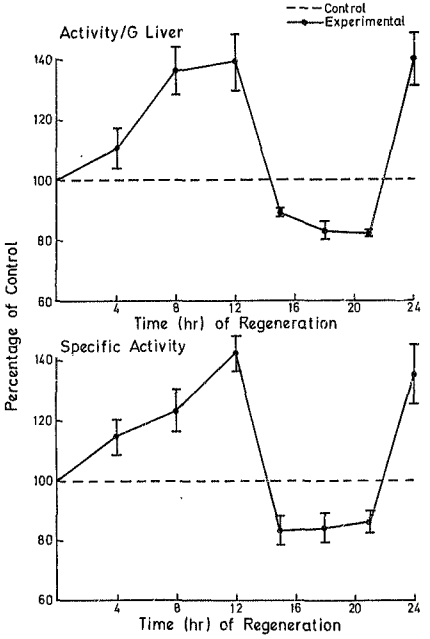


Fig. 3.9 Activities of ASS from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 10% protein diet.

Table 3.11a AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	0,589	0,669	113,58	140,25±	0,00540	0,00560	103,70	120,55±
	1,457	1,538	105,56	2,85	0,01040	0,01240	119,23	4,01
	0,959	1,041	108,55		0,00800	0,00860	107,50	
	1,021	1,253	122,72		0,00820	0,01000	122,00	
	0,304	0,394	124,67		0,00291	0,00429	147,43	
	0,687	0,846	123,14		0,00416	0,00513	123,31	
	1,124	1,506	133,99		0,00337	0,00381	113,06	
	0,639	0,751	117,53		0,00472	0,00578	122,20	
Mean	0,846	1,000			0,00590	0,00700		
SE	0,128	0,145			0,001	0,001		
t	-4,03				-3,92			
P	0,005				0,007			
8 hour	1,026	1,279	124,65	134,788±	0,00620	0,00780	125,81	127,37±
	0,788	0,773	101,94	9,31	0,00720	0,00700	102,86	2,68
	0,883	0,895	101,36		0,00710	0,00720	101,41	
	0,922	1,212	131,45		0,00880	0,01150	130,68	
	0,538	0,525	102,87		0,00359	0,00350	102,57	
	0,320	0,416	130,00		0,00229	0,00429	187,33	
	0,322	0,548	170,19		0,00215	0,00278	129,30	
	0,431	0,431	100,00		0,00278	0,00287	103,23	
Mean	0,650	0,776			0,00500	0,00600		
SE	0,100	0,116			0,001	0,001		
t	-2,83				-2,56			
P	0,025				0,037			

Table 3.11b AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	1,205	1,559	129,38	131,98±	0,01210	0,01560	128,93	131,06±
	1,292	1,250	96,75	7,86	0,00810	0,00780	96,29	7,96
	0,994	1,243	125,06		0,00780	0,00930	119,23	
	0,974	0,960	98,57		0,00780	0,00770	98,71	
	1,034	1,060	149,86		0,00701	0,01200	149,81	
	0,902	0,862	95,57		0,00694	0,00664	95,68	
	0,716	1,180			0,00542	0,00842		
Mean	1,017	1,159			0,0079	0,0096		
SE	0,072	0,086			0,001	0,001		
t	-1,79				-2,19			
P	NS				NS			
18 hour	0,730	0,663	90,82	74,06±	0,00384	0,00358	93,23	74,96±
	1,246	1,107	88,84	4,28	0,00639	0,00540	84,51	4,29
	1,583	0,918	57,59		0,00812	0,00459	56,53	
	1,106	0,827	74,77		0,00714	0,00459	64,29	
	1,165	0,802	68,84		0,00752	0,00617	82,05	
	1,601	1,266	79,08		0,01067	0,00844	79,10	
	1,008	0,721	71,53		0,00747	0,00555	74,30	
	1,199	0,720	60,05		0,00685	0,00450	65,69	
Mean	1,205	0,878			0,0072	0,0054		
SE	0,102	0,074			0,001	0,001		
t	4,93				0,529			
P	0,002				0,001			

Table 3.11c AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	1,769	0,901	50,93	64,05±	0,01106	0,00563	50,90	74,05±
	1,155	0,773	66,93	3,22	0,00797	0,00595	74,65	4,58
	1,414	0,753	53,25		0,00725	0,00558	76,97	
	1,418	0,772	54,44		0,00859	0,00468	54,48	
	1,370	0,715	52,19		0,00741	0,00493	66,53	
	1,399	0,790	56,47		0,00717	0,00479	66,81	
	1,076	0,988	91,82		0,00746	0,00732	94,33	
	1,379	1,011	73,31		0,00919	0,00778	84,66	
Mean	1,373	0,838			0,0083	0,0058		
SE	0,073				0,000	0,000		
t	0,25				4,26			
P	0,0001				0,004			

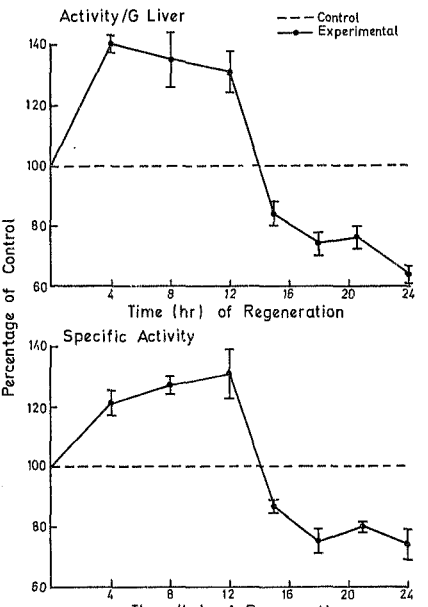


Fig. 3.10 AL activities of regenerating rat livers expressed as a % zero-time controls. Rats were maintained on a 10% protein diet.

Table 3.12a ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn.	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	1028,218	1191,545	108,10	122,83±	9,7926	9,5324	97,34	119,50±
	1473,653	1342,271	109,79	8,5	11,7892	9,5876	122,96	7,09
	994,193	1512,605	152,14		8,2849	12,6050	152,24	
	981,980	985,469	100,36		7,8558	7,8837	100,36	
	572,695	961,013	167,81		3,5793	5,1947	145,13	
	621,675	738,250	118,75		3,7677	4,4742	118,75	
	647,791	754,658	116,50		4,3186	4,5737	105,91	
	635,512	693,710	109,16		4,7075	5,3362	113,36	
Mean	869,463	1022,439			6,7619	7,3984		
SE	109,530	106,640			0,96	0,55		
t	-2,51				-2,48			
P	0,040				0,044			
8 hour	666,436	1044,258	156,69	121,85±	6,0585	9,4933	156,69	123,76±
	1069,075	1018,543	104,96	6,40	9,7189	9,2595	104,96	6,15
	942,867	1189,299	126,14		7,5429	9,5144	126,14	
	1003,544	1159,331	115,52		8,3629	11,0412	132,03	
	860,684	792,828	108,56		5,7379	5,2855	108,73	
	681,826	918,490	134,71		4,8702	6,1233	125,73	
	620,208	781,013	125,93		4,1347	5,3863	130,27	
	687,425	693,710	102,25		4,3769	4,6247	105,66	
Mean	816,507	949,683			6,3504	7,5910		
SE	61,609	64,727			0,711	0,878		
t	-2,38				-2,47			
P	0,049				0,043			

Table 3.12b ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	1183,045	1987,180	167,97	131,89±	11,8305	19,8719	167,97	131,52±
	1037,830	1424,794	137,29	8,3	6,4864	8,9049	137,27	8,40
	1050,012	876,438	119,80		8,4001	7,0115	119,82	
	1050,109	1204,298	114,68		8,4009	9,3344	111,11	
	851,293	891,148	102,31		3,3127	3,4198	103,23	
	856,889	1273,780	146,94		3,3342	4,8992	146,94	
	1001,809	1345,140	134,28		4,7657	6,4054	134,31	
Mean	1004,426	1286,113			6,6472	8,5426		
SE	44,298	141,836			1,182	2,0440		
t	-2,48				-2,69			
P	0,044				0,037			
18 hour	1141,520	759,812	66,56	74,10±	5,7076	4,1071	71,96	73,96±
	1178,276	889,848	75,52	2,97	6,0424	4,4492	73,63	2,24
	861,656	651,918	75,66		4,4188	3,2596	73,77	
	671,295	556,777	82,94		4,3309	3,0932	71,42	
	734,161	641,660	87,40		4,7365	4,1397	87,40	
	1021,949	762,580	74,62		6,8130	5,0839	74,62	
	860,651	534,650	62,12		6,3752	4,1127	64,51	
	970,923	660,636	68,04		5,5481	4,1290	74,42	
Mean	930,053	682,234			5,4178	5,0468		
SE	64,265	41,433			0,288	0,223		
t	6,860				8,020			
P	0,000				0,000			

Table 3.12c ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 10% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	617,482	807,123	130,71	95,91±	3,8593	4,2480	108,83	114,04±
	602,329	715,951	120,52	7,33	4,1540	5,5073	132,58	5,72
	932,804	725,681	77,82		4,7836	5,3749	112,36	
	1153,471	932,434	80,84		6,9907	5,6511	80,84	
	982,683	986,913	100,43		5,3118	6,8063	128,14	
	856,353	817,772	95,50		4,3916	4,9562	112,86	
	824,420	738,174	89,54		4,3391	5,4679	126,01	
	838,497	603,209	71,94		4,1925	4,6401	110,68	
Mean	851,004	803,407			4,7438	5,3315		
SE	64,47	80,557			0,357	0,271		
t	-0,95				-1,88			
P	NS				NS			

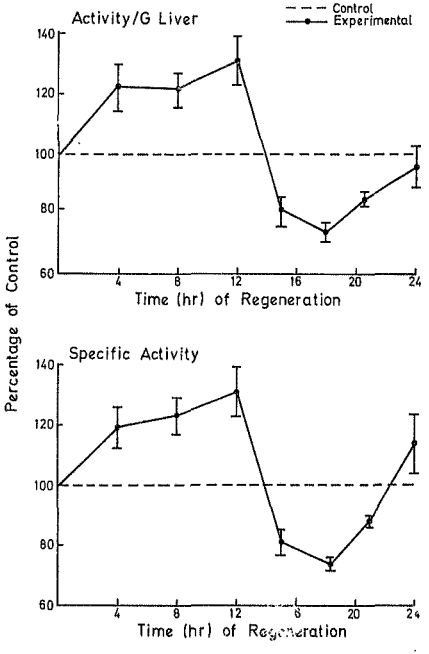


Fig. 3.11 Arginase activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 10% protein diet.

An experiment was conducted to determine the effect of a normal 22% protein diet on urea cycle enzymes following partial hepatectomy. Rats were trained for one week to a diet of normal laboratory pellets prior to experimentation. Rats were operated on at various times and the changes in urea cycle enzyme activities determined after 1, 2, 3, 4, 6, 8, 10, 12, 18 and 24 hours of regeneration following partial hepatectomy.

As can be seen from tables 3.13a to 3.17a and figures 3.12 to 3.16, all the urea cycle enzymes behave in a concerted coordinated way, the pattern of change being qualitatively essentially the same but quantitatively dissimilar. All the enzymes show an initial slight increased activity at one hour postoperatively. The activities then begin to drop and by four hours have reached the nadir after which they begin to increase. By twenty-four hours of regeneration they have returned approximately to control values.

The activity of CPS drops to 50% of control values by 4 hours of regeneration and by 18 hours has returned to control values. OTC drops to 55% of control activities by 4 hours and only returns to the control values by 24 hours after the operation; ASS drops by only 40% to reach 50-60% of control activities by 4 hours after partial hepatectomy, its activity returning to control values by 18 hours and by 24 hours shows a slight increase above the control value (116% of control); AL activities drop to 66% of control by 4 hours but return rapidly to the control value by 12 hours and between 12 and 24 hours of regeneration shows no significant deviation from the control; the activity of arginase drops by less than the other four enzymes studied reaching 65% of the control activity at the nadir which occurs at 4 hours postoperatively. The activity then increases to control values by 12 hours and by 18 hours shows a significant increase above the control (114-119% of control), but by 24 hours of regeneration has returned to control value.

Table 3.13a CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
1 hour	4,150	3,040	73,25	94,77±	0,0330	0,0360	109,10	90,29±
	2,740	2,070	75,54	7,03	0,0270	0,0280	103,71	8,67
	8,440	7,730	91,59		0,0940	0,0930	98,94	
	2,730	2,510	91,94		0,0290	0,0250	86,20	
	4,532	5,055	111,54		0,0283	0,0316	111,66	
	5,314	3,291	61,93		0,0341	0,0235	68,92	
	3,806	3,102	81,50		0,0293	0,0182	62,12	
	2,974	3,002	100,94		0,0198	0,0294	148,49	
Mean	4,335	3,725			0,0368	0,0353		
SE	0,669	0,649			0,008	0,008		
t	1,69				0,58			
P	NS				NS			
2 hour	3,520	2,390	67,89	66,89±	0,0120	0,0101	59,70	59,59±
	4,840	4,290	88,68	7,96	0,0293	0,0187	63,82	1,60
	4,950	2,570	51,92		0,0248	0,0139	56,05	
	5,010	2,960	59,08		0,0251	0,0148	59,20	
	5,320	3,200	60,20		0,0340	0,0240	70,59	
	3,810	3,002	78,80		0,0290	0,0180	62,07	
Mean	4,575	3,069			0,0257	0,0166		
SE	0,297	0,273			0,003	0,002		
t	4,78				6,28			
P	0,005				0,002			

Table 3.13b CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
3 hour	4,640	2,100	45,26	62,88±	0,0480	0,0240	50,00	54,77±
	6,680	5,840	87,42	4,87	0,0520	0,0480	92,30	3,35
	4,860	3,420	70,37		0,0460	0,0260	56,52	
	2,380	1,780	74,78		0,0280	0,0160	57,14	
	5,961	4,124	69,19		0,0351	0,0243	69,23	
	5,908	3,953	66,91		0,0348	0,0168	48,27	
	7,785	3,953	50,78		0,0354	0,0168	47,46	
	6,008	5,423	90,27		0,0363	0,0285	80,73	
Mean	5,598	3,824			0,0394	0,0251		
SE	0,569	0,501			0,002	0,004		
t	3,78				6,43			
P	0,014				0,00			
4 hour	6,160	5,090	82,63	52,28±	0,0257	0,0176	68,48	41,64±
	3,720	2,300	61,82	6,46	0,0219	0,0094	36,58	6,51
	4,550	2,170	47,70		0,0182	0,0086	47,26	
	4,610	2,890	61,70		0,0231	0,0126	54,55	
	4,689	2,660	56,70		0,0391	0,0069	17,65	
	1,919	1,001	52,16		0,0213	0,0125	58,69	
	2,731	0,790	28,92		0,0321	0,0088	27.41	
	2,101	0,560	26,65		0,0247	0,0056	22,67	
Mean	3,810	2,183			0,0258	0,0102		
SE	0,520	0,520			0,002	0,001		
t	9,340				5,090			
P	0,000				0,001			

Table 3.13c CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour	4,156	2,872	69,07	62,61±	0,0194	0,0154	74,23	67,84±
	3,440	2,228	64,75	1,89	0,0172	0,0120	69,77	2,65
	4,560	2,626	57,40		0,0224	0,0142	62,28	
	3,490	2,027	58,09		0,0152	0,0102	67,11	
	4,220	2,565	60,65		0,0184	0,0108	58,70	
	4,370	2,879	65,74		0,0192	0,0144	75,00	
Mean	4,056	2,532			0,0186	0,0126		
SE	0,192	0,140			0,001	0,001		
t	6,060				9,030			
P	0,002				0,000			
8 hour	3,080	2,545	82,63	65,73±	0,0257	0,0196	76,26	66,94±
	3,041	2,432	80,00	5,47	0,0400	0,0293	74,75	2,81
	3,642	2,452	67,33		0,0383	0,0245	63,97	
	3,640	1,872	51,43		0,0383	0,0234	61,10	
	3,835	2,323	60,57		0,0642	0,0387	60,28	
	3,920	2,055	52,42		0,0986	0,0643	65,28	
Mean	3,526	2,279			0,0508	0,0333		
SE	0,153	0,106			0,0108	0,0060		
t	6,100				6,0000			
P	0,002				0,0020			

Table 3.13d CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour	3,532	2,544	69,49	86,26±	0,0176	0,0144	81,82	81,03±
	3,433	3,032	88,37	4,00	0,0154	0,0150	98,68	6,26
	3,809	3,531	92,72		0,0196	0,0190	96,94	
	4,657	4,441	95,43		0,0183	0,0106	57,92	
	3,734	3,505	91,60		0,0136	0,0104	76,47	
	3,041	2,432	80,00		0,0198	0,0148	74,75	
Mean	3,542	3,066			0,0172	0,0140		
SE	0,272	0,328			0,001	0,001		
t	3,140				3,400			
P	0,018				0,020			
12 hour	1,520	1,216	80,00	74,49±	0,0183	0,0153	82,00	76,96±
	2,106	1,567	74,40	2,35	0,0222	0,0165	74,32	1,20
	4,705	3,124	70,42		0,0143	0,0329	74,27	
	4,436	3,485	78,56		0,0739	0,0581	78,62	
	4,704	3,376	71,69		0,0784	0,0596	76,02	
	2,352	1,690	71,90		0,0392	0,0300	76,53	
Mean	3,293	2,410			0,0410	0,0354		
SE	0,599	0,418			0,012	0,008		
t	4,440				4,540			
P	0,007				0,006			

Table 3.13e CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
18 hour	3,445	3,182	92,37	101,95±	0,0181	0,0169	93,99	99,68±
	4,099	4,322	105,44	3,92	0,0261	0,0206	95,37	4,52
	3,501	4,189	119,59		0,0167	0,0200	119,76	
	3,421	3,468	101,37		0,0221	0,0231	104,51	
	3,533	4,008	113,45		0,0221	0,0251	113,58	
	3,697	3,853	104,22		0,0190	0,0193	101,58	
	3,971	3,412	90,00		0,0230	0,0195	84,78	
	4,481	3,993	89,11		0,0280	0,0235	83,93	
Mean	3,746	3,803			0,0219	0,0210		
SE	0,132	0,143			0,001	0,001		
t	-0,390				0,75			
P	NS				NS			
24 hour	3,213	2,980	92,74	76,98±	0,0169	0,0132	78,11	70,74±
	2,550	1,718	67,67	4,53	0,0170	0,0107	62,94	4,36
	3,581	2,570	71,77		0,0224	0,0147	65,63	
	3,532	2,869	81,22		0,0202	0,0169	83,67	
	5,086	3,634	71,49		0,0299	0,0168	56,69	
	3,527	2,280	64,65		0,0172	0,0134	77,90	
Mean	3,582	2,620			0,0206	0,0143		
SE	0,340	0,236			0,002	0,001		
t	4,570				4,130			
P	0,006				0,009			

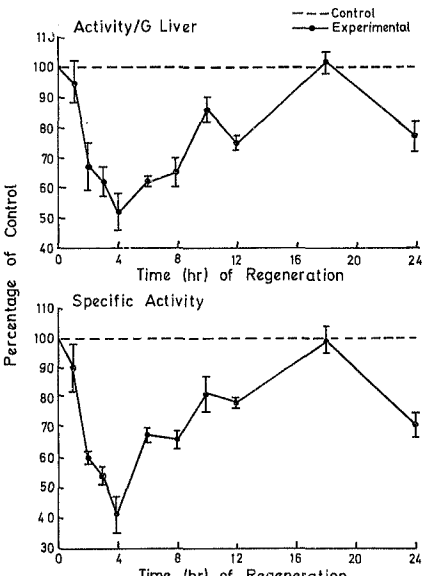


Fig. 3.12 CPS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet.

Table 3.14a OTC Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
2 hours	297,60	194,40	84,82	93,21±	2,125	1,795	84,48	85,84±
	232,00	256,00	101,59	3,73	1,482	1,249	84,28	2,93
	317,00	284,00	89,59		1,695	1,349	90,23	
	475,00	460,00	96,84		2,568	2,167	84,38	
Mean	403,05	299,35			1,917	1,460		
SE	47,21	56,51			0,263	0,212		
t	1,57				4,980			
P	NS				0,010			
3 hours	285,10	161,20	56,55	68,36±	1,140	0,919	80,61	77,57±
	230,14	170,40	74,04	7,13	1,452	1,421	81,10	5,24
	292,61	168,08	57,44		1,961	1,222	62,32	
	199,89	172,35	86,22		1,904	1,642	86,24	
Mean	251,94	168,01			1,614	1,201		
SP	22,02	2,43			0,194	0,153		
t	3,48				2,080			
P	0,025				0,050			

Table 3.14b OTC Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hours	324,00	173,02	53,04	59,29±	1,390	1,041	74,89	69,81±
	233,00	127,60	54,76	3,06	2,805	1,806	64,39	2,40
	267,03	176,07	65,94		1,468	1,066	72,62	
	221,37	139,24	62,90		1,497	1,008	67,34	
Mean	261,34	153,98			1,790	1,231		
SE	23,02	12,12			0,339	0,191		
t	7,01				3,750			
P	0,005				0,025			
6 hours	269,61	251,01	93,11	78,40±	1,284	0,984	76,63	75,82±
	231,65	202,22	87,30	7,30	1,987	1,516	76,30	1,66
	229,80	140,00	60,92		1,683	1,243	73,86	
	187,27	135,28	72,26		1,803	1,379	76,49	
Mean	229,28	182,13			1,689	1,281		
SE	33,68	27,56			0,148	0,113		
t	3,02				10,890			
P	0,03				<0,001			

Table 3.14c OTC Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. *Rats were maintained on a 22% protein diet.*

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
8 hours	247,30	187,40	75,68	81,78 ±	1,337	0,950	71,06	81,97 ±
	224,60	171,00	76,14	5,06	1,774	1,580	89,06	6,73
	245,12	237,40	96,85		2,370	1,620	68,35	
	232,64	182,47	78,43		2,010	1,926	95,82	
Mean	237,42	194,57			1,872	1,570		
SE	5,35	29,37			0,216	0,200		
t	3,61				2,27			1,19
P	0,025				0,05			NS
10 hours	247,60	207,40	83,76	80,48 ±	1,656	1,366	82,49	80,52 ±
	259,71	206,18	79,39	3,23	2,086	1,792	85,91	2,68
	196,29	141,19	71,93		1,570	1,149	73,20	
	207,56	180,29	86,86		1,972	1,588	80,53	
Mean	227,79	176,27			1,821	1,474		
SE	15,31	17,72			0,124	0,139		
t	1,82				10,59			1,87
P	NS				<0,001			0,05

Table 3.14d OTC Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hours	203,20	190,97	93,98	99,62 ±	1,348	1,200	89,00	95,19 ±
	250,81	227,62	90,75	6,08	1,029	1,008	97,95	2,49
	114,87	135,05	117,57		1,572	1,470	93,51	
	262,63	252,84	96,21		1,436	1,440	100,28	
Mean	207,88	201,62			1,347	1,280		
SE	33,55	25,56			0,115	0,108		
t	0,67				1,89			0,22
P	NS				NS			NS
24 hours	221,33	286,91	129,6	107,60 ±	1,115	1,137	101,97	95,30 ±
	255,98	270,61	104,49	7,57	1,250	1,190	95,20	2,86
	226,45	224,41	100,79		1,340	1,184	88,00	
	198,27	189,32	95,49		1,987	1,908	96,02	
Mean	226,01	242,81			1,423	1,355		
SE	12,70	22,21			0,193	0,185		
t	-0,98				1,87			0,26
P	NS				NS			NS

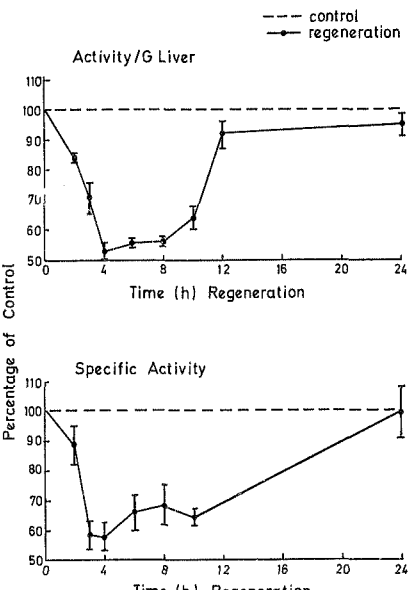


Fig. 3.13 Activities of OTC from regenerating rat livers expressed as a % of zero time controls. Rats were maintained on a 22% protein diet.

Table 3.15a ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
1 hour	3,710	4,148	111,51	99,12±	0,0233	0,0259	111,59	106,24±
	2,976	2,878	96,84	6,22	0,0191	0,0206	107,85	6,91
	3,436	3,785	110,00		0,0164	0,0140	84,47	
	3,481	4,407	127,36		0,0232	0,0259	116,64	
	2,139	1,735	81,30		0,0057	0,0068	119,30	
	2,406	2,148	89,28		0,0076	0,0095	125,00	
	1,614	1,674	103,72		0,0060	0,0070	116,67	
	1,611	1,182	73,37		0,0057	0,0039	68,42	
Mean	2,672	2,779			0,0134	0,0140		
SE	0,299	0,455			0,003	0,003		
t	-0,6				-0,73			
P	NS				NS			
2 hour	1,796	1,372	76,39	79,59±	0,0076	0,0073	96,05	80,20±
	2,736	2,546	92,72	7,19	0,0159	0,0138	86,76	7,89
	2,244	1,914	85,29		0,0098	0,0050	61,22	
	3,422	2,641	77,18		0,0149	0,0102	68,46	
	1,686	1,581	93,77		0,0030	0,0024	80,00	
	2,001	1,158	57,87		0,0040	0,0027	67,50	
	1,632	1,317	80,70		0,0240	0,0160	92,31	
	2,001	1,473	73,61		0,0280	0,0250	89,29	
Mean	2,191	1,750			0,0134	0,0103		
SE	0,216	0,200			0,003	0,003		
t	4,74				3,36			
P	0,002				0,012			

Table 3.15b ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
3 hour	4,009	2,615	65,23	71,09±	0,0236	0,0154	65,25	68,29±
	2,800	2,100	75,00	3,96	0,0165	0,0120	72,73	6,07
	2,652	2,258	85,14		0,0113	0,0103	91,15	
	2,986	2,616	87,61		0,0176	0,0138	78,41	
	2,406	1,036	43,06		0,0084	0,0031	36,91	
	2,529	1,680	66,43		0,0065	0,0047	72,31	
	2,079	1,455	69,99		0,0066	0,0035	53,03	
	2,040	1,527	74,85		0,0080	0,0046	57,50	
Mean	2,658	1,911			0,0123	0,0084		
SE	0,221	0,204			0,002	0,002		
t	5,42				4,95			
P	0,001				0,002			
4 hour	3,315	3,114	93,94	60,37±	0,0276	0,0260	94,20	49,73±
	2,267	2,166	95,54	7,61	0,0241	0,0227	94,19	7,66
	2,367	2,168	91,60		0,0279	0,0241	86,40	
	2,906	2,558	88,02		0,0484	0,0256	52,90	
	3,389	1,428	42,14		0,0161	0,0084	52,17	
	3,519	1,634	46,43		0,0185	0,0067	36,22	
	3,158	2,792	88,41		0,0158	0,0143	90,51	
	3,692	3,148	85,27		0,0166	0,0161	96,99	
	2,580	0,789	30,58		0,0360	0,0090	25,00	
	1,581	0,579	36,44		0,0310	0,0080	25,81	
	1,791	1,152	64,32		0,0230	0,0150	65,22	
	2,475	1,527	61,70		0,0410	0,0220	50,00	
Mean	2,753	1,925			0,0272	0,0165		
SE	0,198	0,251			0,003	0,002		
t	4,16				3,78			
P	0,002				0,003			

Table 3.15c AS. Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour	1,503	1,170	77,84	77,61±	0,0036	0,0027	69,23	76,65±
	2,124	1,569	73,87	5,93	0,0054	0,0052	96,29	8,11
	1,562	1,461	93,53		0,0037	0,0045	82,22	
	1,680	1,095	65,18		0,0041	0,0024	58,85	
	2,160	1,698	78,61		0,0092	0,0067	69,79	
	2,468	1,900	76,98		0,0101	0,0076	75,25	
Mean	1,916	1,482			0,0063	0,0047		
SE	0,159	0,126			0,001	0,001		
t	5,65				3,65			
P	0,002				0,015			
8 hour	2,301	2,254	97,96	92,08±	0,0235	0,0020	86,44	78,13±
	3,144	2,008	53,87	5,95	0,0748	0,0020	81,62	8,10
	1,719	1,710	95,53		0,0052	0,0041	78,84	
	1,360	2,076	75,14		0,0069	0,0031	48,44	
	1,422	1,266	89,03		0,0029	0,0027	93,10	
	1,917	1,794	90,79		0,0053	0,0035	66,04	
Mean	2,010	1,851			0,0085	0,0060		
SE	0,259	0,142			0,003	0,003		
t	0,72				3,30			
P	NS				0,022			

Table 3.15d ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour	1,791	1,403	78,34	82,97±	0,0090	0,0080	88,89	86,21±
	2,509	2,166	84,34	3,08	0,0111	0,0098	88,29	3,90
	2,347	2,033	86,60		0,0146	0,0131	89,73	
	1,924	1,516	78,79		0,0089	0,0070	78,63	
	1,047	0,729	69,63		0,0053	0,0036	67,92	
	1,940	1,714	88,35		0,0097	0,0095	97,94	
	1,865	1,768	94,80		0,0093	0,0088	94,62	
Mean	1,918	1,618			0,0097	0,0085		
SE	0,177	0,179			0,001	0,001		
t	7,41				4,880			
P	0,0001				0,003			
12 hour	2,967	2,358	79,47	82,49±	0,0312	0,0251	80,45	81,21±
	4,182	3,222	77,04	6,90	0,0440	0,0339	77,05	7,63
	3,168	2,152	67,93		0,0528	0,0354	68,00	
	3,341	3,178	95,12		0,0557	0,0557	100,00	
	2,481	2,072	83,51		0,0165	0,0116	70,30	
	2,316	1,666	71,93		0,0070	0,0081	115,71	
	1,572	1,452	92,37		0,0158	0,0145	91,77	
	2,372	1,485	62,61		0,0237	0,0186	78,48	
Mean	2,801	2,198			0,0308	0,0254		
SE	0,280	0,246			0,006	0,006		
t	4,90				2,57			
P	0,002				0,037			

Table 3.15e ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
18 hour	3,731	3,413	91,48	96,54±	0,0196	0,0182	91,84	96,44±
	3,441	3,152	91,60	5,05	0,0181	0,0150	86,62	5,64
	2,973	2,448	82,34		0,0142	0,0117	82,40	
	3,156	3,230	102,34		0,0204	0,0215	105,39	
	2,725	3,456	125,83		0,0170	0,0216	127,06	
	2,921	2,731	93,50		0,0150	0,0156	104,00	
	2,750	2,853	103,75		0,0167	0,0163	97,60	
	3,441	2,806	81,55		0,0215	0,1650	76,74	
Mean	3,142	3,011			0,0178	0,01		
SE	0,129	0,126			0,0001	0,001		
t	-0,89				0,87			
P	NS				NS			
24 hour	2,215	2,342	105,73	114,92±	0,0111	0,0114	102,70	116,66±
	1,602	2,082	129,95	6,85	0,0085	0,0093	109,41	9,57
	0,977	1,788	183,00		0,0065	0,0112	172,31	
	1,788	2,599	144,36		0,0112	0,0149	133,04	
	1,361	1,757	129,10		0,0078	0,0103	132,05	
	2,923	2,753	94,18		0,0172	0,0138	80,23	
	1,783	2,063	115,70		0,0087	0,0124	142,53	
Mean	1,807	2,198			0,0101	0,0119		
SE	0,236	0,145			0,001	0,001		
t	-2,91				-1,67			
P	0,027				NS			

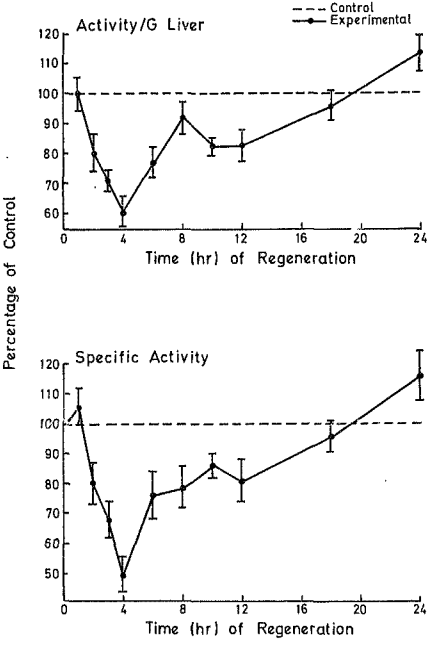


Fig 3.14 ASS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet.

Table 3.16a AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
1 hour	2,578	1,503	58,30	100,79±	0,0206	0,0177	85,92	110,21±
	2,939	1,435	48,83	14,65	0,0280	0,0194	69,29	10,89
	2,120	1,814	85,56		0,0236	0,0227	96,19	
	1,163	0,758	65,18		0,0172	0,0076	44,18	
	2,802	3,528	120,56		0,0174	0,0210	120,69	
	2,022	2,724	133,40		0,0195	0,0291	149,23	
	2,348	3,328	141,74		0,0210	0,0294	140,00	
	2,210	3,439	108,40		0,0183	0,0360	196,72	
Mean	2,363	2,266			0,0207	0,0229		
SE	0,210	0,361			0,001	0,003		
t	0,31				-0,65			
P	NS				NS			
2 hour	2,884	2,837	98,37	85,89±	0,0165	0,0121	73,33	80,81±
	2,506	2,199	87,75	5,21	0,0152	0,0096	63,16	7,84
	2,976	2,482	84,82		0,0139	0,0134	96,40	
	2,063	2,010	97,42		0,0118	0,0101	85,60	
	2,260	0,996	44,07		0,0147	0,0078	53,60	
	2,578	1,892	83,72		0,0207	0,0120	57,97	
	2,104	1,306	62,07		0,0138	0,0090	71,74	
	2,164	2,090	96,58		0,0094	0,0080	85,11	
Mean	2,442	1,977			0,0145	0,0102		
SE	0,125	0,210			0,001	0,001		
t	3,03				4,19			
P	0,019				0,004			

Table 3.16b AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
3 hour	3,056	2,452	80,24	81,39±	0,0322	0,0223	69,25	70,89±
	2,940	1,000	34,01	6,63	0,0226	0,0083	36,73	3,90
	2,011	2,003	99,60		0,0194	0,0155	79,87	
	3,740	2,037	54,47		0,0440	0,0185	42,05	
	3,670	2,684	75,13		0,0216	0,0158	73,15	
	3,788	2,842	75,03		0,0222	0,0136	61,26	
	3,438	3,206	87,36		0,0156	0,0110	70,51	
	1,964	1,826	92,79		0,0192	0,0130	71,88	
Mean	3,076	2,256			0,0246	0,0148		
SE	0,261	0,244			0,003	0,002		
t	3,34				3,88			
P	0,014				0,006			
4 hour	2,181	1,537	70,47	65,67±	0,0190	0,0106	55,79	66,98±
	3,404	3,358	98,65	3,05	0,0200	0,0138	69,00	3,20
	3,050	1,933	63,38		0,0230	0,0152	66,09	
	2,039	1,389	68,12		0,0204	0,0120	58,83	
	2,138	1,308	60,34		0,0102	0,0076	74,83	
	2,740	2,600	94,89		0,0144	0,0134	93,06	
	2,266	1,876	82,79		0,0171	0,0144	82,21	
	2,960	2,336	78,92		0,0192	0,0186	96,88	
	2,960	1,726	58,31		0,0246	0,0182	73,98	
	1,923	0,981	51,02		0,0213	0,0117	54,93	
	2,040	1,220	59,80		0,0360	0,0204	56,67	
	2,379	1,512	63,56		0,0216	0,0168	77,77	
Mean	2,507	1,815			0,0206	0,0144		
SE	0,142	0,194			0,002	0,001		
t	6,660				5,040			
P	0,000				0,000			

Table 3.16c AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour	1,897	1,128	59,46	68,24±	0,0095	0,0066	69,47	68,34±
	2,141	1,439	67,21	7,75	0,0157	0,0085	54,14	6,82
	2,362	1,187	50,25		0,0131	0,0079	60,31	
	1,827	1,166	63,82		0,0085	0,0058	68,24	
	1,266	0,970	76,77		0,0110	0,0098	89,09	
	1,352	0,867	69,81		0,0106	0,0086	71,70	
	1,706	1,082	63,42		0,0073	0,0047	64,38	
	1,700	1,250	73,52		0,0072	0,0050	69,44	
Mean	1,768	1,136			0,0104	0,0071		
SE	0,137	0,061			0,001	0,001		
t	6,48				4,67			
P	0,000				0,002			
8 hour	2,997	1,581	52,75	70,68±	0,0182	0,0076	41,76	70,87±
	2,278	1,504	66,02	5,40	0,0150	0,0134	88,16	8,20
	1,542	0,962	62,39		0,0100	0,0058	58,00	
	3,304	2,480	74,88		0,0276	0,0146	54,90	
	2,392	2,316	96,82		0,0368	0,0356	96,74	
	2,536	1,946	76,74		0,0362	0,0242	66,85	
	2,674	1,412	52,80		0,0314	0,0234	74,52	
	2,564	2,128	83,00		0,0302	0,0194	64,24	
Mean	2,412	1,360			0,0256	0,0180		
SE	0,182	0,198			0,002	0,002		
t	5,50				4,59			
P	0,001				0,003			

Table 3.16d AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour	1,664	1,355	81,43	82,99±	0,0083	0,0075	90,36	78,26±
	1,589	1,500	94,40	3,50	0,0079	0,0075	94,94	7,81
	1,539	1,404	91,23		0,0103	0,0085	82,52	
	1,670	1,300	77,57		0,0105	0,0068	64,76	
	1,336	1,310	98,20		0,0076	0,0066	86,84	
	1,584	1,270	82,04		0,0072	0,0064	88,89	
	1,892	1,368	72,30		0,0108	0,0078	72,22	
	1,724	1,300	75,41		0,0108	0,0065	60,19	
Mean	1,625	1,351			0,0092	0,0072		
SE	0,057	0,026			0,001	0,000		
t	4,240				3,740			
P	0,003				0,007			
12 hour	2,073	2,193	105,79	118,14±	0,0146	0,0183	125,34	101,60±
	2,295	3,84	157,32	5,40	0,0256	0,0192	75,00	3,20
	3,563	4,091	114,82		0,0216	0,0202	95,32	
	2,778	3,282	118,14		0,0185	0,0199	107,57	
	1,740	2,076	119,31		0,0064	0,0072	112,50	
	1,866	1,926	103,22		0,0066	0,0068	103,03	
	1,602	2,259	141,01		0,0134	0,0126	94,03	
	2,580	2,358	91,40		0,0131	0,0131	100,00	
Mean	2,426	2,878			0,0150	0,0147		
SE	0,336	0,393			0,002	0,002		
t	−2,38				0,3			
P	NS				NS			

Table 3.16e AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
18 hour	4,200	3,010	71,67	92,31±	0,0221	0,0161	72,62	107,175±
	1,255	1,530	121,91	7,70	0,0066	0,0073	110,61	7,40
	1,985	1,890	76,41		0,00945	0,0090	95,24	
	1,780	1,360	95,21		0,0115	0,0092	79,57	
	1,985	2,045	103,02		0,0124	0,0141	113,70	
	2,915	3,045	104,46		0,0150	0,0174	116,00	
	2,365	2,220	93,87		0,0144	0,0141	97,92	
	2,945	2,475	84,04		0,0184	0,0146	79,08	
Mean	2,430	2,197	90,41		0,0135	0,0125	92,59	
SE	0,325	0,220	58,31		0,001	0,001		
t	1,42				1,01			
P	NS				NS			
24 hour	2,328	3,189	138,77	82,57±	0,0119	0,0156	135,77	84,49±
	1,116	0,945	84,68	10,3	0,0059	0,0047	79,59	9,2
	1,803	1,329	73,71		0,0120	0,0083	67,83	
	1,734	1,803	103,98		0,0108	0,0103	95,28	
	2,277	1,497	65,74		0,0130	0,0088	67,74	
	2,376	2,286	96,21		0,0140	0,0114	81,76	
	2,268	2,229	98,28		0,0111	0,0078	70,46	
Mean	1,982	1,897			0,0111	0,0096		
SE	0,170	0,282			0,001	0,001		
t	0,43				1,54			
P	NS				NS			

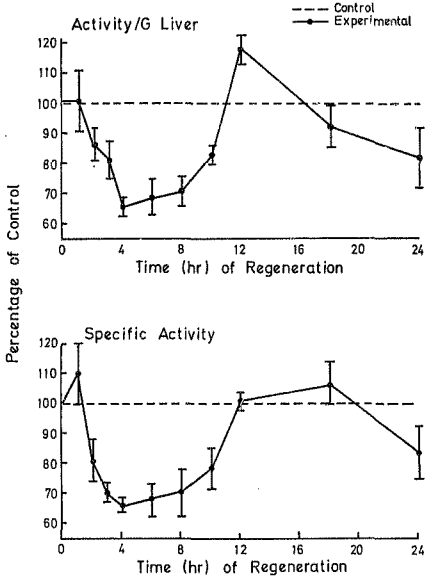


Fig 3.15 AL activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet.

Table 3.17a ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
1 hour	1816,115	1484,749	81,	100,52±	14,5289	17,4559	120,15	106,91±
	1368,603	1765,414	92,46	5,02	13,0343	19,7833	151,78	4,92
	1163,054	1237,269	106,74		12,8895	15,4783	120,10	
	1193,070	1086,193	91,27		12,5277	10,8619	86,71	
	1332,670	1509,040	113,23		8,3292	9,4315	113,23	
	1187,970	1172,000	98,87		6,1520	8,3714	109,93	
	993,970	1182,410	119,32		7,6228	6,9554	91,24	
	951,480	1641,190	172,49		6,3432	9,6541	152,96	
Mean	1249,740	1384,907			10,3051	12,2500		
SE	95,726	87,926			1,146	1,685		
t	-1,23				-2,11			
P	NS				NS			
2 hour	484,690	400,708	92,67	88,93±	2,2055	2,0625	89,85	92,67±
	760,820	718,360	94,42	6,82	3,7927	3,1233	82,35	3,38
	1082,770	985,210	90,99		5,3255	5,1560	96,80	
	903,610	727,320	80,49		3,7650	3,6366	96,59	
	1274,170	945,890	74,23		5,4224	4,8507	89,46	
	1504,340	1205,580	80,14		8,1316	5,1301	63,09	
	1049,680	888,970	84,69		4,5638	4,4449	97,42	
	1596,050	1121,800	70,29		6,1387	4,8774	79,45	
Mean	1082,016	874,229			4,1793	4,1602		
SE	131,771	90,520			0,629	0,395		
t	3,99				3,00			
P	0,005				0,020			

Table 3.17b ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
3 hour	1177,433	927,609	78,78	85,50±	12,3940	8,4328	68,04	82,44±
	1627,738	1304,672	80,15	4,25	12,5211	10,8723	86,83	3,60
	1357,742	1242,604	91,52		12,9309	9,5585	73,92	
	1481,603	1004,409	70,16		16,8424	9,1310	54,86	
	1484,200	1482,400	99,87		8,7306	8,7182	99,85	
	1490,530	1450,710	95,86		8,7678	7,8920	90,00	
	2119,500	2031,680	95,53		9,6341	8,6454	89,74	
	1667,170	1592,630	95,53		9,8098	8,3789	85,42	
Mean	1550,738	1378,613			11,4538	8,9615		
SE	97,505	122,862			0,981	0,325		
t	2,98				2,83			
P	0,021				0,025			
4 hour	857,110	794,415	92,68	61,087±	3,4541	2,9556	85,57	65,82±
	852,540	765,422	89,78	6,35	5,0149	3,1242	62,30	5,15
	762,307	632,040	82,91		2,8766	2,4786	86,16	
	829,100	828,650	99,95		4,1455	3,6028	86,90	
	1362,070	751,130	49,65		6,4860	4,4184	68,12	
	1309,410	650,230	31,91		6,8916	3,3345	48,38	
	1099,570	350,920	44,35		5,4979	1,7996	32,73	
	1346,870	599,510	44,51		5,9342	3,1553	53,17	
	1363,339	557,691	40,91		11,3612	5,8704	51,67	
	855,217	577,026	65,18		9,8357	5,7703	58,67	
	1315,361	859,836	65,36		13,1536	9,3315	70,94	
	1384,857	1184,219	85,51		13,8486	11,8422	83,51	
Mean	1108,743	719,425			7,3746	4,8071		
SE	75,750	59,860			1,0850	0,8670		
t	4,270				5,500			
P	0,001				0,000			

Table 3.17c ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour	1675,839	879,904	52,50	69,4033±	12,8911	6,0545	45,96	69,66±
	1250,657	948,571	75,85	3,38	9,6204	7,4857	77,81	11,11
	1000,457	645,443	64,51		7,6958	5,6125	72,93	
	1358,824	1059,223	67,95		11,5468	7,0615	61,16	
	1644,580	1074,442	65,30		8,2229	5,3721	65,33	
	1591,420	1112,220	69,90		7,9571	5,5611	69,89	
	1010,629	669,064	66,20		5,4629	3,9397	72,04	
	1045,872	687,566	69,90		5,9671	3,1980	45,90	
Mean	1347,284	884,553			8,7955	8,5356		
SE	106,445	68,783			0,8630	0,5080		
t	8,000				5,310			
P	0,000				0,001			
8 hour	1181,761	829,714	70,21	70,17±	10,7433	5,9265	55,16	74,30±
	1124,232	653,216	58,21	3,37	6,6131	6,5322	98,78	5,78
	827,447	647,439	78,81		5,2997	3,9239	74,04	
	1665,064	1385,444	83,18		13,8805	8,1498	58,71	
	1029,672	676,601	61,92		10,9267	6,7660	61,92	
	1683,148	859,032	51,04		16,8305	10,7409	63,04	
	1461,348	1060,601	72,58		14,6135	10,6060	72,57	
	1952,946	1293,884	66,25		10,9541	10,1786	91,015	
Mean	1361,165	925,741			11,2327	7,8527		
SE	46,229	28,611			1,3805	0,8811		
t	5,97				4,12			
P	0,001				0,004			

Table 3.17d ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour	1051,814	985,523	93,68	93,99±	5,0951	4,7695	93,61	93,08±
	1296,108	1126,167	86,89	1,59	5,1022	5,0548	99,07	2,54
	999,399	900,000	90,05		5,7065	5,0899	89,19	
	1337,238	1318,091	98,57		6,6076	5,5133	77,99	
	1095,401	1025,394	93,61		10,5151	9,8533	93,71	
	1020,437	1009,166	98,90		1,8544	1,6201	97,67	
	1114,130	1017,976	91,37		5,5823	5,2941	94,84	
	1059,221	1047,530	98,90		5,4581	5,4921	100,62	
Mean	1121,718	1053,7[illegible]			7,5335	5,3652		
SE	44,650	43,829			1,390	0,768		
t	3,520				3,240			
P	0,010				0,012			
12 hour	710,006	813,201	114,53	96,76±	14,2001	13,5524	95,45	95,87±
	771,752	657,853	89,90	3,86	15,4550	18,7398	89,03	3,29
	879,731	743,118	84,47		11,7297	12,3855	105,59	
	903,282	827,432	91,60		18,0656	15,0442	83,27	
	475,660	513,648	107,99		5,0069	5,4068	107,99	
	608,129	618,050	101,63		9,9918	8,4963	85,03	
	599,509	509,775	85,03		8,7880	8,6990	98,99	
	527,263	521,935	98,99		10,7892	9,9521	92,24	
Mean	684,416	650,627			11,6971	11,5371		
SE	56,002	46,774			1,4520	1,5040		
t	1,140				0,2400			
P	NS				NS			

Table 3.17e ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
18 hour	400,203	488,973	117,18	114,33± 4,24	2,106	2,344	111,33	119,89± 10,76
	390,146	521,028	131,15		2,053	2,742	133,55	
	439,978	575,729	130,09		2,095	3,838	183,19	
	484,344	518,343	107,02		3,124	3,239	103,67	
	499,600	586,195	117,13		3,122	3,350	107,29	
	460,609	515,412	111,90		2,362	3,031	128,35	
	576,777	565,844	98,10		3,495	2,760	78,96	
	551,401	565,844	102,63		3,446	3,835	112,79	
Mean	475,382	542,171			2,725	3,142		
SE	23,372	12,494			0,223	0,187		
t	-3,36				-1,70			
P	0,009				NS			
24 hour	1145,167	897,706	78,39	105,88± 7,37	5,586	4,775	85,48	108,08± 6,79
	1048,466	1133,727	108,18		4,659	5,398	115,85	
	963,654	908,313	94,24		6,022	6,065	100,54	
	897,539	1149,920	128,12		5,127	7,187	140,58	
	1112,747	1116,825	124,43		6,545	6,381	97,49	
	986,759	863,490	87,51		4,933	4,934	100,00	
	804,180	963,035	120,30		4,718	5,503	116,62	
Mean	994,050	1005,003			5,370	5,749		
SE	45,315	46,947			0,268	0,322		
t	-0,17				-1,09			
P	NS				NS			

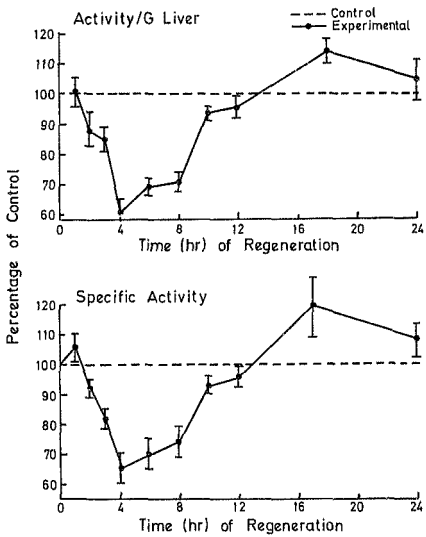


Fig. 3.16 Arginase activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet.

The effect of a high (75%) protein diet on the activities of hepatic CPS, ASS, AL and arginase at 4, 8, 12, 18 and 24 hours of regeneration after partial hepatectomy was studied. All experimental conditions were as described previously except that the rats were trained to a diet containing 75% protein for one week prior to experimentation. Results are shown in tables 3.18a to 3.21c and graphically in figures 3.17 to 3.20.

As can be seen from these tables and figures there is an initial decrease in activity in the regenerating liver compared with zero time controls, the activities increasing to values above those of controls between eight and twelve hours postoperatively. By four hours the activity of CPS drops by only 20% to reach 80% of control values and by eight hours has shown a significant overshoot above the control (120%) reaching 130% of control by twelve hours postoperatively (see fig. 3.17). The activity of ASS drops by 35% at eight hours and increases to 120% by twelve hours (see fig 3.18). The activity of AL drops by about 35% at four hours and has significantly overshot the control activity by 12 hours (see fig. 3.19). Arginase shows the smallest drop, falling by only 15% at four hours of regeneration and by eight hours has increased above control value. The activity is 140% of the control by twelve hours which is a significant increase (see fig 3.20).

From the above it can be seen that the urea cycle enzymes studied behave in a concerted way. Since the results are similar whether expressed as activity/gram liver or specific activity, this argues against a nonspecific effect on these enzymes through an alteration in liver components such as carbohydrate or total water content.

Table 3.18a CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	14,419	9,165	63,56	79,63±	0,0874	0,0458	52,40	76,45±
	13,839	11,949	86,34	5,20	0,0748	0,0724	96,79	6,70
	12,967	7,124	54,93		0,0894	0,0432	48,32	
	11,164	10,346	92,76		0,0677	0,0668	98,67	
	13,913	11,332	85,89		0,1210	0,0944	78,01	
	11,256	10,597	94,14		0,1126	0,0883	78,41	
	13,818	9,612	69,56		0,1063	0,0739	69,52	
	11,653	10,464	89,79		0,0900	0,0805	89,44	
Mean	12,879	10,130			0,0936	0,0707		
SE	0,469	0,512			0,007	0,007		
t	3,34				3,770			
P	0,006				0,007			
8 hour	9,001	10,487	116,50	119,44±	0,0450	0,0488	108,44	117,61±
	7,358	11,284	153,35	5,9	0,0433	0,0664	153,34	,40
	10,100	11,044	109,34		0,0561	0,0614	109,44	
	8,740	10,354	118,46		0,0795	0,0941	118,36	
	17,243	22,860	132,57		0,1437	0,1905	132,56	
	16,032	16,591	103,48		0,1032	0,1069	103,59	
	13,014	15,419	118,48		0,1183	0,1402	118,51	
	11,551	11,936	103,33		0,0889	0,0918	103,26	
Mean	11,630	13,747			0,0848	0,100		
SE	1,258	1,539			0,013	0,016		
t	-3,13				-2,82			
P	0,013				0,026			

Table 3.18b CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	10,792	15,966	147,94	138,43±	0,0654	0,0798	122,01	133,62±
	7,892	10,917	138,32	12,10	0,0478	0,0622	130,12	11,50
	6,116	11,142	182,17		0,0489	0,0768	157,05	
	6,740	12,471	185,02		0,0408	0,0805	197,30	
	15,393	15,993	103,89		0,1139	0,1278	112,20	
	21,072	21,187	100,54		0,1505	0,1513	100,53	
	15,809	16,211	102,54		0,1216	0,1247	102,54	
	13,627	20,044	147,09		0,1048	0,1542	147,13	
Mean	12,180	15,570			0,0867	0,1090		
SE	1,948	1,406			0,0015	0,012		
t	-3,18				-3,66			
P	0,007				0,008			
18 hour	6,925	3,243	46,83	69,09±	0,0346	0,0162	46,82	68,71±
	6,652	4,579	68,80	4,53	0,0459	0,0269	58,60	6,90
	7,60	3,654	48,01		0,0412	0,0183	44,41	
	7,073	4,951	69,99		0,0416	0,0257	66,74	
	7,090	5,311	74,90		0,0364	0,0287	78,84	
	6,862	5,537	80,69		0,0280	0,0293	104,64	
	11,525	8,055	69,89		0,0630	0,0503	79,84	
	11,431	6,124	53,57		0,0572	0,0383	69,95	
Mean	8,146	5,182			0,0435	0,0294		
SE	0,734	0,531			0,004	0,004		
t	6,19				5,11			
P	0,0001				0,001			

Table 3.18c CPS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	14,491	9,056	62,41	71,17±	0,0580	0,0412	71,03	90,72±
	16,568	10,177	61,42	5,80	0,0543	0,0509	93,73	8,30
	8,891	4,642	52,21		0,0349	0,0211	60,45	
	8,175	6,589	80,56		0,0256	0,0285	106,75	
	7,319	6,269	85,65		0,0244	0,0267	109,42	
	6,864	5,820	84,79		0,0269	0,0277	102,97	
Mean	10,385	7,092			0,0375	0,0327		
SE	1,673	0,855			0,006	0,005		
t	3,4				1,42			
P	0,019				0,216			

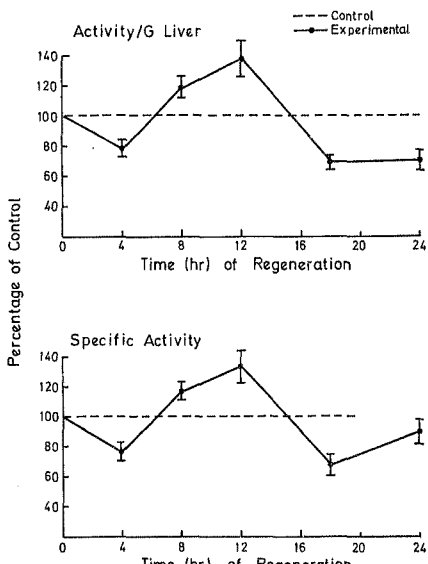


Fig.3.17 GPS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 75% protein diet.

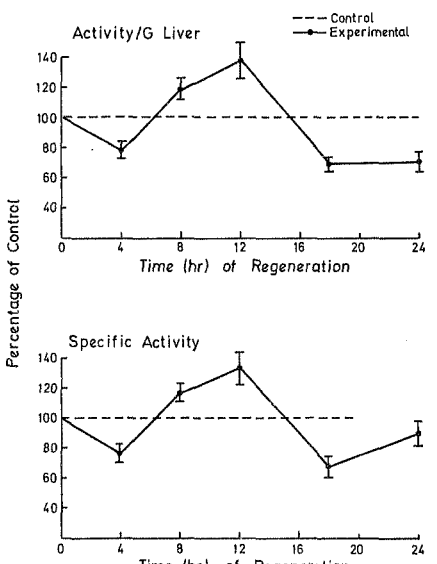


Fig.3.17 CPS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 75% protein diet.

Table 3.19a ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	10,191	7,953	78,03	74,53± 1,90	0,0485	0,0388	78,30	70,11± 3,90
	8,737	6,703	76,71		0,0437	0,0279	63,84	
	9,154	7,112	77,69		0,0591	0,0508	85,95	
	9,042	6,875	76,03		0,0624	0,0355	56,89	
	9,637	6,236	64,70		0,0838	0,0542	64,67	
	9,146	6,257	68,41		0,0915	0,0521	56,93	
	8,263	6,680	80,84		0,0636	0,0514	80,81	
	9,127	6,772	73,83		0,0702	0,0521	74,21	
Mean	9,162	6,824			0,0653	0,0453		
SE	0,202	0,192			0,0060	0,0030		
t	11,74				5,16			
P	<0,0001				0,001			
8 hour	11,685	7,737	66,19	68,64± 3,0	0,0584	0,0360	61,64	65,53± 1,67
	9,968	7,263	72,86		0,0586	0,0404	68,94	
	10,393	6,336	60,97		0,0577	0,0352	61,00	
	9,577	8,233	86,14		0,0888	0,0549	61,82	
	10,128	6,889	68,06		0,0844	0,0574	68,00	
	10,579	6,850	64,75		0,0705	0,0428	60,70	
	9,205	6,418	69,73		0,0837	0,0584	69,77	
	8,389	6,516	72,48		0,0692	0,0501	72,39	
Mean	10,066	7,030			0,0714	0,0469		
SE	0,303	0,238			0,008	0,003		
t	9,47				13,54			
P	< 0,0001				< 0,0001			

Table 3.19b ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	10,731	11,688	108,91	118,05±	0,0650	0,0708	108,92	119,21±
	9,968	10,103	101,35	5,60	0,0604	0,0612	101,32	5,60
	9,540	10,393	108,94		0,0717	0,0763	106,41	
	9,557	9,768	102,2.		0,0579	0,0630	108,80	
	7,498	9,402	125,39		0,0625	0,0815	130,88	
	6,909	10,030	145,17		0,0494	0,0716	144,93	
	6,713	9,048	134,78		0,0516	0,0696	134,88	
	7,243	8,518	117,60		0,0557	0,0655	117,58	
Mean	8,520	9,868			0,0593	0,0700		
SE	0,556	0,338			0,003	0,002		
t	-3,66				-3,76			
P	0,008				0,007			
18 hour	6,000	5,431	90,51	78,70±	0,0500	0,0426	85,20	84,57±
	6,418	4,286	66,78	3,13	0,0430	0,0252	4,70	
	6,120	4,793	78,31		0,0360	0,0266	73,88	
	6,259	5,292	84,55		0,0321	0,0286	89,09	
	6,541	5,582	85,33		0,0267	0,0232	86,89	
	6,243	4,300	68,87		0,0272	0,0269	98,89	
	5,746	4,400	76,57		0,0287	0,0275	95,15	
Mean	6,190	4,867			0,0348	0,0287		
SE	0,10u	0,211			0,0030	0,0020		
t	6,320				2,69			
P	0,001				0,036			

Table 3.19c ASS Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	6,110	6,064	99,24	93,66±	0,0244	0,0276	113,11	104,61±
	6,918	5,683	82,14	5,60	0,0276	0,0247	89,49	7,30
	5,906	4,862	82,32		0,0268	0,0243	,67	
	5,224	5,668	108,49		0,0209	0,0258	,44	
	7,693	5,652	73,46		0,0308	0,0246	79,87	
	5,356	5,208	97,23		0,0214	0,0222	103,73	
	5,066	5,710	112,71		0,0197	0,0260	131,97	
Mean	6,039	5,550			0,0245	0,0250		
SE	0,036	0,148			0,002	0,001		
t	1,32				-0,3			
P	NS				NS			

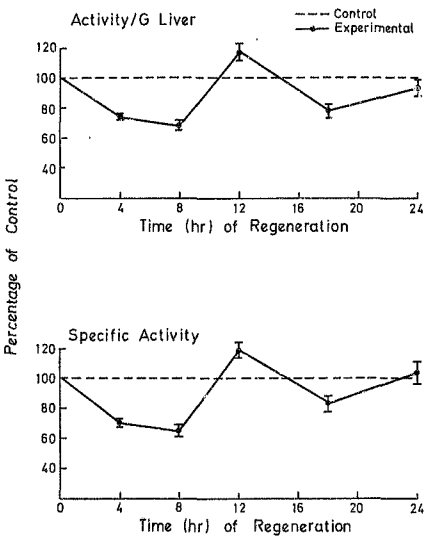


Fig. 3.18 ASS activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 75% protein diet.

Table 3.20a AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	6,030	2,505	41,50	66,20 ± 5,7	0,0303	0,0126	41,50	61,01 ± 5,3
	7,719	6,540	84,70		0,0387	0,0273	70,50	
	5,217	3,546	67,90		0,0336	0,0252	75,00	
	3,996	2,847	71,20		0,0276	0,0138	50,00	
	6,108	3,396	64,40		0,0531	0,0327	61,50	
	6,194	4,186	67,50		0,0476	0,0322	67,60	
Mean	5,877	3,837			0,0385	0,0270		
SE	0,501	0,591			0,004	0,003		
t	5,370				3,200			
P	0,003				0,024			
8 hour	4,808	3,530	73,40	70,63 ± 5,90	0,0282	0,0208	73,70	66,63 ± 6,30
	5,420	4,190	77,30		0,0494	0,0280	56,90	
	11,050	6,390	57,80		0,0736	0,0400	54,30	
	7,256	3,944	54,30		0,0660	0,0360	54,50	
	7,682	5,176	67,30		0,0600	0,0400	66,60	
	7,378	5,918	80,21		0,0614	0,0476	77,52	
Mean	7,266	4,858			0,0564	0,0354		
SE	0,894	0,470			0,007	0,004		
t	4,290				5,270			
P	0,008				0,003			

Table 3.20b AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	5,764	6,740	116,90	142,29± 13,90	0,0348	0,0408	117,20	142,26± 15,00
	4,100	4,172	101,70		0,0248	0,0252	101,60	
	4,000	4,848	121,20		0,0320	0,0336	105,00	
	4,160	7,044	169,30		0,0252	0,0456	180,90	
	3,319	3,807	114,70		0,0277	0,0331	119,40	
	4,568	8,588	188,00		0,0328	0,0616	187,80	
	4,516	8,320	184,20		0,0248	0,064	183,90	
Mean	4,347	6,217			0,0303	0,0434		
SE	0,283	0,738			0,002	0,006		
t	-3,30				-2,74			
P	0,024				0,034			
18 hour	6,945	6,468	93,13	73,96± 4,80	0,0347	0,0293	84,44	73,09± 5,50
	7,387	5,685	74,30		0,0523	0,0344	65,70	
	7,494	4,890	65,20		0,0405	0,0245	60,30	
	6,543	3,285	50,20		0,0385	0,0182	47,30	
	7,440	6,195	93,27		0,0382	0,0293	76,70	
	7,788	6,177	79,31		0,0318	0,0262	82,39	
	8,803	3,972	60,10		0,0287	0,0247	86,10	
	3,849	2,880	74,80		0,0220	0,0180	81,80	
Mean	6,781	4,944			0,0358	0,0257		
SE	0,450	0,490			0,003	0,002		
t	5,50				4,37			
P	0,001				0,003			

Table 3.20c AL Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	4,720	4,414	93,52	84,30±	0,0163	0,0130	79,80	30,04±
	4,852	3,390	70,00	4,32	0,0194	0,0147	75,77	4,10
	4,380	4,190	95,66		0,0172	0,0140	81,40	
	4,722	4,390	93,00		0,0157	0,0123	78,34	
	4,646	3,648	81,72		0,0190	0,0155	81,68	
	4,350	3,113	71,90		0,0171	0,0142	83,23	
Mean	4,610	3,860			0,0174	0,0139		
SE	0,082	0,223			0,001	0,0001		
t	3,70				13,76			
P	0,019				<0,0001			

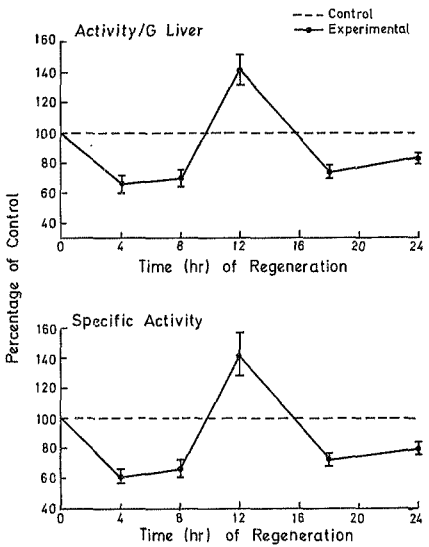


Fig. 3.19 AL activities from regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 75% protein diet.

Table 3.21a ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
4 hour	1424,074	987,488	69,34	84,89± 3,8	6,7813	4,8176	71,04	85.63± 4,3
	1272,291	1253,371	98,51		6,3615	5,2307	82,22	
	1321,039	1273,298	96,39		9,4360	8,7814	93,06	
	1318,568	1259,117	95,59		9,0936	8,3941	92,31	
	1950,032	1524,401	78,17		12,7033	9,5220	74,96	
	1602,522	1390,618	86,68		13,9062	13,8544	99,62	
	1698,964	1391,751	82,03		10,7058	10,5536	98,58	
	1698,629	1423,224	83,90		13,0664	10,5423	80,68	
Mean	1523,506	1318,875			10,1749	8,6920		
SE	87,729	56,897			1,019	1,006		
t	3,24				3,22			
P	0,014				0,015			
8 hour	1279,612	1366,016	107,51	120,12± 7,7	6,3531	6,3535	100,01	107,23± 10,8
	1187,242	1482,890	124,90		6,9838	5,4922	78,64	
	1355,680	1626,816	120,05		9,0794	9,9814	109,98	
	1418,030	1583,324	111,66		12,8936	10,5555	81,87	
	1577,521	2027,957	128,55		13,1460	16,8997	128,55	
	1600,370	1713,129	107,05		10,6691	10,7071	100,36	
	1572,032	1585,501	100,86		14,2912	14,4137	100,86	
	1221,051	1957,297	160,30		9,3927	15,0563	160,30	
Mean	1335,698	1667,865			9,8299	11,0696		
SE	86,15	79,78			1,236	1,456		
t	-3,34				-1,21			
P	0,012				NS			

Table 3.21b ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
12 hour	1193,706	1392,696	116,67	147,58±	7,2346	6,9635	96,25	142,60±
	1113,00	2059,756	185,06	10,9	6,0162	10,4834	174,25	13,8
	1141,687	1685,762	147,66		9,1335	11,6258	127,29	
	1113,783	2021,401	181,48		6,7052	13,0413	193,40	
	1512,340	2140,953	141,75		13,1334	17,8413	135,83	
	1984,122	3307,913	166,71		14,1724	23,6280	166,70	
	1913,325	2000,982	104,98		14,7179	15,3920	104,58	
	2253,805	3926,079	174,20		17,3370	30,2001	174,10	
Mean	1527,,7	2316,941			11,0565	16,3969		
SE	16?,032	302,025			1,521	2,619		
t	-?,14				-3,14			
P	0,004				0,011			
18 hour	922,170	951,153	103,14	102,29±	4,6188	3,7300	80,90	96,21±
	921,109	793,949	89,91	5,9	6,3732	5,4755	85,91	11,80
	943,478	877,348	92,50		5,1270	1,8870	36.81	
	832,857	726,549	87,24		4,8992	4,0363	82,39	
	978,252	883,296	90,29		5,0167	4,7746	95,17	
	801,461	805,026	100,44		4,1101	4,3515	105,87	
	822,319	1009,479	122,76		4,6990	6,3092	134,26	
	687,479	907,788	132,05		3,9285	5,6731	144,41	
Mean	861,639	869,322			4,8456	4,5296		
SE	34,061	32,263			0,264	0,488		
t	0,1				-0,1			
P	NS				NS			

Table 3.21c ARG Activities of control vs. regenerating rat livers for the first 24 hours after partial hepatectomy. Rats were maintained on a 75% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
24 hour	1402,015	1116,015	79,60	84,20 ± 3,07	5,6081	5,0720	90,45	91,74 ± 3,40
	1202,736	931,361	77,37		4,8150	4,0494	84,10	
	1202,426	1170,815	97,37		5,8655	5,8541	99,81	
	1296,208	999,046	77,07		5,0871	4,5411	89,27	
	1150,163	946,932	82,30		5,1118	4,1171	80,54	
	1309,818	1200,844	91,68		5,5737	5,1100	91,68	
	1219,040	1060,629	87,01		5,3106	5,4583	106,39	
Mean	1254,770	1060,840			5,3388	4,8848		
SE	32,368	40,327			0,138	0,256		
t	3,12				3,01			
P	0,002				0,024			

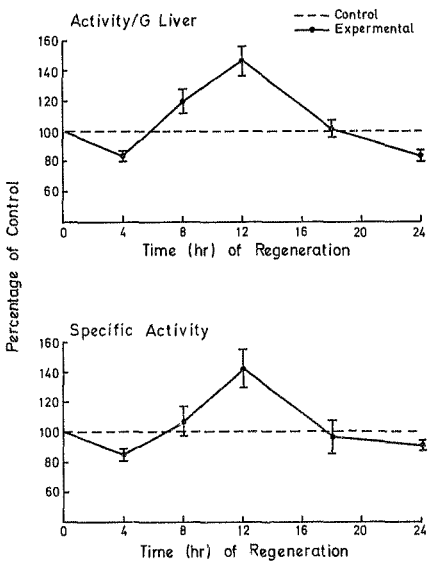


Fig. 3.20 *Arginase activities from regenerating* rat livers expressed as a % of zero-time controls. Rats were maintained on a 7% protein diet.

Animals were sham operated four hours prior to sacrifice and their hepatic urea cycle enzymes were assayed and activities compared to those of nonoperated controls, to test whether the shock of the operation or the anaesthetic were responsible for the drop observed in the regenerating livers after partial hepatectomy.

As can be seen from tables 3.22a and 3.22b there is no significant difference between the sham operated animals and controls indicating that the observed drop in regenerating liver is specific and not due to the handling of the animals, the anaesthetic nor the operation itself.

Table 3.22a A comparison of urea cycle enzyme activities in control vs. sham operated animals. Enzymes were assayed at four hours post-operatively.

	CONTROL		SHAM OPERATED	
CPS.	Activity/g liver	Specific Activity	Activity/gliver	Specific Activity
	5,8300	0,0248	5,2060	0,0347
	5,3110	0,0351	4,3745	0,0190
	4,7900	0,0212	4,2690	0,0334
	4,8400	0,0358	5,9428	0,0282
Mean±	5,1930	0,0292	4,9479	0,0289
S.E.	0,548	0,0036	0,4250	0,0050
OTC.	250,0122	1,0638	280,6061	1,2353
	249,5010	1,4176	225,5250	1,4152
	257,7200	1,1897	160,9810	1,2540
	270,0111	1,0185	220,2250	1,0102
Mean±	256,8111	1,1720	221,8330	1,2286
S.E.	14,1100	0,0830	22,4200	0,1400
ASS.	1,2500	0,0130	2,3436	0,0260
	2,4500	0,0170	2,8125	0,0200
	2,8248	0,0151	2,5586	0,0290
	3,7500	0,0340	2,5311	0,0330
Mean±	2,5685	0,0212	2,5615	0,0270
S.E.	0,2201	0,0020	0,2122	0,0023
AL.	1,7500	0,0071	1,2500	0,0071
	2,2500	0,0050	2,0800	0,0075
	1,6000	0,0110	2,0400	0,0082
	1,0100	0,0057	1,6500	0,0064
Mean±	1,6530	0,0075	1,7550	0,0073
S.E.	0,0430	0,0004	0,2020	0,0010

Table 3.22b A comparison of urea cycle enzyme activities in control vs. sham operated animals. Enzymes were assayed four hours post-operatively.

	CONTROL		SHAM OPERATED	
	Activity/g	Specific Activity	Activity/g	Specific Activity
ARG.	648,750	2,761	825,011	2,705
	795,000	2,259	1080,024	2,348
	1061,250	2,358	660,715	2,276
	702,001	2,933	642,513	1,967
Mean±	824,250	2,578	802,066	2,324
S.E.	76,471	0 ·24	85,717	0,151

The results described in earlier experiments were due to the interaction of a number of effects. In order to shed more light on mechanism of these changes in enzyme levels, effects of some inhibitors of RNA synthesis were tested.

The effect of the RNA synthesis inhibitor, actinomycin D (AMD) on hepatic CPS, ASS, AL and arginase was determined in 35-day old rats maintained on a 22% protein diet and an 8-16 controlled feeding schedule for one week prior to sacrifice. For the experimental group of animals partial hepatectomy was performed at 24:00 hours and 04:00 hours and the animals sacrificed at 10:00 hours for the ten and six hour point respectively. An i.p. injection of AMD (50µg/50g) was administered to these animals at zero time and the activity of the urea cycle enzymes was assayed at zero time and at six and ten hours postoperatively. The results of these experiments were compared with non-AMD treated controls. Results are expressed as activity / g liver and specific activity and are shown in tables 3.23a to 3.26b and a graphic representation of % differences is shown in figs 3.21 to 3.24. The significance of the differences was determined using the paired t-test. Differences with P values of less than 5% are considered to be significant.

The activity of CPS drops to 65% of control in the non-AMD-treated animals at six hours but in the AMD-treated animal increase to 120% of the control, an effective increase of 55%. At ten hours in the nontreated animals the activity of CPS is about 80% of the control value while in the AMD-treated animals it is 150%, an effective increase of 70% (see tables 3.23a, b; fig 3.21).

At six hours the activity of ASS in nontreated regenerating liver is 74% of controls but in the AMD-treated animals it is about equal to the zero time controls, an effective increase of 76%. At ten hours the activity in the nontreated regenerating liver is about 85% of the zero time control, but in the AMD-treated animals, it is about 135%, an effective increase of 50% (see tables 3.24a, b; figure 3.22).

The activity of AL by six hours of regeneration in nontreated liver is about 65% of the zero time control and in the treated livers increases to about 78%, an increase of about 13%. By ten hours postoperatively, the activity of AL in nontreated regenerating liver is 76% of the zero time control but in the AMD-treated regenerating livers it increased to 113% of control, an increase of about 37% (see tables 3.25a,b and figure 3.23).

Arginase shows a similar pattern of change, its activity being about 20% greater in AMD treated compared with nontreated regenerating livers by six hours postoperatively and 60% greater by ten hours postoperatively (see tables 3.26 a, b and fig 3.24).

In summary, AMD inhibits the initial drop in levels of urea cycle enzymes, and accentuatesthe subsequent increase normally seen by 10 hours postoperatively.

Table 3.23a CPS Activities from control and Actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour Control	2,0784	1,4356	69,07	62,61± 1,89	0,0097	0,0072	74,23	67,84± 2,65
	1,7206	1,1141	64,75		0,0086	0,0060	69,77	
	2,2824	1,3130	57,40		0,0114	0,0071	62,28	
	1,7432	1,0137	58,09		0,0076	0,0051	67,11	
	2,1146	1,2825	60,65		0,0092	0,0054	58,70	
	2,1894	1,4393	65,74		0,0096	0,0072	75,00	
Mean	2,0218	1,2664			0,0093	0,0063		
SE	0,096	0,070			0,001	0,000		
t	14,020				9,030			
P	0,001				0,001			
6 hour AMD	1,6208	1,6728	102,53	104,51± 6,61	0,0067	0,0096	143,28	124,96± 7,15
	1,2302	1,6978	138,01		0,0062	0,0086	139,00	
	1,6302	1,4592	89,51		0,0082	0,0088	103,32	
	1,7884	2,0744	115,99		0,0072	0,0104	144,44	
	1,9008	1,8104	95,23		0,0076	0,0091	119,74	
	2,1224	1,8742	88,31		0,0095	0,0092	97,89	
	2,1250	2,1682	102,03		0,0085	0,0108	127,06	
Mean	1,7754	1,8224			0,0077	0,0101		
SE	0,119	0,092			0,000	0,000		
t	-0,49			6,10	-2,940			7,50
P	NS			0,001	0,026			0,001

Table 3.23b CPS Activities from control and Actinomycin D treated regenerating rat livers. *Rats were maintained on a 22% protein diet.*

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour Control	1,7658	1,2270	69,49	86,27±	0,0088	0,0072	81,82	81,09±
	1,7165	1,3168	88,37	3,99	0,0076	0,0075	98,68	6,21
	1,9043	1,7657	92,72		0,0098	0,0095	96,94	
	2,2270	2,2221	95,43		0,0183	0,0106	57,92	
	1,3670	1,2523	91,61		0,0068	0,0052	76,47	
	1,5222	1,2160	80,00		0,0099	0,0074	74,75	
Mean	1,7670	1,5333			0,0102	0,0079		
SE	0,156	0,164			0,002	0,001		
t	3,220				2,920			
P	0,018				0,026			
10 hour AMD	2,2270	2,5130	112,84	121,44±	0,0097	0,0123	126,80	150,36±
	1,5325	2,0190	123,68	6,22	0,0071	0,0135	190,14	12,40
	1,5470	1,7120	110,67		0,0077	0,0114	148,05	
	1,7023	1,7878	105,02		0,0077	0,0076	98,70	
	2,1665	2,5695	118,60		0,0072	0,0105	145,83	
	1,9538	2,4348	123,98		0,0065	0,0099	152,31	
	1,6260	2,5288	155,34		0,0054	0,0103	190,74	
Mean	1,8372	2,2360			0,0073	0,0108		
SE	0,105	0,147			0,000	0,001		
t	-3,930			4,76	-4,550			4,99
P	0,008			0,001	0,004			0,001

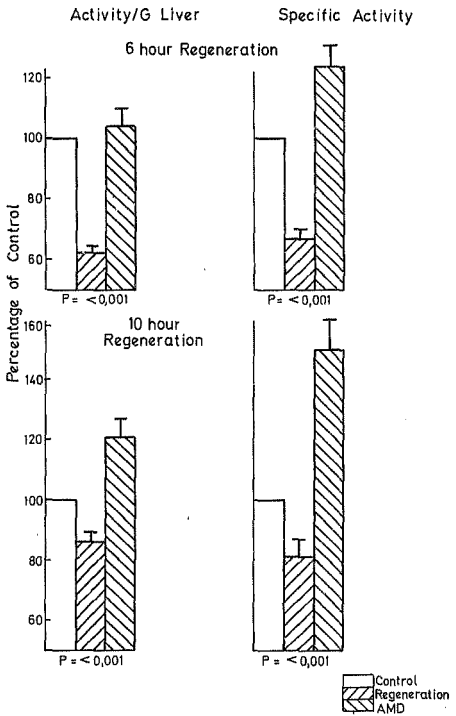


Fig. 3.21 Activity/g liver and specific activity of CPS from 6 hour and 10 hour untreated regenerating rat livers and AMD-treated regenerating rat livers expressed as a % of zero time controls. Rats were maintained on a 22% protein diet.

Table 3.24a ASS Activities from control and Actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour Control	2,794	1,740	62,28	74,25± 1,35	0,0123	0,0087	70,73	74,62± 2,33
	3,379	2,256	66,77		0,0147	0,0122	82,99	
	2,537	2,172	86,64		0,0117	0,0087	74,36	
	2,150	1,698	78,61		0,0092	0,0067	69,79	
	2,468	1,900	76,98		0,0101	0,0076	75,25	
Mean	2,662	1,953			0,0116	0,0088		
SE	0,206	0,112			0,001	0,001		
t	4,430				12,950			
P	0,011				0,001			
6 hour AMD	2,909	2,547	87,55	84,16± 7,03	0,0119	0,0146	122,69	101,74± 12,33
	2,212	2,077	98,34		0,0106	0,0154	145,28	
	2,273	2,562	112,71		0,0114	0,0155	135,96	
	2,773	2,546	91,78		0,0139	0,0127	91,37	
	2,271	1,483	65,30		0,0114	0,0094	82,46	
	1,724	1,220	70,77		0,0086	0,0061	70,93	
	2,145	1,345	62,70		0,0107	0,0068	63,55	
Mean	2,315	1,969			0,0112	0,0112		
SE	0,153	0,230			0,001	0,002		
t	2,320			1,20	0,000			2,16
P	NS			NS	NS			0,03

Table 3.24b ASS Activities from control and Actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour	1,791	1,403	78,34	82,97±	0,0090	0,0080	88,89	86,57±
Control	2,509	2,166	84,34	3,08	0,0111	0,0098	88,29	3,85
	2,347	2,052	86,60		0,0146	0,0131	89,73	
	1,924	1,516	78,79		0,0089	0,0070	78,65	
	1,047	0,729	69,63		0,0053	0,0036	67,92	
	1,040	1,714	88,35		0,0097	0,0095	97,94	
	1,865	1,768	94,80		0,0093	0,0088	94,62	
Mean	1,918	1,618			0,0097	0,0086		
SE	0,177	0,179			0,001	0,001		
t	7,410				5,090			
P	0,001				0,002			
10 hour	2,286	2,629	115,00	118,34±	0,0100	0,0150	150,00	138,22±
AMD	3,148	3,776	119,95	3,62	0,0134	0,0252	188,06	10,45
	1,498	1,750	116,82		0,0094	0,0159	169,15	
	1,621	1,824	112,52		0,0074	0,0078	105,41	
	1,135	1,445	127,31		0,0045	0,0059	131,11	
	1,280	1,727	134,92		0,0051	0,0066	129,41	
	1,890	1,894	100,21		0,0076	0,0078	102,63	
	1,500	1,800	120,00		0,0060	0,0078	130,00	
Mean	1,795	2,106			0,0079	0,0115		
SE	0,231	0,267			0,001	0,002		
t	-4,860			7,45	-2,53			4,64
P	0,002			0,001	0,039			0,001

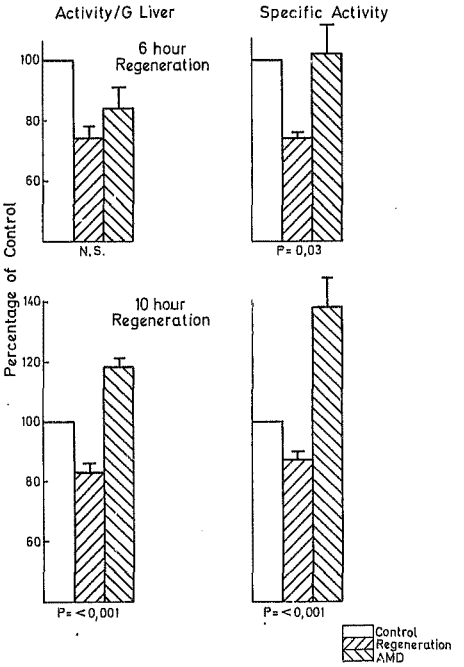


Fig. 3.22 Activity/g liver and specific activity of ASS from 6 hour and 10 hour untreated regenerating rat livers and AMD-treated regenerating rat livers expressed as a % of zero time controls. Rats were maintained on a 22% protein diet.

Table 3.25a AL Activities from control and Actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour Control	1,827	1,166	63,82	65,52±	0,0085	0,0058	68,24	66,75±
	1,266	0,970	76,61	3,93	0,0055	0,0049	89,09	4,39
	2,088	0,542	40,32		0,0097	0,0046	47,42	
	0,242	0,867	69,81		0,005	0,0038	71,83	
	1,706	1,082	63,42		0,0073	0,0047	64,38	
	1,700	1,250	73,53		0,0072	0,0050	69,44	
	2,141	1,439	67,21		0,0157	0,0085	54,14	
	1,897	1,128	59,46		0,0095	0,0066	69,47	
Mean	1,733	1,095			0,0085	0,0055		
SE	0,188	0,070			0,0010	0,0005		
t	6,120				4,17			
P	0,000				0,004			
6 hour AMD	1,707	1,276	97,63	75,92±	0,0073	0,0053	68,00	78,56±
	1,[illegible]	1,076	60,76	6,12	0,0089	0,0080	89,89	4,65
	1,[illegible]	0,695	61,83		0,0056	0,0042	75,00	
	1,[illegible]27	0,667	67,65		0,0058	0,0034	58,62	
	1,478	0,930	62,92		0,0059	0,0047	79,66	
	0,989	0,860	86,96		0,0050	0,0043	86,00	
	1,069	1,002	93,73		0,0054	0,0050	92,78	
Mean	1,270	0,929			0,0059	0,0053		
SE	0,103	0,081			0,0004	0,0007		
t	3,44			1,43	3,36			1,85
P	0,014			NS	0,019			0,04

Table 3.25b AL Activities from control and Actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour Control	1,734	1,310	98,20	81,47±	0,0076	0,0066	86,84	76,50±
	1,892	1,368	72,30	4,50	0,0108	0,0078	72,22	5,18
	1,724	1,300	75,41		0,0108	0,0065	60,19	
	1,512	1,201	79,43		0,0076	0,0057	75,00	
	1,584	1,270	82,04		0,0072	0,0064	88,89	
Mean	1,609	1,290			0,0088	0,0065		
SE	0,946	0,273			0,0008	0,0004		
t	3,830				3,480			
P	0,019				0,025			
10 hour AMD	1,636	1,168	71,40	82,06±	0,0071	0,0067	94,37	112,67±
	1,176	0,944	80,27	3,15	0,0054	0,0063	116,67	9,42
	1,368	1,088	79,53		0,0062	0,0099	159,68	
	1,718	1,124	79,27		0,0065	0,0056	86,15	
	1,296	1,252	96,60		0,0043	0,0051	118,60	
	1,551	1,272	82,01		0,0052	0,0049	94,23	
	1,030	0,955	90,95		0,0035	0,0042	120,00	
Mean	1,356	1,114			0,0054	0,0061		
SE	0,772	0,493			0,0005	0,0007		
t	4,570			0,11	-1,12			0,36
P	0,004			NS	NS			0,003

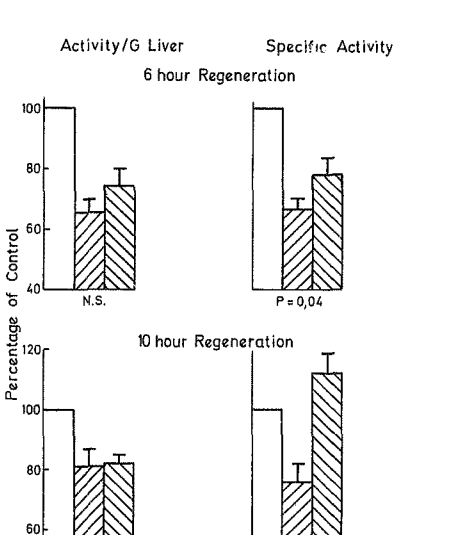


Fig. 3.23 Activity/g liver and specific activity of AL from 6 hour and 10 hour untreated regenerating rat livers and AMD-treated regenerating rat livers expressed as a % of zero time controls. Rats were maintained on a 22% protein diet.

Table 3.26a Arginase Activities from control and Actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour Control	1692,973	1444,248	85,30	87,82±	7,8743	6,2794	79,75	74,36±
	1034,613	909,722	87,93	3,12	4,8122	3,9553	82,19	2,69
	1484,916	1126,625	75,87		6,9006	4,8984	70,92	
	1288,240	1146,066	88,96		5,4819	3,5814	65,33	
	1246,597	1112,231	89,22		5,3047	3,7074	69,89	
	1445,196	1440,754	99,69		6,1498	4,8025	78,09	
Mean	1365,421	1196,606			6,0882	4,5374		
SE	92,639	85,208			0,465	0,415		
t	3,420				9,170			
P	0,019				0,001			
6 hour AMD	957,688	906,001	94,60	87,85±	4,7984	5,1771	107,89	99,71±
	1010,478	827,707	81,11	2,13	5,1020	6,1312	120,17	4,81
	1352,229	1181,403	87,36		6,7511	7,1600	106,06	
	1132,330	1029,808	92,93		5,4716	5,1690	94,10	
	1363,267	1105,340	80,78		6,8413	5,5267	80,78	
	1367,719	1264,118	92,42		6,8386	6,3356	92,64	
	1383,460	1186,616	85,77		6,9172	5,9331	85,77	
Mean	1225,619	1071,570			6,1029	5,9161		
SE	69,557	60,188			0,354	0,270		
t	5,720			0,01	0,590			4,60
P	0,001			NS	NS			0,001

Table 3.26b Arginase Activities from control and Actinomycin D treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour	1051,814	983,323	93,68	93,99±	5,0951	4,7659	93,61	93,08±
Control	1296,108	1126,167	86,89	1,59	5,1022	5,0548	99,07	2,54
	999,399	900,000	90,05		5,7065	5,0499	89,19	
	1337,238	1318,091	98,57		6,6076	5,5133	77,99	
	1095,401	1025,394	93,61		10,5151	9,8533	93,71	
	1020,137	1009,166	98,90		1,8544	1,6201	97,67	
	1114,130	1017,976	91,37		5,5823	5,2941	94,84	
	1059,221	1047,530	98,90		5,4521	5,4921	100,62	
Mean	1121,718	1053,705			7,5335	5,3652		
SE	44,65	43,829			1,390	0,768		
t	5,970				3,240			
P	0,001				0,012			
10 hour	1386,90	1813,566	130,76	125,16±	6,0300	10,3632	171,86	161,94±
AMD	977,787	1471,428	150,49	5,88	4,1608	9,9095	238,16	16,24
	1341,708	1537,101	114,56		8,3957	13,9336	165,96	
	1220,835	1458,354	119,46		5,5493	6,2058	111,83	
	1172,522	1349,837	115,12		3,7223	5,5095	148,01	
	1315,519	1403,483	106,69		4,3851	5,1021	116,35	
	1195,094	1662,229	139,08		3,9836	7,2271	181,42	
Mean	1231,479	1527,998			5,1752	8,3215		
SE	52,524	60,743			0,623	1,216		
t	-4,900			5,12	-3,860			4,19
P	0,003			0,001	0,008			0,001

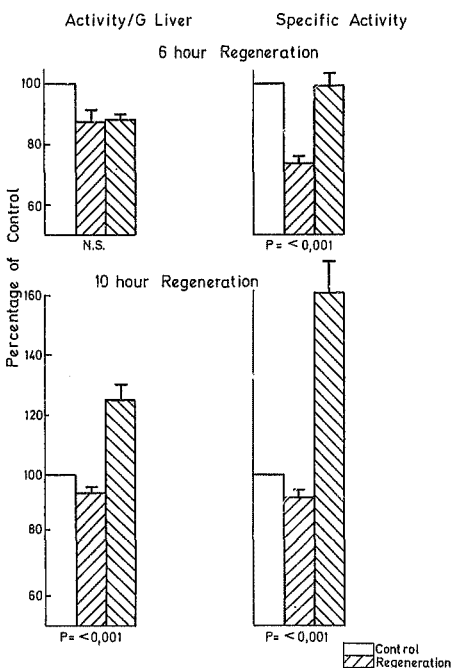


Fig. 3.24 Activity/g liver and specific activity of ARG from 6 hour and 10 hour untreated regenerating rat livers and AMD-treated regenerating rat livers on a 22% protein diet.

The effect of ·oin, the inhibitor of RNA synthesis was tested on hepatic CPS, and arginase. The experimental conditions and methods e: ed were identical to those in the previous experiment except that an i.p. of cordecepin (1mg/100g) was injected at zero time.

In inhibitor-untreated regenerating liver at six hours postoperatively the activity of CPS drops by 35% but in cordecepin-treated livers it drops by 8% only; an effective increase of 73%. At 10 hours of regeneration the activity in the cordecepin-treated regenerated liver is 66% greater than in the untreated control (see tables 3.27a, b; fig 3.25).

The activity of ASS is 75% greater in cordecepin-treated than nontreated livers by six hours and by ten hours is 40% greater (see tables 3.28a, b; fig 3.26).

The activity of AL in the cordecepin-treated livers is 30% greater compared to the untreated livers by six hours postoperatively. By ten hours the activi..es differ by 46% with the cordecepin-treated livers having the greater activity (see tables 3.29a, b; fig 3.27).

For arginase the difference in activity between the inhibitor-treated and untreated regenerating livers is 70% and 30% for six hours and ten hours of regeneration respectively, the inhibitor-treated livers having greater arginase activity in both instances (see tables 3.30a, b; fig 3.28).

In conclusion the effect of cordecepin is similar to that of *Actinomycin D on the inhibition of* the initial drop in urea cycle enzyme activity following partial hepa·ectomy as well as in accentuating the subsequent increase.

Table 3.27a CPS Activities of control and Cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour Control	4,3700	2,8790	65,74	62,61±	0,0192	0,0144	75,00	67,84±
	4,1560	2,8720	69,07	1,89	0,0194	0,0154	74,23	2,81
	3,4400	2,2280	64,75		0,0172	0,0120	69,77	
	4,5600	2,6260	57,40		0,0224	0,0142	62,28	
	3,4900	2,0270	58,09		0,0152	0,0102	67,11	
	4,2200	2,5650	60,65		0,0184	0,0108	58,70	
Mean	4,0560	2,5320			0,0186	0,0126		
SE	0,1920	0,140			0,001	0,001		
t	6,060				9,030			
P	0,002				0,001			
6 hour Corde-cepin	2,8150	2,0960	74,46	95,30±	0,0188	0,0161	85,64	91,81±
	1,4705	1,3775	68	8,06	0,0098	0,0095	96,94	2,36
	2,1445	2,429	9		0,0172	0,0157	91,28	
	2,3060	2,500	6		0,0167	0,0156	93,41	
Mean	2,2340	2,100[illegible]			0,0156	0,0142		
SE	0,289	0,257			0,002	0,002		
t	0,63			3,95	2,800			6,53
P	NS			0,004	0,013			0,001

Table 3.27b CPS Activities of control and Cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour Control	3,5320	2,5440	69,49	86,26 ± 4,00	0,0178	0,0144	81,82	81,03 ± 6,26
	3,4330	3,0320	88,37		0,0154	0,0150	98,68	
	3,8090	3,5310	92,72		0,0196	0,0190	96,94	
	4,6570	4,4410	95,43		0,0183	0,0106	57,92	
	3,7340	3,5050	91,60		0,0136	0,0104	76,47	
	3,0410	2,4320	80,00		0,0198	0,0148	74,75	
Mean	3,5340	3,0660			0,0172	0,0140		
SE	0,272	0,328			0,001	0,001		
t	3,440				3,400			
P	0,018				0,020			
10 hour Cordecepin	2,5960	2,7130	104,51	141,71 ± 26,46	0,0130	0,0160	123,08	147,09 ± 20,57
	2,1765	4,7050	216,20		0,0115	0,0240	208,70	
	1,8845	2,6910	142,80		0,0094	0,0122	129,51	
	2,6550	2,7435	103,33		0,0144	0,0183	127,08	
Mean	2,3280	3,2131			0,0121	0,0165		
SE	0,182	0,497			0,002	0,003		
t	-1,55			2,07	-1,510			3,07
P	NS			0,03	NS			0,006

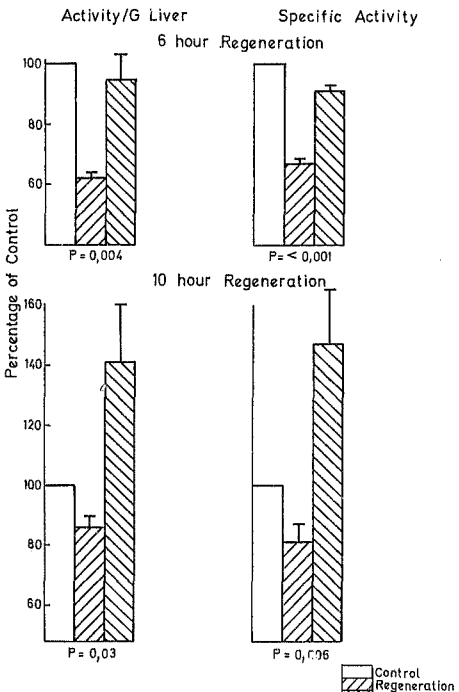


Fig. 3.25 Activity/g liver and specific activity of CPS from 6 hour and 10 hour untreated regenerating rat livers and cordecepin-treated regenerating rat livers expressed as a % of zero time control livers. Rats were maintained on a 22% protein diet.

Table 3.28a ASS Activities from control and Cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour Control	1,664	1,445	86,84	77,61± 5,93	0,0110	0,0085	77,27	76,65± 6,3
	1,425	1,255	88,07		0,0095	0,0074	77,89	
	2,124	1,569	77,84		0,0054	0,0052	96,29	
	1,503	1,170	77,84		0,0039	0,0027	69,23	
	1,562	1,461	93,53		0,0037	0,0045	82,22	
	1,680	1,095	65,18		0,0041	0,0024	58,85	
	2,160	1,698	78,61		0,0092	0,0067	69,79	
	2,468	1,900	76,98		0,0101	0,0076	75,25	
Mean	1,916	1,682			0,0063	0,0047		
SE	0,159	0,126			0,001	0,001		
t	5,650				3,650			
P	0,002				0,015			
6 hour Corde-cepin	1,530	1,520	99,35	103,83± 4,16	0,0120	0,0012	100,00	103,80± 4,12
	1,289	1,283	99,53		0,0086	0,0085	98,83	
	1,275	1,554	121,88		0,0080	0,0082	102,50	
	1,878	1,563	83,23		0,0117	0,0115	98,29	
	1,293	1,216	94,04		0,0072	0,0066	91,67	
	1,333	1,394	104,58		0,0099	0,0096	96,97	
	1,834	1,805	98,42		0,0096	0,0125	130,21	
	1,581	1,835	116,07		0,0105	0,0102	97,14	
	1,012	1,188	117,39		0,0075	0,0089	118,67	
Mean	1,447	1,467			0,0101	0,0098		
SE	0,094	0,090			0,001	0,001		
t	-0,340			3,62	0,410			3,61
P	NS			0,004	NS			0,004

Table 3.28b ASS Activities from control and Cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour Control	1,940	1,714	88,35	82,97± 3,08	0,0097	0,0095	97,94	86,57± 3,90
	1,863	1,768	94,80		0,0093	0,0088	94,62	
	1,791	1,403	78,34		0,0090	0,0080	88,89	
	2,309	2,166	84,34		0,0111	0,0098	88,29	
	2,347	2,033	86,60		0,0146	0,0131	89,73	
	1,047	0,729	69,63		0,0089	0,0070	78,63	
Mean	1,918	1,618			0,0053	0,0036	67,92	
SE	0,177	0,179			0,0097	0,0085		
t	7,410				0,001	0,001		
P	0,001				4,880			
10 hour Corde-cepin	1,442	1,700	117,89	110,15± 3,14	0,0072	0,0100	138,89	127,29± 5,66
	1,703	1,736	102,99		0,0090	0,0091	101,11	
	2,051	2,100	102,39		0,0103	0,0140	135,92	
	1,381	1,419	102,75		0,0071	0,0075	105,64	
	1,393	1,687	121,11		0,0084	0,0121	144,05	
	0,910	1,167	128,24		0,0062	0,0073	117,74	
	0,898	1,065	118,60		0,0062	0,0073	117,74	
	1,154	1,341	116,20		0,0096	0,0154	160,42	
	1,354	1,317	97,27		0,0071	0,0088	123,94	
	1,132	1,128	99,65		0,0078	0,0086	110,26	
	1,492	1,560	104,56		0,0083	0,0120	144,58	
Mean	1,356	1,476			0,0079	0,0102		
SE	0,101	0,095			0,000	0,001		
t	-3,460			-6,18	-4,180			5,93
P	0,006			0,001	0,002			0,001

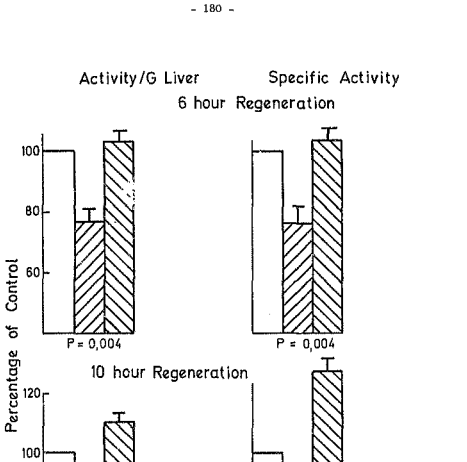


Fig. 3.2 Activity/g liver and specific activities of ASS from 6 hour and 10 hour regenerating rat livers from control and cordecepin-treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet.

Table 3.29a AL Activities from control and cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour Control	1,897	1,128	59,46	65,51± 2,94	0,0095	0,0066	69,47	68,34± 3,60
	2,141	1,439	67,21		0,0157	0,0085	54,14	
	2,362	1,187	50,25		0,0131	0,0079	60,31	
	1,827	1,166	63,82		0,0085	0,0058	68,24	
	1,268	0,970	76,61		0,0110	0,0098	89,09	
	1,242	0,867	69,81		0,0106	0,0086	71,70	
	1,706	1,082	63,42		0,0073	0,0047	64,38	
	1,700	1,250	73,53		0,0072	0,0050	69,44	
Mean	1,768	1,136			0,0103	0,0075		
SE	0,137	0,061			0,0010	0,0006		
t	6,450				4,67			
P	0,000				0,002			
6 hour Corde- cepin	1,964	1,069	54,43	93,92± 6,22	0,0131	0,0085	64,89	97,32± 3,48
	2,259	1,355	60,04		0,0137	0,0110	93,97	
	1,589	1,244	78,29		0,0106	0,0099	93,40	
	1,760	1,355	76,99		0,0117	0,0108	92,31	
	1,424	1,564	109,83		0,0086	0,0082	95,35	
	2,316	2,066	89,21		0,0140	0,0125	99,29	
	2,061	2,295	111,35		0,0115	0,0124	107,83	
	2,008	2,195	104,33		0,0134	0,0133	99,25	
	1,920	2,031	105,78		0,0116	0,0123	106,03	
	2,093	2,427	115,96		0,0140	0,0135	96,43	
	2,530	2,726	107,78		0,0141	0,0151	107,91	
	1,800	2,000	111,11		0,0090	0,0100	111,11	
Mean	1,976	1,777			0,0121	0,0114		
SE	0,089	0,158			0,005	0,006		
t	1,35			4,13	1,47			5,78
P	NS			0,001	NS			0,001

Table 3.29b AL Activities from control and cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour Control	1,664	1,355	81,42	84,07± 3,33	0,0083	0,0075	90,36	80,09± 4,52
	1,589	1,500	94,40		0,0079	0,0075	94,94	
	1,539	1,404	91,23		0,0103	0,0085	82,52	
	1,670	1,300	77,57		0,0105	0,0068	64,76	
	1,334	1,370	98,20		0,0076	0,0066	86,84	
	1,584	1,270	82,04		0,0072	0,0064	88,89	
	1,892	1,368	72,30		0,0108	0,0078	72,22	
	1,724	1,300	75,41		0,0108	0,0065	60,19	
Mean	1,654	1,350			0,0091	0,0072		
SE	0,067	0,026			0,0005	0,0003		
t	4,31				3,74			
P	0,004				0,007			
10 hour Corde- cepin	2,103	2,008	95,08	117,11± 11,18	0,0140	0,0154	110,00	126,25± 4,46
	2,114	2,903	137,32		0,0141	0,0215	152,25	
	1,931	2,008	103,99		0,0129	0,0149	115,50	
	1,964	1,806	91,96		0,0131	0,0144	109,92	
	2,091	2,296	109,80		0,0110	0,0139	126,36	
	1,736	2,856	148,96		0,0107	0,0144	134,58	
	2,204	2,849	129,26		0,0122	0,0158	129,51	
	1,864	2,803	150,38		0,0129	0,0156	120,93	
	1,986	2,811	141,54		0,0124	0,0156	125,81	
	1,602	2,176	135,83		0,0134	0,0145	108,21	
	2,008	2,613	130,13		0,0138	0,0174	126,09	
	2,000	2,600	130,00		0,0111	0,0173	155,86	
Mean	1,966	2,454			0,0126	0,0158		
SE	0,048	0,109			0,0003	0,0006		
t	-4,43			2,830	-5,88			7,270
P	0,001			0,006	0,0001			0,001

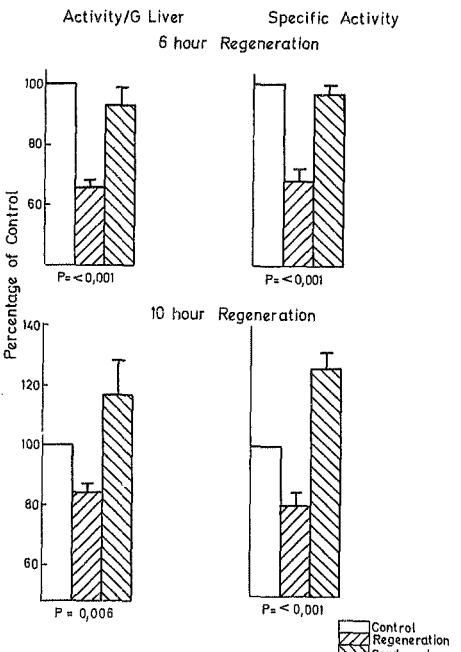


Fig. 3.27 Activity/g liver and specific activity of AL from 6 hour and 10 hour untreated and cordecepin-treated regenerating rat livers expressed as a % of zero-time controls. Rats were maintained on a 22% protein diet.

Table 3.30: ARG Activities from control and Cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
6 hour Control	1018,013	674,775	66,28	67,04± 0,95	4,7349	3,9693	83,83	71,00± 8,76
	1010,629	669,064	66,20		5,4629	3,9357	72,04	
	1045,071	687,566	65,79		6,9671	3,1980	45,90	
	921,872	644,350	69,90		4,6094	3,7905	82,23	
Mean	998,896	668,938			5,446	3,772		
SE	26,718	9,059			0,541	0,179		
t	18,490				9,050			
P	0,001				0,001			
6 hour Corde-cepin	1075,796	940,233	87,40	102,81± 5,10	7,1720	6,2682	87,32	91,52± 4,60
	1056,052	949,029	89,87		7,0403	6,3269	89,87	
	923,673	897,150	97,19		6,1538	5,9808	97,19	
	973,640	920,000	94,49		6,491	5,7500	98,58	
	1118,316	811,986	72,61		6,7777	4,6399	68,48	
	1253,350	1240,916	99,01		7,5961	5,4132	71,26	
	912,269	1173,355	128,62		5,7017	6,3425	111,24	
	860,563	1042,999	121,20		6,3745	7,1931	112,84	
	1020,487	1087,766	106,59		6,8032	7,2518	106,59	
	875,397	996,797	113,87		6,4844	5,5378	85,40	
	838,221	1006,560	120,08		6,4480	5,0327	78,05	
Mean	991,558	1006,069			6,6410	5,9761		
SE	38,397	37,635			0,155	0,247		
t	-0,290			6,89	2,150			2,07
P	NS			0,001	NS			0,03

Table 3.30b ARG Activities from control and Cordecepin treated regenerating rat livers. Rats were maintained on a 22% protein diet.

	Activity/g Liver				Specific Activity			
	Control	Regn	% of Control	Mean ± SE	Control	Regn	% of Control	Mean ± SE
10 hour Control	1095,450	1025,394	93,60	95,73±	5,0951	4,7693	93,61	91,30±
	1020,437	1009,166	98,90	1,93	5,1022	5,0458	98,98	3,29
	1114,130	1017,976	91,37		5,7065	5,0899	89,19	
	1057,221	1047,530	99,08		6,6076	5,5133	83,44	
Mean	1071,799	1025,016			5,628	5,105		
SE	20,823	8,220			0,357	0,154		
t	2,160				2,800			
P	NS				0,013			
10 hour Cordecepin	959,889	982,287	102,33	119,95±	4,7944	5,7782	120,52	121,32±
	1072,111	1092,993	101,95	2,54	5,6427	5,7526	101,95	5,68
	881,778	1005,706	113,67		4,4239	6,7047	151,56	
	1031,542	1113,865	107,98		5,4786	5,5693	101,65	
	917,771	979,811	106,60		5,5622	5,9382	106,76	
	1042,954	1139,710	109,28		6,3209	8,1408	128,79	
	930,608	1026,813	110,34		5,1700	6,4176	124,13	
	941,791	1026,813	109,09		6,4951	7,0816	109,03	
	904,791	1223,047	135,17		6,2399	7,8906	126,45	
	1082,546	1244,450	113,110		7,4658	8,1630	109,34	
	959,816	1103,963	115,02		6,6194	7,3500	111,04	
	741,462	882,109	118,97		4,1192	6,7855	164,73	
Mean	952,437	1063,962			5,6943	6,7977		
SE	27,719	31,777			0,283	0,272		
t	-4,540			7,59	-4,540			4,58
P	0,001			0,001	0,001			0,001

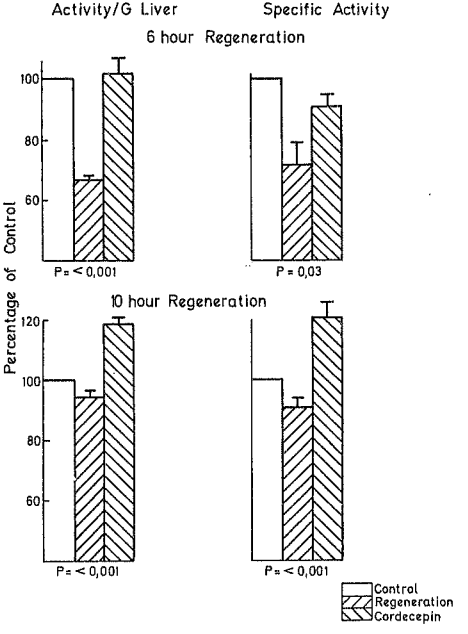


Fig. 3.28 Activity/g liver and specific activity of ARG from 6 hour and 10 hour untreated regenerating rat livers and cordecepin-treated regenerating rat livers expressed as a % of zero time control livers. Rats were maintained on a 22% protein diet.

3.4 Urea cycle enzymes in neoplasia

The effect of the administration of different hormones was studied in normal liver, host livers and hepatomas of 35 day old rats maintained on the various protein diets. The hormones studied included β-methasone (100 µg /50g), glucagon (250 µg /50g), dBcAMP (250µg / 50 g) and combination of β-methasone and glucagon. Animals were trained to an 8-16 controlled feeding regime and to a particular diet for one week prior to sacrifice. Groups of eight animals were chosen randomly from the tumour- and nontumour-bearing animals and given a single i.p. injection of a particular hormone or combination of hormones, twenty four hours prior to sacrifice. A further group of nonhormone-treated animals trained to the appropriate diet were used as controls for comparison. All animals were sacrificed at 10:00 hours.

From tables 3.31a to 3.40b it can be seen that the urea cycle enzymes of the control livers do not respond significantly to the hormone stimulus irrespective of diet on which they are maintained. This is in agreement with the experiments described earlier wherein livers of 35 day old rats are not responsive to glucagon induction. The results are similar when described as activity/gram liver or as specific activity.

The hormonal effects on host livers of 35-day old tumour-bearing animals are described in figures 3.35, 3.36, 3.39, 3.40, 3.43, 3.44, 3.47, 3.48, 3.51, and 3.52. From these histograms it can be seen that the urea cycle enzymes in these livers are significantly responsive to hormonal induction irrespective of the diet on which the animals were maintained. All of the enzymes behave in a concerted way suggesting some common control mechanism. The effects of β-methasone and glucagon are very similar and in combination are not additive suggesting that β-methasone is not permissive for nor synergistic with glucagon. In general, the effect of dBcAMP is larger than that of the other hormones tested. In some instances, the activities of the urea cycle enzymes in the host liver are induced by the hormones studied to levels above those of control livers. The results are similar when described either

as activity / g liver or as specific activity. This suggests that the effects are specific to urea cycle enzymes and not due to a nonspecific alteration in some liver component such as total liver protein content, water or carbohydrate content.

In the hepatoma the pattern of inducibility is similar to that of host livers. All five enzymes show similar concerted patterns of change being responsive to hormone treatment irrespective of the dietary protein level. CPS appears to be the most responsive of the enzymes studied (see figures 3.35 to 3.54).

Table 3.31. A comparison of CPS Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1,467	2,098	2,407	1,994	1,695
	1,231	2,011	2,127	2,730	1,686
	1,600	1,692	1,677	2,123	1,980
	1,692	1,097	1,572	1,608	1,016
	1,240	2,537	1,919	2,496	1,135
	2,227	1,479	2,107	2,121	1,076
	1,240	1,959	1,918	2,349	1,135
	2,076	1,517	2,127	2,102	1,016
Mean	1,597	1,735	1,982	2,063	1,342
SE	0,136	0,124	0,095	0,113	0,135
t		0,750	2,320	2,630	-1,330
P		NS	0,038	0,020	NS
Host	1,270	1,226	1,930	1,184	1,359
	1,183	1,140	1,856	1,097	1,823
	1,053	1,500	1,356	1,184	1,477
	1,226	1,500	1,930	1,097	1,448
	1,212	1,880	2,089	2,102	1,652
	1,177	1,959	1,900	1,196	1,737
	1,177	1,772	1,974	1,768	1,547
	1,014	1,830	2,112	1,811	1,418
Mean	1,164	1,602	1,894	1,430	1,546
SE	0,109	0,031	0,083	0,141	0,056
t	3,101	3,860	8,240	2,630	5,950
P	0,001	0,005	0,001	0,020	0,001
Hepatoma	0,016	0,008	0,021	0,023	0,016
	0,011	0,015	0,026	0,021	0,017
	0,013	0,015	0,039	0,028	0,019
	0,011	0,015	0,024	0,044	0,019
	0,016	0,019	0,018	0,020	0,021
	0,017	0,012	0,025	0,020	0,021
	0,019	0,016	0,014	0,024	0,018
	0,017	0,011	0,020	0,025	0,018
Mean	0,014	0,015	0,023	0,026	0,018
SE	0,001	0,001	0,003	0,003	0,001
t		0,700	2,890	3,610	2,800
P		NS	0,018	0,006	0,016

Table 3.31b A comparison of CPS Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0122	0,0175	0,0195	0,0161	0,0142
	0,0101	0,0158	0,0154	0,0182	0,0140
	0,0160	0,0141	0,0129	0,0153	0,0118
	0,0141	0,0091	0,0121	0,0124	0,0102
	0,0103	0,0192	0,0160	0,0177	0,0101
	0,0185	0,0129	0,0162	0,0188	0,0108
	0,0103	0,0173	0,0148	0,0188	0,0114
	0,0138	0,0134	0,0142	0,0146	0,0102
Mean	0,0132	0,0143	0,0159	0,0162	0,0162
SE	0,001	0,001	0,001	0,001	0,001
t		0,810	1,920	1,340	1,320
P		NS	NS	NS	NS
Host	0,0121	0,0117	0,0168	0,0103	0,0113
	0,0103	0,0109	0,0151	0,0110	0,0130
	0,0092	0,0111	0,0129	0,0113	0,0123
	0,0107	0,0123	0,0158	0,0105	0,0120
	0,0101	0,0164	0,0182	0,0183	0,0118
	0,0115	0,0170	0,0215	0,0179	0,0124
	0,0098	0,0157	0,0215	0,0177	0,0129
	0,0080	0,0179	0,0184	0,0181	0,0119
Mean	0,0122	0,0149	0,0175	0,0144	0,0143
SE	0,001	0,001	0,001	0,001	0,001
t	0,680	2,840	3,900	2,310	2,310
P	NS	0,013	0,002	0,040	0,040
Hepatoma	0,10027	0,00013	0,00035	0,00038	0,00027
	0,10018	0,00025	0,00043	0,00035	0,00028
	0,10022	0,00025	0,00065	0,00047	0,00032
	0,10018	0,00025	0,00040	0,00073	0,00032
	0,10027	0,00031	0,00030	0,00033	0,00034
	0,10028	0,00020	0,00042	0,00034	0,00035
	0,10032	0,00027	0,00023	0,00041	0,00030
	0,10028	0,00020	0,00033	0,00042	0,00031
Mean	0,0023	0,0025	0,0039	0,0040	0,0030
SE	0,000	0,000	0,000	0,000	0,000
t	19,390	-0,560	2,360	5,120	2,310
P	0,001	NS	0,016	0,000	0,046

Table 3.32a A comparison of OTC Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

	Activity/g Liver				
	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	114,310	48,430	91,909	84,135	84,304
	84,214	88,831	87,640	100,713	90,845
	57,263	61,192	94,636	121,815	93,381
	76,962	45,145	91,282	116,829	86,380
	89,438	101,126	129,017	59,627	81,241
	115,900	103,400	141,310	72,274	87,801
	93,890	75,890	94,840	90,251	84,836
	80,000	70,390	115,967	69,221	87,585
Mean	90,936	74,300	105,857	81,620	87,049
SE	6,750	7,890	7,188	7,032	1,350
t		- 1,60	1,51	-0,96	-0,56
P		NS	NS	NS	NS
Host	71,092	85,814	119,950	90,527	76,379
	71,634	73,068	140,812	65,814	76,708
	45,011	79,179	98,709	69,601	70,366
	61,192	78,470	140,812	84,924	99,525
	77,926	107,272	113,329	77,994	105,240
	79,188	84,810	99,018	97,012	81,854
	72,668	79,328	83,440	111,123	88,292
	74,810	71,694	95,852	107,072	95,567
Mean	64,315	81,698	111,490	90,501	86,817
SE	4,103	3,045	7,494	4,748	4,408
t	3,590	3,40	5,83	4,64	4,20
P	0,003	0,005	0,001	0,001	0,001
Hepatoma	1,192	1,480	1,284	1,308	1,126
	1,240	1,152	1,280	1,180	1,138
	1,336	1,416	1,680	1,060	1,046
	1,144	1,252	1,628	1,200	1,138
	1,153	1,087	1,848	1,183	1,077
	1,290	1,393	1,694	1,015	1,094
	1,431	1,304	1,035	1,110	1,130
	0,999	1,130	1,650	1,600	1,230
Mean	1,223	1,273	1,512	1,207	1,109
SE	0,047	0,052	0,098	0,065	0,012
t		0,770	2,660	-0,200	-2,010
P		NS	0,024	NS	NS

Table 3.32b A comparison of OTC Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,9526	0,6210	0,7070	0,8013	0,7664
	0,7018	0,8076	0,7621	0,9592	0,9085
	0,5453	0,5828	0,9013	0,9370	0,7781
	0,6996	0,6515	0,8695	1,1127	0,7198
	0,8624	0,8794	0,9751	0,5863	0,7254
	0,9659	0,8618	0,9776	0,7241	0,7848
	0,7825	0,6717	0,7903	0,9392	0,7070
	0,8000	0,6831	0,9658	0,6923	0,7820
Mean	0,7888	0,7199	0,8686	0,7717	0,7712
SE	0,050	0,040	0,037	0,053	0,022
t		-1,080	1,290	-0,230	0,320
P		NS	NS	NS	NS
Host	0,6771	0,7151	1,0361	0,8628	0,7538
	0,7163	0,6959	1,2245	0,8581	0,7671
	0,4287	0,7541	0,9401	0,7960	0,7037
	0,5828	0,7473	1,1734	0,8492	0,9294
	0,6097	0,9291	0,7552	0,7750	0,7517
	0,6108	0,7375	0,8610	0,7471	0,7441
	0,6056	0,6811	0,8345	0,7417	0,8027
	0,6645	0,7160	0,9696	0,7142	0,7964
Mean	0,6119	0,7360	0,9868	0,7930	0,7811
SE	0,051	0,031	0,052	0,020	0,024
t	3,030	2,84	6,200	4,920	4,360
P	0,009	0,013	0,001	0,001	0,001
Hepatoma	0,0200	0,0247	0,0214	0,0218	0,0205
	0,0207	0,0192	0,0213	0,0197	0,0207
	0,0223	0,0236	0,0280	0,0177	0,0190
	0,0191	0,0287	0,0271	0,0200	0,0207
	0,0192	0,0181	0,0308	0,0197	0,0196
	0,0215	0,0233	0,0282	0,0180	0,0198
	0,0239	0,0217	0,0173	0,0190	0,0206
	0,0167	0,0185	0,0275	0,0270	0,0204
Mean	0,0204	0,0225	0,0252	0,0204	0,0202
SE	0,001	0,001	0,002	0,000	0,000
t	13,960	1,360	2,640	-0,050	-0,320
P	0,001	NS	0,025	NS	NS

Table 3.33a A comparison of ASS Activities from untreated control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1,467	1,805	1,113	1,617	2,416
	2,775	1,838	1,958	1,214	1,715
	1,641	1,329	1,333	1,113	2,048
	1,600	1,923	2,350	2,403	1,579
	1,340	1,482	1,621	1,169	1,656
	2,230	1,293	2,227	2,215	1,795
	1,712	1,325	1,887	1,730	1,490
	1,883	1,478	2,805	2,659	1,662
Mean	1,831	1,559	1,914	1,890	1,795
SE	0,165	0,091	0,194	0,193	0,106
t		-1,440	0,330	0,230	-0,180
P		NS	NS	NS	NS
Host	1,178	1,768	1,700	1,750	1,890
	1,300	1,413	1,382	2,008	1,873
	1,366	1,730	2,130	2,330	1,718
	1,200	1,808	1,552	1,830	1,553
	1,269	1,981	1,486	2,160	1,529
	1,388	1,739	2,019	1,822	1,878
	2,090	2,398	1,869	1,695	1,879
	1,296	1,727	2,632	1,565	2,211
Mean	1,261	1,821	1,864	1,889	1,818
SE	0,035	0,099	0,145	0,092	0,077
t	3,370	5,310	3,920	6,390	6,580
P	0,005	0,001	0,004	0,001	0,001
Hepatoma	0,132	0,130	0,182	0,136	0,202
	0,124	0,172	0,138	0,138	0,187
	0,128	0,186	0,174	0,160	0,223
	0,108	0,186	0,156	0,272	0,157
	0,205	0,218	0,187	0,202	0,200
	0,156	0,124	0,187	0,155	0,185
	0,140	0,265	0,186	0,256	0,202
	0,100	0,333	0,189	0,200	0,167
Mean	0,137	0,202	0,170	0,190	0,190
SE	0,012	0,025	0,008	0,018	0,007
t		2,38[0]	2,340	2,450	3,910
P		0,039	0,036	0,031	0,002

Table 3.33b A comparison of ASS Activities from untreated control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0122	0,0157	0,0148	0,0154	0,0201
	0,0185	0,0153	0,0151	0,0116	0,0172
	0,0137	0,0127	0,0127	0,0153	0,0171
	0,0133	0,0148	0,0181	0,0195	0,0132
	0,0112	0,0129	0,0135	0,0102	0,0148
	0,0186	0,0113	0,0196	0,0198	0,0149
	0,0143	0,0117	0,0157	0,0144	0,0124
	0,0157	0,0131	0,0187	0,0222	0,0148
Mean	0,0147	0,0134	0,0160	0,0160	0,0156
SE	0,001	0,001	0,001	0,001	0,001
t		-1,100	1,020	0,770	0,670
P		NS	NS	NS	NS
Host	0,0112	0,0158	0,0148	0,0152	0,0158
	0,0130	0,0145	0,0140	0,0175	0,0156
	0,0100	0,0165	0,0185	0,0203	0,0143
	0,0120	0,1572	0,0135	0,0159	0,0141
	0,0106	0,0165	0,0135	0,0180	0,0139
	0,0125	0,0143	0,0168	0,0152	0,0134
	0,0130	0,0200	0,0156	0,0141	0,0157
	0,0104	0,0144	0,0219	0,0131	0,0184
Mean	0,0116	0,0160	0,0161	0,0169	0,0151
SE	0,000	0,001	0,001	0,001	0,001
t	2,940	5,670	4,020	4,780	4,950
P	0,011	0,001	0,003	0,001	0,001
Hepatoma	0,0022	0,0022	0,0030	0,0023	0,0032
	0,0021	0,0029	0,0023	0,0023	0,0030
	0,0021	0,0031	0,0022	0,0027	0,0035
	0,0018	0,0031	0,0026	0,0029	0,0025
	0,0034	0,0036	0,0032	0,0034	0,0032
	0,0026	0,0021	0,0032	0,0026	0,0030
	0,0023	0,0044	0,0032	0,0043	0,0032
	0,0017	0,0056	0,0032	0,0033	0,0027
Mean	0,0023	0,0034	0,0029	0,0030	0,0030
SE	0,000	0,000	0,000	0,000	0,000
t		2,44	2,420	2,300	3,480
P		0,035	0,031	0,038	0,005

Table 3.34a A comparison of AL Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1,217	1,037	1,313	0,960	1,193
	0,937	0,819	1,121	1,466	1,021
	0,806	0,940	1,164	1,493	1,262
	0,928	0,803	1,191	1,002	0,813
	0,977	0,984	1,048	0,967	1,083
	1,130	0,781	1,143	0,944	1,142
	1,125	1,101	1,135	0,992	1,055
	0,957	1,051	1,200	1,014	1,055
Mean	1,010	0,938	1,142	1,115	1,078
SE	0,044	0,048	0,047	0,078	0,047
t		-1,10	-2,60	1,15	1,000
P		NS	0,029	NS	NS
Host	0,996	0,994	1,450	1,088	1,855
	1,088	1,040	1,450	1,180	1,709
	0,786	1,134	1,361	0,996	1,567
	0,780	0,948	1,380	0,996	1,502
	1,202	1,128	1,183	0,762	1,597
	0,973	1,078	1,195	1,303	1,927
	1,1[illegible]	1,243	1,293	1,277	1,567
	1,000	0,929	1,322	1,128	1,550
Mean	0,994	1,074	1,330	1,153	1,659
SE	0,053	0,033	0,036	0,032	0,055
t	0,22	1,28	5,240	2,570	8,710
P	NS	NS	0,001	0,026	0,001
Hepatoma	0,062	0,046	0,076	0,060	0,062
	0,080	0,064	0,098	0,100	0,061
	0,042	0,082	0,056	0,079	0,057
	0,036	0,042	0,051	0,035	0,072
	0,064	0,059	0,074	0,084	0,073
	0,072	0,056	0,092	0,090	0,057
	0,064	0,071	0,087	0,106	0,080
Mean	0,060	0,061	0,080	0,082	0,067
SE	0,005	0,005	0,007	0,006	0,003
t		-0,14	2,22	2,54	1,000
P		NS	0,045	0,023	NS

Table 3.34bA comparison of AL Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0101	0,0090	0,0101	0,0128	0,0107
	0,0085	0,0074	0,0097	0,0113	0,0102
	0,0073	0,0090	0,0111	0,0115	0,0105
	0,0084	0,0107	0,0113	0,0095	0,0074
	0,0081	0,0086	0,0070	0,0084	0,0097
	0,0090	0,0079	0,0076	0,0092	0,0102
	0,0094	0,0101	0,0076	0,0096	0,0088
	0,0080	1,0210	0,0080	0,0098	0,0094
Mean	0,0085	0,0093	0,0090	0,0105	0,0096
SE	0,001	0,000	0,001	0,001	0,000
t		1,270	0,74	2,81	2,11
P		NS	NS	0,016	NS
Host	0,0095	0,0093	0,0138	0,0080	0,0155
	0,0109	0,0099	0,0138	0,0118	0,0122
	0,0079	0,0108	0,0118	0,0100	0,0131
	0,0078	0,0113	0,0120	0,0120	0,0125
	0,0100	0,0098	0,0118	0,0076	0,0114
	0,0081	0,0094	0,0120	0,0130	0,0138
	0,0094	0,0108	0,0129	0,0127	0,0131
	0,0080	0,0093	0,0132	0,0113	0,0129
Mean	0,0090	0,0101	0,0127	0,0111	0,0131
SE	0,000	0,000	0,000	0,001	0,000
t	-0,860	2,390	7,260	3,030	6,930
P	NS	0,039	0,001	0,010	0,001
Hepatoma	0,00103	0,00077	0,00127	0,00100	0,0010
	0,00133	0,00107	0,00153	0,00167	0,0009
	0,00070	0,00137	0,00093	0,00132	0,0709
	0,00060	0,00070	0,00085	0,00092	0,0010
	0,00107	0,00098	0, 123	0,00142	0,0012
	0,00120	0,00933	0,00152	0,00150	0,0009
	0,00107	0,00118	0,00145	0,00180	0,0012
Mean	0,0010	0,0010	0,0013	0,0014	0,0010
SE	0,000	0,000	0,000	0,000	0,000
t	13,660	-0,120	2,230	2,510	0,250
P	0,001	NS	0,044	0,026	NS

Table 3.35a A comparison of ARC Activities from untreated control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	665,894	837,962	842,189	790,900	735,923
	791,923	674,255	713,921	1023,939	692,850
	491,642	457,877	555,551	850,635	636,336
	603,964	678,966	791,972	878,809	729,910
	657,260	784,148	658,816	403,378	596,726
	747,046	597,804	662,645	552,329	674,343
	600,179	500,424	669,424	531,974	720,985
	600,500	455,440	758,677	715,930	739,563
Mean	644,641	623,384	706,648	718,484	630,829
SE	33,190	51,578	31,860	73,605	18,392
t		-0,350	1,350	0,910	1,220
P		NS	NS	NS	NS
Host	734,360	717,032	885,688	746,476	736,915
	707,676	556,257	837,967	586,377	911,107
	548,115	710,093	604,950	822,003	811,295
	500,980	726,742	850,500	670,948	741,561
	455,847	783,262	604,106	690,436	709,562
	403,153	819,619	645,049	508,436	782,396
	625,051	551,782	653,714	584,689	858,837
	478,996	626,537	662,196	673,968	843,064
Mean	507,646	686,415	717,999	661,163	799,341
SE	24,014	35,077	41,908	35,159	24,521
t	3,340	4,210	4,360	3,610	8,500
P	0,005	0,001	0,001	0,004	0,001
Hepatoma	2,258	2,653	2,003	1,577	1,246
	1,781	2,704	1,684	2,216	1,691
	1,944	2,337	1,487	1,549	1,049
	2,254	1,703	2,641	2,574	1,244
	3,959	1,030	1,028	1,106	2,320
	1,094	1,260	1,874	1,452	2,388
	0,825	1,028	1,302	1,866	1,967
	0,632	1,184	1,532	2,000	2,125
Mean	1,843	1,737	1,694	1,793	1,754
SE	0,376	0,256	0,174	0,165	0,185
t		-0,23	-0,36	-0,12	-0,12
P		NS	NS	NS	NS

Table 3.35b A comparison of ARG Activities from untreated control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a zero% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	5,5491	7,2866	5,4784	5,8696	6,1327
	5,3994	5,6188	5,2080	7,8765	5,7735
	4,0972	4,3607	5,2907	5,5433	5,3028
	5,0329	5,9041	5,8867	5,7601	6,0826
	5,4772	5,8187	5,2986	4,0338	4,9727
	4,8197	4,9817	5,7621	5,2603	6,0209
	5,0015	4,7659	5,6512	4,0921	6,0082
	6,0050	4,5544	7,2255	5,5072	5,6889
Mean	5,1977	4,6840	5,7263	5,4929	5,7478
SE	0,209	0,748	0,231	0,423	0,147
t		-0,660	1,700	0,630	2,060
P		NS	NS	NS	NS
Host	5,1197	5,8289	7,7016	5,4911	6,1410
	5,1308	5,2977	7,2867	5,8638	6,5100
	4,7711	5,7628	6,0494	7,1479	6,7608
	4,3563	5,9214	7,3957	5,8343	6,1797
	4,3414	7,4596	5,2357	6,3951	5,9130
	3,8396	5,3048	5,6091	5,0844	5,5884
	4,0326	5,7981	5,6845	5,0929	7,1570
	4,8000	6,2654	5,7858	5,9643	7,0256
Mean	4,5489	5,7548	6,3430	5,8592	6,4059
SE	0,170	0,243	0,339	0,242	0,194
t	2,41	4,740	4,720	4,430	7,190
P	0,030	0,001	0,001	0,001	0,001
Hepatoma	0,0381	0,0442	0,0334	0,0263	0,0198
	0,0297	0,0451	0,0281	0,0369	0,0269
	0,0324	0,0390	0,0248	0,0260	0,0166
	0,0376	0,0284	0,0441	0,0430	0,0198
	0,2599	0,0167	0,0171	0,0184	0,0368
	0,0182	0,0210	0,0150	0,0121	0,0379
	0,0138	0,0171	0,0207	0,0072	0,0312
	0,0105	0,0197	0,0128	0,0200	0,0337
Mean	0,0283	0,0289	0,0245	0,0238	0,0279
SE	0,004	0,005	0,004	0,004	0,003
t		0,090	-0,610	-0,690	-0,06
P		NS	NS	NS	NS

Table 3.36a A comparison of CPS Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	3,375	3,015	2,941	3,122	3,557
	3,160	3,926	3,232	3,203	2,552
	2,863	3,304	2,717	3,374	2,281
	2,753	3,304	3,304	3,087	2,445
	2,753	3,261	2,753	2,715	2,445
	2,786	2,829	3,385	3,504	2,980
	3,304	2,829	2,867	2,774	2,287
	2,560	2,941	2,941	2,900	2,270
Mean	2,944	3,176	3,018	3,085	2,727
SE	0,105	0,128	0,090	0,098	0,172
t		1,400	0,530	0,980	-1,080
P		NS	NS	NS	NS
Host	2,177	3,279	3,470	2,414	2,751
	2,177	3,281	3,450	3,361	2,518
	1,893	3,308	3,312	4,083	3,034
	1,607	4,224	3,215	3,820	2,661
	1,774	3,293	3,126	2,466	2,679
	2,057	3,833	2,893	2,718	2,896
	2,066	3,109	3,345	2,839	2,628
	2,160	3,338	3,140	3,119	2,597
Mean	1,989	3,458	3,244	3,102	2,721
SE	0,075	0,137	0,068	0,217	0,060
t	7,430	9,680	12,470	4,850	7,650
P	0,001	0,000	0,000	0,001	0,001
Hepacoma	0,077	0,096	0,080	0,073	0,090
	0,073	0,072	0,073	0,098	0,064
	0,065	0,065	0,073	0,075	0,111
	0,074	0,055	0,075	0,090	0,087
	0,081	0,086	0,078	0,092	0,093
	0,061	0,085	0,115	0,106	0,090
	0,063	0,109	0,110	0,083	0,088
	0,066	0,087	0,152	0,080	0,090
Mean	0,070	0,082	0,095	0,087	0,089
SE	0,003	0,006	0,010	0,004	0,004
t		1,790	2,35	3,580	3,700
P		NS	0,047	0,004	0,003

Table 3.36b A comparison of CFS Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0338	0,0402	0,0290	0,0312	0,0339
	0,0351	0,0393	0,0308	0,0320	0,0243
	0,0286	0,0441	0,0272	0,0450	0,0252
	0,0273	0,0413	0,0367	0,0412	0,0272
	0,0196	0,0408	0,0306	0,0339	0,0272
	0,0222	0,0377	0,0271	0,0367	0,0284
	0,0315	0,0377	0,0329	0,0300	0,0254
	0,0320	0,0392	0,0245	0,0363	0,0252
Mean	0,0288	0,0400	0,0298	0,0358	0,0271
SE	0,002	0,001	0,001	0,002	0,001
t		5,410	0,460	2,630	-0,750
P		0,000	NS	0,020	NS
Host	0,0200	0,0328	0,0347	0,0284	0,0367
	0,0199	0,0329	0,0350	0,0374	0,0240
	0,0237	0,0308	0,0332	0,0453	0,0405
	0,0145	0,0403	0,0322	0,0332	0,0355
	0,0177	0,0329	0,0313	0,0290	0,0447
	0,0187	0,0383	0,0289	0,0453	0,0396
	0,0243	0,0311	0,0335	0,0315	0,0396
	0,0206	0,0322	0,0314	0,0284	0,0433
Mean	0,0199	0,0339	0,0325	0,0348	0,0380
SE	0,001	0,001	0,001	0,003	0,002
t	4,290	8,450	9,520	5,400	7,140
P	0,001	0,000	0,000	0,000	0,000
Hepatoma	0,0070	0,0090	0,0070	0,0086	0,0090
	0,0070	0,0090	0,0090	0,0163	0,0080
	0,0060	0,0090	0,0090	0,0065	0,0110
	0,0070	0,0080	0,0090	0,0086	0,0090
	0,0090	0,0120	0,0130	0,0130	0,0090
	0,0070	0,0120	0,0140	0,0150	0,0090
	0,0090	0,0160	0,0140	0,0100	0,0090
	0,0070	0,0170	0,0190	0,0130	0,0090
Mean	0,0070	0,0110	0,0120	0,0110	0,0089
SE	0,000	0,000	0,000	0,000	0,000
t		3,260	3,020	3,450	2,630
P		0,012	0,017	0,007	0.020

Table 3.37a A comparison of OTC Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	167,808	133,996	134,927	154,052	158,399
	146,365	158,595	134,196	138,991	128,203
	182,231	122,931	133,426	138,540	114,831
	177,003	126,257	146,416	138,540	118,775
	142,362	115,227	131,271	140,972	118,870
	117,371	144,537	136,242	146,010	126,664
	122,024	125,692	131,794	128,279	128,006
	135,550	130,857	128,059	151,115	121,469
Mean	148,839	132,261	134,541	142,062	126,902
SE	4,828	8,662	1,917	2,887	4,820
t		-1,670	-1,610	-0,740	-2,210
P		NS	NS	NS	NS
Host	109,034	129,538	138,764	138,764	121,631
	110,064	146,348	145,116	145,115	125,767
	108,011	151,144	145,730	145,730	128,230
	99,559	151,578	150,435	150,435	124,807
	102,499	125,993	141,746	129,671	125,052
	99,038	124,506	124,510	123,355	138,714
	105,469	122,381	123,861	124,298	123,856
	103,786	135,177	122,290	123,993	124,958
Mean	104,676	135,958	136,556	133,932	126,627
SE	1,485	4,262	3,991	3,414	1,845
t	5,020	6,930	7,490	7,860	9,270
P	0,002	0,001	0,001	0,001	0,001
Hepatoma	3,3631	5,0783	3,6091	3,6770	3,4135
	3,4028	3,5247	3,5851	3,4041	3,8102
	2,9048	3,3550	3,3289	3,4041	4,1085
	2,9883	3,5612	3,6277	3,1550	3,8597
	2,9095	3,9599	3,9965	3,8219	3,9690
	2,7423	4,0750	3,9692	3,7353	4,4880
	2,8792	3,7577	3,9705	4,0854	3,7368
	2,8260	3,2590	3,9493	4,0998	3,7806
Mean	3,0024	3,8217	3,7545	3,676	3,896
SE	0,087	0,205	0,088	0,110	0,110
t		3,680	6,080	4,57	6,370
P		0,005	0,001	0,001	0,001

Table 3.37b A comparison of OTC Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1,237	1,787	1,327	2,054	1,509
	1,626	2,115	1,491	1,390	1,425
	1,822	1,639	1,483	1,847	1,276
	1,686	1,683	1,539	1,846	1,320
	1,780	1,536	1,459	1,880	1,132
	1,467	1,927	1,514	1,883	1,407
	1,162	1,676	1,318	1,603	1,423
	1,694	1,636	1,281	1,890	1,350
Mean	1,559	1,750	1,427	1,799	1,355
SE	0,087	0,066	0,036	0,073	0,041
t		1,740	-1,410	2,110	-2,12
P		NS	NS	NS	NS
Host	0,948	1,295	1,542	1,633	1,622
	0,957	1,464	1,452	1,707	1,498
	1,150	1,440	1,457	1,619	1,710
	0,905	1,444	1,308	1,308	2,270
	1,025	1,260	1,575	1,525	2,084
	0,900	1,245	1,245	1,431	1,850
	1,141	1,224	1,239	1,381	2,064
	0,988	1,287	1,223	1,378	2,083
Mean	1,002	1,332	1,380	1,500	1,898
SE	0,034	0,035	0,051	0,051	0,095
t	5,940	6,710	6,180	8,140	8,840
P	0,001	0,000	0,000	0,000	0,000
Hepatoma	0,0454	0,0726	0,0722	0,0518	0,0411
	0,0387	0,0504	0,0717	0,0749	0,0544
	0,0393	0,0479	0,0564	0,0485	0,0587
	0,0404	0,0712	0,0544	0,0501	0,0552
	0,0393	0,0567	0,0800	0,0538	0,0567
	0,0371	0,0582	0,0794	0,0526	0,0641
	0,0389	0,0538	0,0673	0,0575	0,0548
	0,0382	0,0652	0,0494	0,0651	0,0541
Mean	0,0397	0,0595	0,0663	0,0568	0,0549
SE	0,001	0,003	0,004	0,003	0,002
t		5,850	6,360	5,210	6,200
P		0,000	0,000	0,001	0,000

Table 3.38a A comparison of ASS Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

	Activity/g Liver				
	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1,504	1,373	1,116	1,645	1,636
	1,095	1,612	1,216	1,756	1,130
	1,442	1,315	1,474	1,448	1,344
	1,585	1,367	1,572	1,729	1,515
	1,136	1,382	1,344	1,630	1,298
	1,758	1,611	1,654	1,311	1,137
	1,448	1,354	1,331	1,687	1,733
	1,400	1,643	1,309	1,450	1,490
Mean	1,421	1,456	1,377	1,582	1,410
SE	0,077	0,049	0,064	0,056	0,078
t		0,380	-0,440	1,680	-0,100
P		NS	NS	NS	NS
Host	1,068	1,659	2,175	2,070	2,512
	1,079	1,652	2,787	2,767	1,761
	1,278	2,040	2,835	2,243	2,603
	1,370	2,618	1,893	2,874	2,747
	1,178	1,730	2,991	2,701	1,605
	0,976	2,940	2,424	2,640	1,525
	0,972	2,402	2,791	2,170	3,112
	1,059	2,555	2,446	2,422	1,580
Mean	1,124	2,200	2,536	2,487	2,181
SE	0,050	0,176	0,138	0,107	0,223
t	3,230	5,890	9,650	11,550	4,630
P	0,006	0,000	0,000	0,000	0,002
Hepatoma	0,182	0,209	0,185	0,230	0,350
	0,159	0,199	0,255	0,208	0,282
	0,196	0,126	0,240	0,255	0,328
	0,114	0,239	0,240	0,302	0,204
	0,182	0,174	0,264	0,288	0,206
	0,131	0,273	0,298	0,269	0,268
	0,218	0,251	0,240	0,297	0,202
	0,243	0,269	0,278	0,257	0,202
Mean	0,181	0,243	0,251	0,263	0,255
SE	0,016	0,017	0,012	0,012	0,021
t		2,620	3,520	4,140	2,770
P		0,020	0,004	0,001	0,016

Table 3.38b A comparison of ASS Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,2000	0,0193	0,0106	0,0166	0,0156
	0,0122	0,0215	0,0116	0,0176	0,0126
	0,0144	0,0175	0,0164	0,0193	0,0103
	0,0151	0,0171	0,0157	0,0173	0,0169
	0,0108	0,0194	0,0149	0,0217	0,0124
	0,0141	0,0145	0,0132	0,0109	0,0125
	0,0138	0,0191	0,0107	0,0211	0,0133
	0,0156	0,0117	0,0109	0,0183	0,0156
Mean	0,0145	0,0175	0,0130	0,0166	0,0136
SE	0,001	0,001	0,001	0,001	0,001
t		2,060	-1,170	1,330	-0,690
P		NS	NS	NS	NS
Host	0,0072	0,0166	0,0242	0,0244	0,0335
	0,0072	0,0165	0,0279	0,0241	0,0235
	0,0085	0,0195	0,0284	0,0249	0,0248
	0,0091	0,0249	0,0189	0,0250	0,0252
	0,0080	0,1730	0,0310	0,0235	0,0268
	0,0076	0,0294	0,0242	0,0230	0,0210
	0,0084	0,0239	0,0279	0,0189	0,0260
	0,0071	0,0242	0,0213	0,0211	0,0253
Mean	0,0079	0,0208	0,0255	0,0231	0,0258
SE	0,000	0,001	0,001	0,001	0,001
t	6,660	9,540	12,090	19,220	13,790
P	0,001	0,000	0,000	0,000	0,000
Hepatoma	0,0021	0,0030	0,0037	0,0033	0,0034
	0,0018	0,0029	0,0051	0,0029	0,0027
	0,0026	0,0046	0,0041	0,0032	0,0032
	0,0012	0,0034	0,0042	0,0038	0,0021
	0,0017	0,0025	0,0053	0,0041	0,0021
	0,0013	0,0039	0,0060	0,0038	0,0026
	0,0021	0,0036	0,0041	0,0037	0,0021
	0,0021	0,0040	0,0035	0,0041	0,0025
Mean	0,0019	0,0035	0,0045	0,0036	0,0026
SE	0,000	0,000	0,000	0,000	0,000
t		5,580	7,570	7,790	3,000
P		0,000	0,000	0,000	0,010

Table 3.39aA comparison of AL Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dbcAMP
Control	1,052	0,895	1,193	1,035	1,103
	0,818	0,752	1,215	1,135	1,084
	1,488	1,186	1,097	1,097	1,053
	1,187	1,128	1,069	1,068	1,060
	0,898	0,960	1,187	1,244	1,051
	0,961	1,227	1,158	1,055	1,082
	1,069	0,914	1,069	1,049	0,952
	1,127	1,128	0,977	1,265	1,021
Mean	1,070	1,024	1,121	1,116	1,051
SE	0,069	0,059	0,029	0,030	0,017
t		-0,51	0,680	0,610	-0,270
P		NS	NS	NS	NS
Host	1,030	1,351	1,487	1,407	1,178
	0,992	1,258	1,327	1,435	1,250
	0,915	1,269	1,663	1,683	1,654
	1,008	1,461	1,708	1,401	1,130
	0,884	1,341	1,364	1,244	1,131
	1,055	1,461	2,093	1,429	1,132
	1,085	1,201	2,023	1,128	1,119
	0,979	1,308	1,623	1,898	1,367
Mean	0,994	1,306	1,661	1,431	1,245
SE	0,024	0,046	0,099	0,086	0,066
t	1,050	5,990	6,540	5,120	3,600
P	NS	0,000	0,000	<0,001	0,006
Hepatoma	0,113	0,168	0,182	0,117	0,104
	0,115	0,110	0,128	0,116	0,104
	0,099	0,107	0,107	0,130	0,117
	0,107	0,105	0,101	0,124	0,114
	0,105	0,122	0,130	0,101	0,126
	0,117	0,131	0,123	0,114	0,144
	0,107	0,108	0,123	0,140	0,155
	0,114	0,110	0,118	0,135	0,135
Mean	0,110	0,120	1,265	0,122	0,125
SE	0,002	0,008	0,009	0,004	0,007
t		1,340	2,540	2,510	2,200
P		NS	0,023	0,031	NS

Table 3.39b A comparison of AL Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0140	0,0119	0,0114	0,0138	0,0123
	0,0091	0,0125	0,0135	0,0114	0,0120
	0,0149	0,0154	0,0110	0,0146	0,0081
	0,0113	0,0150	0,0120	0,0142	0.0118
	0,0090	0,0128	0,0095	0,0148	0,0100
	0,0097	0,0150	0,0093	0,0141	0,0130
	0,0102	0,0122	0,0080	0,0087	0,0106
	0,0141	0,0150	0,0109	0,0158	0,0113
Mean	0,0115	0,0137	0,0107	0,0134	0,0110
SE	0,001	0,001	0,001	0,001	0,000
t		2,16	-0,790	1,600	-0,53
P		NS	NS	NS	NS
Host	0,0100	0,0150	0,0165	0,0156	0,0157
	0,0086	0,0140	0,0133	0,0239	0,0119
	0,0114	0,0115	0,0166	0,0187	0,0221
	0,0101	0,0134	0,0145	0,0122	0,0205
	0,0088	0,0149	0,0152	0,0139	0,0189
	0,0096	0,0162	0,0209	0,0143	0,0151
	0,0112	0,0110	0,0202	0,0113	0,0187
	0,0093	0,0120	0,0141	0,0155	0,0228
Mean	0,0099	0,0135	0,0164	0,0156	0,0182
SE	0,000	0,001	0,001	0,001	0,001
t	2,79	4,860	6,220	3,900	6,090
P	0,020	0,001	∠0,000	0,005	∠0,000
Hepatoma	0,0013	0,0019	0,0021	0,0014	0,0014
	0,0013	0,0013	0,0015	0,0019	0,0014
	0,0013	0,0012	0,0012	0,0013	0,0016
	0,0012	0,0012	0,0012	0,0012	0,0015
	0,0010	0,0017	0,0026	0,0014	0,0017
	0,0011	0,0020	0,0025	0,0016	0,0019
	0,0009	0,0015	0,0021	0,0018	0,0021
	0,0011	0,0014	0,0015	0,0021	0,0018
Mean	0,0011	0,0016	0,0018	0,0016	0,0017
SE	0,000	0,000	0,000	0,000	0,000
t	3,690	3,26	3,350	3,510	5,090
P	∠0,001	0,009	0,001	0,006	∠0,000

Table 3.40a A comparison of ARC Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	794,185	1137,267	848,941	952,091	889,107
	757,613	960,215	818,80[illegible]	981,355	821,284
	1064,688	1078,826	876,742	971,443	819,965
	943,550	728,408	881,762	1007,374	84[illegible],415
	1014,809	833,274	754,976	905,860	85[illegible],793
	1116,311	1045,691	1032,983	971,314	976.167
	855,754	1066,988	749,691	1048,132	1143,17[illegible]
	955,055	837,783	825,047	969,095	1045,976
Mean	938,369	961,030	848,617	975,726	923,983
SE	51,592	45,148	31,592	14,592	42,227
t		0,330	-1,630	0,790	-0,230
P		NS	NS	NS	NS
Host	624,509	757,908	993,771	805,087	820,229
	583,365	1054,403	1088,970	1056,248	777,820
	650,574	932,347	86[illegible] 75	879,458	867,432
	555,928	1065,171	1116,928	952,705	999,744
	593,654	1044,285	974,265	837,696	791,546
	609,574	963,783	834,165	855,219	812,765
	599,354	913,875	881,587	902,412	795,406
	665,438	1053,032	781,598	837,696	885,506
Mean	610,298	971,850	917,069	890,815	843,804
SE	12,637	37,563	54,863	28,573	25,904
t	7,000	9,120	5,450	8,980	8,100
P	0,001	0,000	0,001	0,000	0,001
Hepatoma	3,359	4,400	4,008	5,566	3,759
	4,808	7,180	5,717	5,031	5,444
	3,037	3,521	4,642	5,603	4,401
	2,713	3,521	4,327	5,060	5,409
	2,280	5,209	2,932	3,880	4,453
	2,280	3,145	3,145	4,401	5,409
	2,289	7,051	4,401	4,405	5,409
	2,283	5,803	4,401	7,520	4,525
Mean	2,881	4,984	4,197	5,183	4,851
SE	0,311	0,564	0,309	0,395	0,229
t		3,260	3,000	4,580	5,100
P		0,008	0,010	0,001	,001

Table 3.40b A comparison of ARG Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 10% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	10,589	10,339	9,085	9,521	9,468
	8,418	12,002	9,698	9,814	9,125
	10,647	10,788	9,742	9,831	8,200
	9,986	9,712	8,818	10,074	9,345
	7,249	10,416	8,389	9,059	9,500
	9,931	11,619	11,478	9,713	8,135
	9,150	10,670	7,497	10,481	8,167
	10,938	10,472	7,500	10,767	9,368
Mean	9,614	10,752	8,851	9,908	8,914
SE	0,448	0,259	0,455	0,190	0,222
t		2,200	-1,040	0,600	-1,400
P		0,050	NS	NS	NS
Host	5,431	7,579	11,109	9,472	10,936
	5,073	10,544	10,890	12,425	10,371
	5,914	8,784	8,652	9,772	11,566
	5,054	10,145	10,161	9,527	13,330
	5,937	10,443	10,083	9,855	13,192
	5,542	9,638	8,342	10,161	10,837
	6,659	8,704	8,816	10,027	13,270
	6,338	10,029	7,820	7,284	14,758
Mean	5,744	9,446	9,485	9,815	12,033
SE	0,204	0,380	0,436	0,493	0,437
t	7,360	8,590	7,770	7,640	13,050
P	0,001	0,000	0,000	0,000	0,000
Hepatoma	0,0382	0,0629	0,0802	0,0784	0,0531
	0,0546	0,1026	0,1143	0,0786	0,0656
	0,0410	0,0303	0,0767	0,0789	0,0530
	0,0292	0,0704	0,0540	0,0804	0,0652
	0,0213	0,0744	0,0586	0,0547	0,0537
	0,0440	0,0500	0,0629	0,0620	0,0652
	0,0210	0,1007	0,0746	0,0621	0,0660
	0,0278	0,1161	0,0551	0,0940	0,0550
Mean	0,0346	0,0784	0,0720	0,0736	0,0596
SE	0,004	0,009	0,007	0,005	0,002
t		4,460	4,340	6,310	5,260
P		0,001	0,00	0,000	0,000

Table 3.41a A comparison of CPS Activities from untreated control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	5,146	5,599	4,274	5,893	5,544
	5,511	6,666	6,355	5,636	5,252
	5,024	4,683	5,790	5,669	6,438
	5,028	4,921	5,146	6,613	5,968
	5,131	5,514	6,691	5,231	6,389
	6,134	6,402	5,131	6,234	5,940
	5,514	6,199	6,199	5,691	5,716
	6,134	4,387	6,134	5,614	5,370
Mean	5,403	5,546	5,715	5,825	5,824
SE	0,164	0,296	0,284	0,150	0,157
t		0,280	0,800	1,680	1,640
P		NS	NS	NS	NS
Host	3,366	4,022	5,862	5,046	5,311
	3,366	3,386	5,958	4,446	5,978
	2,916	3,386	6,150	6,246	4,572
	2,772	5,862	6,150	4,846	4,574
	3,136	4,110	5,960	5,700	5,804
	2,982	5,044	5,740	5,304	4,502
	2,676	5,464	5,350	5,084	5,026
	2,832	5,206	5,360	5,026	5,156
Mean	3,006	4,560	5,816	5,212	5,115
SE	0,092	0,338	0,112	0,196	0,200
t	13,030	4,430	19,390	10,250	9,590
P	0,001	0,002	0,001	0,001	0,001
Hepatoma	0,076	0,073	0,157	0,118	0,125
	0,073	0,083	0,198	0,134	0,100
	0,070	0,096	0,085	0,105	0,158
	0,077	0,084	0,086	0,102	0,078
	0,069	0,120	0,092	0,069	0,110
	0,078	0,097	0,086	0,070	0,078
	0,050	0,136	0,100	0,084	0,101
	0,077	0,098	0,066	0,072	0,106
Mean	0,071	0,098	0,109	0,095	0,107
SE	0,003	0,007	0,016	0,009	0,009
t		3,400	2,320	2,530	3,710
P		0,007	0,049	0,032	0,005

Table 3.41b A comparison of GPS Activities from untreated control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0343	0,0431	0,0342	0,0393	0,0370
	0,0394	0,0444	0,0410	0,0377	0,0457
	0,0305	0,0360	0,0374	0,0380	0,0415
	0,0372	0,0379	0,0332	0,0389	0,0412
	0,0342	0,0368	0,0432	0,0399	0,0399
	0,0396	0,0427	0,0331	0,0367	0,0371
	0,0347	0,0413	0,0400	0,0379	0,0346
	0,0454	0,0338	0,0396	0,0374	0,0358
Mean	0,0376	0,0395	0,0378	0,0382	0,0391
SE	0,002	0,001	0,001	0,001	0,001
t		0,900	0,060	0,380	0,730
P		NS	NS	NS	NS
Host	0,0269	0,0309	0,0468	0,0400	0,0531
	0,0270	0,0292	0,0477	0,0342	0,0629
	0,0233	0,0295	0,0492	0,0490	0,0381
	0,0222	0,0451	0,0490	0,0439	0,0391
	0,0250	0,0343	0,0477	0,0388	0,0464
	0,0238	0,0388	0,0477	0,0408	0,0360
	0,0191	0,0421	0,0428	0,0407	0,0402
	0,0202	0,0405	0,0429	0,0402	0,0516
Mean	0,0234	0,0363	0,0467	0,0409	0,0459
SE	0,001	0,002	0,002	0,001	0,003
t	7,390	5,360	17,230	9,600	6,500
P	0,001	0,001	0,001	0,001	0,001
Hepatoma	0,0089	0,0086	0,0132	0,0110	0,0110
	0,0073	0,0097	0,0170	0,0120	0,0090
	0,0075	0,0110	0,0102	0,0100	0,0140
	0,0070	0,0100	0,0103	0,0160	0,0100
	0,0081	0,1070	0,0111	0,0090	0,0100
	0,0078	0,0087	0,0100	0,0090	0,0089
	0,0073	0,0120	0,0091	0,0105	0,0092
	0,0070	0,0100	0,0080	0,0090	0,0094
Mean	0,0070	0,0110	0,0110	0,0100	0,0100
SE	0,000	0,002	0,000	0,000	0,000
t		3,200	3,340	4,750	3,710
P		0,010	0,009	0,001	0,002

Table 3.42aA comparison of OTC Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	203,428	187,019	230,981	207,291	172,236
	198,118	210,018	193,773	194,224	164,543
	185,266	168,688	176,307	211,124	186,622
	187,019	185,071	193,573	179,390	179,929
	192,944	151,937	184,175	170,588	188,720
	166,123	195,656	156,812	183,174	185,234
	208,393	187,570	200,692	171,613	178,829
	190,943	156,812	204,378	175,025	196,413
Mean	191,529	180,346	192,586	186,550	181,566
SE	4,584	6,983	7,673	5,613	3,530
t		-1,340	0,120	-0,690	-1,720
P		NS	NS	NS	NS
Host	170,520	204,741	236,430	197,833	210,820
	170,520	222,689	219,295	189,487	197,974
	161,937	188,496	201,132	213,012	231,173
	161,413	211,124	183,663	203,428	205,334
	173,452	230,532	309,658	288,879	220,127
	177,243	294,616	266,606	231,278	223,706
	166,930	243,661	253,595	285,729	212,762
	153,786	242,548	236,943	219,742	223,175
Mean	166,975	229,801	238,414	228,673	215,509
SE	9,673	11,409	13,926	13,586	3,907
t	4,620	5,360	5,040	4,460	10,240
P	0,001	0,001	0,001	0,002	0,001
Hepatoma	6,129	8,444	7,320	7,691	6,698
	7,115	8,106	6,560	6,100	6,515
	6,646	7,216	6,[illegible]8	6,851	6,456
	6,646	6,967	[illegible]491	7,769	6,577
	6,549	5,891	6,605	7,624	8,862
	6,064	6,401	6,127	5,738	7,167
	5,693	5,993	6,574	6,962	7,145
	7,166	6,554	6,962	5,127	6,600
Mean	6,521	6,912	6,647	6,733	7,005
SE	0,177	0,332	0,124	0,349	0,283
t		1,040	0,580	0,540	1,450
P		NS	NS	NS	NS

Table 3.42b A comparison of OTC Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1,356	1,439	1,444	1,382	1,378
	1,415	1,400	1,491	1,295	1,431
	1,123	1,298	1,355	1,408	1,204
	1,385	1,426	1,291	1,380	1,241
	1,286	1,170	1,416	1,312	1,180
	1,329	1,505	1,206	1,221	1,425
	1,345	1,443	1,338	1,320	1,376
	1,414	1,205	1,277	1,296	1,309
Mean	1,344	1,426	1,362	1,328	1,318
SE	0,023	0,051	0,037	0,022	0,035
t		1,460	0,410	-0,510	-0,620
P		NS	NS	NS	NS
Host	1,312	1,575	1,991	1,522	2,108
	1,312	1,713	1,754	1,458	2,084
	1,012	1,450	1,609	1,639	1,926
	1,291	1,624	1,459	1,565	1,643
	1,239	1,775	2,064	1,926	1,751
	1,108	2,266	2,133	1,779	1,598
	1,012	1,874	2,029	1,905	1,520
	1,099	1,866	1,896	1,690	2,232
Mean	1,173	1,767	1,867	1,685	1,856
SE	0,046	0,088	0,084	0,061	0,095
t	3,320	6,000	7,230	6,690	6,460
P	0,005	0,001	0,001	0,001	0,001
Hepatoma	0,0621	0,08444	0,07320	0,07700	0,0638
	0,0691	0,08107	0,06600	0,07000	0,0621
	0,0626	0,07217	0,07000	0,06835	0,0647
	0,0604	0,06969	0,06485	0,07869	0,0667
	0,0624	0,05895	0,06610	0,07624	0,0844
	0,0575	0,06500	0,06175	0,06018	0,0683
	0,0542	0,06000	0,06600	0,07010	0,0680
	0,0652	0,06600	0,07100	0,05130	0,0639
Mean	0,0617	0,06970	0,06740	0,06850	0,0676
SE	0,004	0,003	0,001	0,003	0,003
t	3,870	2,660	5,700	2,230	1,967
P	0,001	0,020	0,001	0,040	NS

Table 3.43a A comparison of ASS Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

	Activity/g Liver				
	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	2,338	1,858	1,358	2,078	2,269
	2,208	2,090	2,228	1,858	1,439
	1,483	1,340	1,893	2,070	1,863
	1,750	1,750	1,495	2,078	2,100
	1,576	2,182	1,641	1,821	1,576
	1,573	2,267	1,467	2,394	2,203
	1,646	1,524	1,430	2,254	1,375
	1,700	1,984	2,162	1,977	1,586
Mean	1,797	1,875	1,709	1,970	1,801
SE	0,108	0,114	0,121	0,140	0,126
t		0,500	-0,540	0,980	0,030
P		NS	NS	NS	NS
Host	1,320	2,764	3,452	2,692	2,188
	2,224	3,304	2,916	3,528	2,742
	1,700	3,220	3,684	2,236	2,762
	2,168	2,236	2,220	3,200	3,173
	1,134	2,197	2,157	2,123	2,268
	1,509	1,877	2,541	2,173	2,827
	1,128	2,754	2,124	2,188	2,380
	1,084	2,262	2,039	2,500	2,516
Mean	1,533	2,577	2,642	2,587	2,607
SE	0,163	0,182	0,226	0,191	0,117
t	1,350	4,270	3,980	4,190	5,370
P	NS	0,001	0,002	0,001	0,001
Hepatoma	0,083	0,158	0,173	0,106	0,176
	0,066	0,106	0,114	0,096	0,174
	0,097	0,113	0,111	0,117	0,255
	0,075	0,169	0,082	0,110	0,296
	0,084	0,102	0,141	0,106	0,134
	0,125	0,143	0,092	0,140	0,146
	0,131	0,209	0,172	0,131	0,118
	0,081	0,187	0,172	0,172	0,140
Mean	0,093	0,148	0,132	0,122	0,180
SE	0,008	0,018	0,013	0,009	0,022
t		3,460	2,520	2,430	3,660
P		0,005	0,029	0,028	0,005

Table 3.43b A comparison of ASS Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0180	0,0143	0,0113	0,0160	0,0151
	0,0170	0,0161	0,0150	0,0143	0,0131
	0,0114	0,0104	0,0146	0,0159	0,0113
	0,0135	0,0135	0,0115	0,0160	0,0156
	0,0121	0,0156	0,0125	0,0140	0,0143
	0,0137	0,0170	0,0133	0,0137	0,0142
	0,0127	0,0117	0,0130	0,0130	0,0125
	0,0131	0,0153	0,0144	0,0152	0,0132
Mean	0,0138	0,0142	0,0132	0,0148	0,0137
SE	0,001	0,001	0,001	0,001	0,001
t		0,370	-0,670	1,010	-0,150
P		NS	NS	NS	NS
Host	0,0098	0,0213	0,0276	0,0210	0,0175
	0,0127	0,0254	0,0233	0,0235	0,0289
	0,0097	0,0248	0,0295	0,0172	0,0230
	0,0124	0,0172	0,0178	0,0213	0,0254
	0,0076	0,0169	0,0173	0,0163	0,0181
	0,0084	0,0144	0,0203	0,0170	0,0202
	0,0094	0,0212	0,0170	0,0171	0,0190
		0,0174	0,0164	0,0192	0,0201
Mean		0,0203	0,0211	0,0191	0,0215
SE		0,002	0,002	0,001	0,001
t		5,780	5,880	8,200	7,560
P	0,001	0,001	0,001	0,001	0,001
Hepatoma	0,0010	0,0014	0,0021	0,0013	0,0016
	0,0008	0,0010	0,0011	0,0012	0,0016
	0,0010	0,0013	0,0011	0,0012	0,0024
	0,0008	0,0015	0,0011	0,0010	0,0026
	0,0009	0,0010	0,0103	0,0013	0,0012
	0,0012	0,0013	0,0103	0,0013	0,0013
	0,0012	0,0019	0,0016	0,0012	0,0012
	0,0007	0,0017	0,0016	0,0016	0,0013
Mean	0,0009	0,0014	0,0037	0,0013	0,0016
SE	0,000	0,000	0,001	0,000	0,000
t		3,400	1,880	3,930	3,310
P		0,006	NS	0,003	0,004

Table 3.44A comparison of AL Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1,143	1,258	1,093	1,473	1,834
	2,029	1,651	1,895	1,800	1,520
	1,627	1,341	1,613	2,113	1,533
	1,500	1,900	1,448	1,712	1,121
	1,461	1,664	1,614	2,093	1,502
	1,355	1,589	1,699	1,695	1,581
	1,602	1,564	1,760	1,699	1,560
	1,485	1,739	1,190	1,693	1,481
Mean	1,525	1,487	1,539	1,660	1,516
SE	0,089	0,081	0,098	0,099	0,069
t		-0,131	0,110	1,010	-0,070
P		NS	NS	NS	NS
Host	1,422	2,013	1,852	2,468	1,757
	1,347	2,051	1,852	2,291	1,805
	1,760	2,531	2,194	2,093	1,935
	1,500	1,688	1,920	1,900	1,950
	1,299	2,020	2,374	2,029	2,300
	1,178	2,071	2,176	2,160	1,786
	1,338	1,847	2,114	2,986	1,974
	1,256	1,736	2,505	1,829	2,010
Mean	1,388	1,997	2,124	2,220	1,940
SE	0,063	0,093	0,085	0,131	0,061
t	1,250	5,420	6,940	5,730	6,250
P	NS	0,001	0,001	0,001	0,001
Hepatoma	0,334	0,343	0,257	0,257	0,311
	0,265	0,260	0,303	0,342	0,501
	0,292	0,257	0,327	0,281	0,401
	0,280	0,263	0,349	0,280	0,315
	0,242	0,312	0,340	0,362	0,342
	0,286	0,282	0,298	0,256	0,315
	0,252	0,312	0,312	0,260	0,428
	0,294	0,322	0,270	0,324	0,320
Mean	0,281	0,294	0,307	0,295	0,367
SE	0,010	0,012	0,011	0,015	0,025
t		0,86	1,730	0,820	3,210
P		NS	NS	NS	0,011

Table 3.44b A comparison of AL Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0076	0,0090	0,0073	0,0089	0,0118
	0,0145	0,0110	0,0115	0,0109	0,0132
	0,0099	0,0100	0,0098	0,0128	0,0099
	0,0111	0,0127	0,0096	0,0104	0,0077
	0,0097	0,0110	0,0108	0,0127	0,0094
	0,0087	0,0106	0,0113	0,0103	0,0100
	0,0146	0,0104	0,0106	0,0103	0,0095
	0,0110	0,0105	0,0079	0,0103	0,0129
Mean	0,0109	0,0107	0,0098	0,0108	0,0105
SE	0,001	0,001	0,001	0,001	0,001
t		-0,190	-0,090	-0,060	-0,300
P		NS	NS	NS	NS
Host	0,0174	0,0155	0,0148	0,0165	0,0023
	0,0108	0,0158	0,0150	0,0153	0,0019
	0,0110	0,0195	0,0176	0,0161	0,0161
	0,0120	0,0130	0,0154	0,0146	0,0156
	0,0104	0,0156	0,0190	0,0156	0,0184
	0,0094	0,0160	0,0175	0,0166	0,0128
	0,0081	0,0142	0,0169	0,0200	0,0141
	0,0089	0,0134	0,0200	0,0141	0,0161
Mean	0,0102	0,0155	0,0170	0,0161	0,0170
SE	0,001	0,001	0,001	0,001	0,001
t	0,63	6,040	8,410	7,560	5,970
P	NS	0,001	0,001	0,001	0,001
Hepatoma	0,0029	0,0034	0,0031	0,0031	0,0037
	0,0026	0,0029	0,0031	0,0035	0,0046
	0,0028	0,0031	0,0033	0,0033	0,0036
	0,0026	0,0032	0,0035	0,0029	0,0036
	0,0029	0,0031	0,0034	0,0037	0,0041
	0,0028	0,0029	0,0030	0,0030	0,0030
	0,0027	0,0032	0,0032	0,0031	0,0040
	0,0026	0,0033	0,0029	0,0033	0,0029
Mean	0,0027	0,0031	0,0032	0,0032	0,0037
SE	0,000	0,000	0,000	0,000	0,000
t	3,870	5,150	5,280	4,760	4,660
P	0,001	0,001	0,001	0,001	0,001

Table 3.45aA comparison of ARG Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1005,194	1526,520	1233,717	1200,402	1022,328
	1268,552	1200,629	1157,857	1318,781	1238,005
	1122,482	1191,251	1612,099	1278,522	1197,598
	1100,000	1100,000	1168,673	1224,782	1081,883
	1189,420	1442,896	1330,448	1275,795	1341,777
	1349,669	1282,890	1368,805	1405,120	1354,737
	1305,392	1444,603	1323,427	1644,560	1339,896
	1500,050	1391,395	1314,664	1500,717	1207,226
Mean	1230,094	1322,522	1313,710	1356,084	1222,930
SE	55,914	53,275	50,742	53,767	43,999
t		1,200	1,110	1,620	-0,100
P		NS	NS	NS	NS
Host	1027,899	1398,243	1723,573	1453,145	1355,786
	1027,889	1346,142	1537,814	1425,298	1399,497
	1215,840	1321,690	1564,662	1449,480	1650,619
	1299,539	1571,355	1335,708	1459,858	1403,308
	1110,018	1237,713	1354,251	1259,602	1223,918
	1150,014	1238,588	1278,151	1274,323	1611,359
	967,196	1310,359	1295,722	1179,567	1439,717
	1192,242	1297,066	1347,788	1178,428	1354,155
Mean	1123,827	1340,130	1429,706	1334,961	1429,793
SE	37,978	39,610	56,449	44,091	49,474
t	1,550	3,940	4,440	3,560	4,830
P	NS	0,001	0,001	0,003	0,001
Hepatoma	10,414	8,174	6,082	12,639	12,880
	9,718	5 658	7,545	10,834	9,429
	9,816	13,036	12,240	9,819	10,374
	10,000	10,226	10,834	10,050	10,100
	9,129	7,342	10,226	10,504	10,122
	10,430	8,584	12,566	10,226	10,700
	11,036	9,658	8,784	10,632	9,660
	10,834	10,632	10,924	10,778	9,778
Mean	10,173	9,164	9,900	10,680	10,388
SE	0,222	0,796	0,802	0,308	0,382
t		-1,220	-0,33	1,340	0,490
P		NS	NS	NS	NS

Table 3.45b A comparison of ARG Activities from control vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 22% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	9,3766	10,1768	9,8970	8,0027	9,1786
	9,0611	10,4403	9,2529	8,7919	10,7653
	8,0177	7,6855	10,0756	8,5235	7,7264
	8,1500	7,8570	9,3494	8,1652	9,6551
	7,9295	9,0181	9,8700	10,2064	8,3861
	9,7075	8,0181	9,1254	9,3675	8,4671
	9,6696	9,0288	9,8228	9,9570	9,2407
	11,1115	9,280	9,7644	10,0048	9,9424
Mean	9,172	8,938	9,645	9,127	9,170
SE	0,370	0,366	0,124	0,309	0,343
t		-0,450	1,240	-0,090	-0,010
P		NS	NS	NS	NS
Host	6,9526	13,3167	11,4905	11,1780	10,8463
	9,2231	10,7691	11,9293	10,9638	14,7315
	7,5990	10,5735	12,0359	11,1500	13,7552
	9,8363	12,5708	10,2747	11,2297	11,2265
	9,8801	9,9017	10,4173	9,8893	10,1993
	9,2001	9,9087	9,9319	9,8025	11,5097
	7,7376	10,4829	9,9671	9,9736	10,2837
	8,5160	10,3765	10,3676	10,7130	10,8332
Mean	8,6180	10,9880	10,8020	10,6250	11,6730
SE	0,387	0,446	0,309	0,214	0,589
t	1,030	4,020	4,410	4,540	4,340
P	NS	0,001	0,001	0,001	0,001
Hepatoma	0,0947	0,0743	0,0716	0,1149	0,1171
	0,0884	0,0636	0,0895	0,0985	0,0943
	0,0892	0,1185	0,1113	0,1155	0,1108
	0,0901	0,0930	0,0985	0,0914	0,0918
	0,0830	0,0854	0,0930	0,0950	0,0920
	0,0948	0,1010	0,1143	0,0940	0,0973
	0,1003	0,1136	0,1034	0,0966	0,1137
	0,0985	0,1257	0,0993	0,0980	0,1151
Mean	0,0925	0,0971	0,0976	0,1005	0,1040
SE	0,002	0,008	0,005	0,003	0,004
t		0,590	0,990	2,070	2,610
P		NS	NS	NS	0,021

Table 3.46a A comparison of CPS Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	6,288	8,340	5,079	6,783	9,813
	11,763	9,895	6,506	6,945	9,833
	9,220	10,791	6,240	10,924	7,960
	9,693	9,644	8,193	7,265	9,270
	8,340	11,684	10,390	10,917	7,438
	10,524	9,784	8,343	8,549	7,255
	6,899	8,483	6,240	7,940	9,084
	6,377	9,645	6,240	8,467	6,366
Mean	8,638	9,784	7,145	8,4[illegible]	8,377
SE	0,714	0,388	0,601	0583	0,460
t		1,410	-1,590	-0,19	-0,310
P		NS	NS	NS	NS
Host	5,449	10,776	6,108	6,696	8,109
	4,765	6,249	5,412	6,528	5,818
	5,387	5,553	7,941	6,696	7,481
	5,252	6,249	11,370	9,066	5,600	
	4,582	8,109	5,690	7,505	5,600
	5,008	5,818	8,100	6,700	6,794
	4,583	,600	5,812	7,053	6,862
	5,243	362	7,490	7,781	6,589
Mean	74	, 7	7,240	7,253	6,607
SE	0,123	0,602	0,700	0,302	0,321
t	4,980	3,240	3,110	6,790	4,571
P	0,001	0,012	0,017	0,001	0,001
Hepatoma	0,288	0,450	0,325	0,300	0,317
	0,294	0,25[illegible]	0,425	0,325	0,324
	0,282	0,420	0,325	0,327	0,448
	0,280	0,350	0,295	0,333	0,520
	0,309	0,357	0,309	0,299	0,360
	0,307	0,308	0,312	0,401	0,377
	0,300	0,400	0,372	0,408	0,368
	0,276	0,325	0,317	0,379	0,350
Mean	0,292	0,358	0,335	0,347	0,384
SE	0,004	0,023	0,015	0,015	0,024
t		2,840	2,730	3,400	2,760
P		0,022	0,026	0,009	0,007

Table 3.46b A comparison of CPS Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Specific Activity

	Control	β -methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0406	0,0556	0,0376	0,0522	0,0554
	0,0759	0,0706	0,0492	0,0334	0,0656
	0,0738	0,0771	0,0462	0,0840	0,0578
	0,0646	0,0643	0,0607	0,0582	0,0620
	0,0556	0,0615	0,0769	0,0840	0,0750
	0,0702	0,0652	0,0618	0,0658	0,0691
	0,0552	0,0579	0,0500	0,0627	0,0606
	0,0510	0,0771	0,0510	0,0651	0,0606
Mean	0,0609	0,0602	0,0544	0,0657	0,0634
SE	0,004	0,003	0,004	0,004	0,0020
t		1,010	-1,040	0,780	0,520
P		NS	NS	NS	NS
Host	0,0419	0,0830	0,0510	0,0515	0,0772
	0,0341	0,0508	0,0481	0,0502	0,0484
	0,0450	0,0427	0,0659	0,0558	0,0524
	0,0477	0,0569	0,0948	0,0756	0,0700
	0,0416	0,0872	0,0474	0,0577	0,0533
	0,0401	0,0485	0,0675	0,0515	0,0850
	0,0306	0,0510	0,0484	0,0588	0,0572
	0,0420	0,0624	0,0624	0,0648	0,0549
Mean	0,0404	0,0596	0,0607	0,0582	0,0623
SE	0,002	0,006	0,006	0,003	0,005
t	4,290	3,040	3,370	4,970	4,290
P	0,001	0,016	0,005	0,001	0,001
Hepatoma	0,0440	0,0560	0,0410	0,0430	0,0450
	0,0450	0,0320	0,0530	0,0330	0,0450
	0,0340	0,0560	0,0430	0,0350	0,0640
	0,0300	0,0470	0,0330	0,0420	0,0660
	0,0480	0,0450	0,0390	0,0430	0,0510
	0,0470	0,0390	0,0450	0,0410	0,0540
	0,0360	0,0530	0,0500	0,0460	0,0530
	0,0300	0,0430	0,0370	0,0470	0,0440
Mean	0,0390	0,0470	0,0430	0,0410	0,0530
SE	0,000	0,000	0,000	0,000	0,000
t		1,770	1,040	0,630	3,360
P		NS	NS	NS	0,005

Table 3.47a A comparison of OTC Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	252,898	264,969	311,718	306,516	363,076
	311,128	342,830	231,847	290,973	376,924
	377,571	401,572	273,028	298,269	368,846
	362,515	340,302	299,291	312,225	396,732
	457,284	441,392	367,542	316,856	355,383
	371,558	387,549	413,036	263,375	390,386
	425,312	311,718	341,317	403,543	395,771
	373,451	343,848	374,510	425,312	305,387
Mean	366,414	354,272	626,536	326,996	369,063
SE	22,351	19,436	20,906	20,028	28,411
t		-0,140	-1,300	-1,310	1,290
P		NS	NS	NS	NS
Host	273,333	401,629	347,390	359,496	375,967
	315,504	405,610	367,542	393,776	344,738
	331,121	343,211	363,748	354,743	328,921
	331,997	366,743	383,212	381,218	347,117
	279,220	375,072	375,967	395,752	367,043
	291,327	335,073	395,094	367,077	359,522
	287,865	389,980	388,022	376,700	339,304
	280,654	365,073	360,077	365,955	342,204
Mean	286,377	375,299	372,632	374,339	350,102
SE	15,936	7,842	5,611	5,372	5,590
t	2,92	5,010	5,110	5,230	3,770
P	0,011	0,001	0,001	0,001	0,004
Hepatoma	8,505	9,456	9,147	9,907	7,428
	7,943	9,134	9,908	9,807	7,027
	9,658	9,208	9,340	9,585	9,560
	9,134	10,070	10,020	10,001	9,637
	9,030	9,355	9,046	9,800	10,556
	8,962	9,030	9,818	9,787	10,683
	8,480	9,198	9,249	9,485	10,006
	8,478	10,003	9,898	9,934	11,537
Mean	8,764	9,432	9,553	9,791	9,554
SE	0,189	0,140	0,140	0,063	0,556
t		2,840	3,360	5,15	1,350
P		0,014	0,005	0,001	NS

Table 3.47b A comparison of OTC Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	2,020	2,208	2,309	2,358	2,420
	2,074	2,856	2,275	2,645	2,513
	2,517	2,769	2,022	2,712	2,459
	2,417	2,936	2,216	2,838	2,545
	3,049	3,044	2,723	2,881	2,369
	2,477	2,673	2,754	2,394	2,503
	2,835	2,598	2,529	3,104	2,262
	2,490	2,865	2,774	2,835	2,036
Mean	2,485	2,820	2,450	2,721	2,393
SE	0,122	0,144	0,101	0,089	0,061
t		1,780	-0,220	1,560	-0,680
P		NS	NS	NS	NS
Host	2,103	3,347	2,672	2,996	3,581
	2,254	3,380	2,827	3,281	2,552
	2,365	2,620	3,031	2,667	2,741
	2,371	3,056	3,193	3,177	3,119
	2,538	3,126	2,892	3,298	3,496
	2,331	2,959	3,039	3,059	3,424
	1,919	2,790	3,234	2,932	2,928
	2,245	3,042	3,005	3,050	2,852
Mean	2,266	3,040	2,987	3,058	3,087
SE	0,066	0,091	0,066	0,072	0,134
t	1,580	6,870	7,710	8,050	5,470
P	NS	0,001	0,001	0,001	0,001
Hepatoma	0,118	0,114	0,124	0,14	0,106
	0,114	0,142	0,114	0,123	0,101
	0,113	0,125	0,128	0,120	0,136
	0,134	0,134	0,118	0,125	0,112
	0,116	0,113	0,123	0,140	0,151
	0,113	0,140	0,140	0,122	0,153
	0,123	0,123	0,126	0,120	0,143
	0,133	0,132	0,117	0,124	0,145
Mean	0,121	0,128	0,127	0,127	0,133
SE	0,004	0,003	0,003	0,003	0,006
t		1,470	1,420	1,480	1,830
P		NS	NS	NS	NS

Table 3.48a A comparison of ASS Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were *maintained on a 75% protein diet.*

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dbcAMP
Control	2,397	3,077	2,911	3,118	3,056
	3,197	2,872	2,488	2,766	2,048
	2,773	2,570	3,261	3,145	2,730
	2,276	3,057	2,648	2,429	3,396
	3,156	2,259	3,556	3,053	3,577
	2,259	2,561	2,432	2,519	3,536
	3,190	2,965	2,835	2,364	3,100
	3,298	2,715	3,118	3,330	3,806
Mean	2,818	2,760	2,906	2,841	3,194
SE	0,159	0,101	0,138	0,131	0,214
t		-0,310	0,420	0,110	1,410
P		NS	NS	NS	NS
Host	1,649	3,465	3,653	3,244	2,960
	2,460	3,037	3,344	3,642	3,062
	2,251	3,123	3,274	3,258	3,378
	1,962	3,135	3,724	3,246	2,516
	2,400	3,546	3,563	3,424	3,008
	2,466	4,032	3,434	3,462	3,053
	2,270	4,123	3,427	2,587	3,278
	1,679	4,135	4,052	3,000	3,595
Mean	2,141	3,575	3,559	3,233	3,106
SE	0,199	0,165	0,089	0,114	0,114
t	3,420	7,060	9,580	6,630	5,870
P	0,004	0,001	0,001	0,001	0,001
Hepatoma	0,214	0,258	0,233	0,183	0,234
	0,183	0,283	0,214	0,185	0,243
	0,124	0,287	0,224	0,201	0,275
	0,225	0,218	0,254	0,240	0,240
	0,123	0,263	0,274	0,253	0,263
	0,156	0,357	0,244	0,307	0,357
	0,234	0,216	0,205	0,287	0,267
	0,175	0,285	0,265	0,250	0,240
Mean	0,179	0,276	0,236	0,245	0,265
SE	0,015	0,014	0,007	0,017	0,014
t		4,720	3,340	2,810	4,110
P		0,001	0,008	0,014	0,001

Table 3.48b A comparison of ASS Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0155	0,0205	0,0194	0,0240	0,0204
	0,0213	0,0205	0,0194	0,0213	0,0164
	0,0222	0,0194	0,0217	0,0210	0,0182
	0,0182	0,0204	0,0196	0,0186	0,0180
	0,0204	0,0188	0,0209	0,0204	0,0202
	0,0181	0,0205	0,0190	0,0229	0,0241
	0,0206	0,0212	0,0193	0,0215	0,0177
	0,0213	0,0217	0,0183	0,0222	0,0218
Mean	0,0197	0,0204	0,0197	0,0215	0,0191
SE	0,001	0,002	0,001	0,001	0,001
t		1,020	-0,040	1,820	-0,550
P		NS	NS	NS	NS
Host	0,0127	0,0224	0,0281	0,0271	0,0282
	0,0176	0,0196	0,0257	0,0304	0,0236
	0,0188	0,0223	0,0273	0,0245	0,0282
	0,0164	0,0202	0,0266	0,0271	0,0252
	0,0218	0,0229	0,0274	0,0285	0,0296
	0,0197	0,0288	0,0264	0,0289	0,0291
	0,0151	0,0295	0,0286	0,0215	0,0274
	0,0134	0,0295	0,0289	0,0226	0,0299
Mean	0,0169	0,0244	0,0274	0,0263	0,0276
SE	0,001	0,001	0,001	0,001	0,001
t	2,22	4,030	8,840	5,960	7,880
P	0,030	0,001	0,001	0,001	0,001
Hepatoma	0,0026	0,0032	0,0029	0,0025	0,0033
	0,0022	0,0035	0,0031	0,0025	0,0035
	0,0014	0,0038	0,0032	0,0025	0,0035
	0,0025	0,0032	0,0032	0,0030	0,0030
	0,0015	0,0032	0,0034	0,0035	0,0037
	0,0019	0,0043	0,0035	0,0033	0,0036
	0,0026	0,0031	0,0030	0,0036	0,0039
	0,0019	0,0038	0,0033	0,0031	0,0300
Mean	0,0021	0,0035	0,0032	0,0030	0,0034
SE	0,000	0,000	0,000	0,000	0,000
t		6,380	6,150	3,960	3,250
P		0,001	0,001	0,001	0,006

Table 3.49a A comparison of AL Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	4,546	4,488	4,747	3,587	3,942
	4,637	4,231	4,501	4,922	4,320
	5,644	5,808	5,682	3,812	3,921
	4,226	4,909	5,252	4,990	4,982
	4,315	5,067	3,487	4,792	5,018
	4,533	5,041	5,175	4,324	4,935
	5,425	4,650	4,337	4,734	4,794
	5,064	5,780	4,792	4,753	4,823
Mean	4,799	4,997	4,747	4,489	4,592
SE	0,184	0,201	0,236	0,187	0,163
t		0,730	-0,17	-1,180	-0,840
P		NS	NS	NS	NS
Host	3,088	3,957	3,795	3,950	3,706
	3,344	4,092	4,159	4,070	3,541
	3,212	4,170	4,435	4,094	3,457
	3,064	4,055	4,140	4,123	4,359
	3,245	4,197	3,668	3,940	4,094
	3,204	3,926	4,237	3,787	4,419
	3,434	4,171	4,816	3,926	5,249
	3,370	4,902	4,138	3,934	4,009
Mean	3,245	4,185	4,174	3,986	3,979
SE	0,047	0,109	0,126	0,039	0,131
t	8,180	7,950	6,920	12,210	5,270
P	0,001	0,001	0,001	0,001	0,001
Hepatoma	0,612	0,625	0,566	0,615	0,634
	0,533	0,622	0,582	0,566	0,612
	0,667	0,688	0,592	0,576	0,632
	0,595	0,630	0,617	0,655	0,638
	0,630	0,654	0,632	0,623	0,630
	0,595	0,632	0,630	0,603	0,638
	0,612	0,612	0,638	0,623	0,638
	0,613	0,634	0,601	0,621	0,635
Mean	0,610	0,632	0,607	0,610	0,632
SE	0,014	0,008	0,009	0,010	0,003
t		1,700	-0,200	0,020	1,580
P		NS	NS	NS	NS

Table 3.49b A comparison of AL Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	0,0303	0,0359	0,0352	0,0325	0,0394
	0,0371	0,0339	0,0333	0,0380	0,0346
	0,0376	0,0387	0,0379	0,0346	0,0373
	0,0338	0,0393	0,0351	0,0394	0,0332
	0,0345	0,0362	0,0258	0,0370	0,0335
	0,0363	0,0360	0,0345	0,0393	0,0353
	0,0352	0,0372	0,0321	0,0364	0,0344
	0,0338	0,0395	0,0355	0,0366	0,0322
Mean	0,0348	0,0371	0,0337	0,0367	0,0350
SE	0,001	0,001	0,001	0,001	0,001
t		2,100	-0,760	1,630	0,140
P		NS	NS	NS	NS
Host	0,0238	0,0330	0,0292	0,0329	0,0353
	0,0240	0,0341	0,0320	0,0339	0,0274
	0,0268	0,0298	0,0370	0,0315	0,0290
	0,0280	0,0338	0,0345	0,0344	0,0363
	0,0296	0,0350	0,0282	0,0328	0,0400
	0,0256	0,0327	0,0326	0,0320	0,0421
	0,0230	0,0321	0,0371	0,0327	0,0437
	0,0270	0,0377	0,0345	0,0333	0,0334
Mean	0,0260	0,0336	0,0331	0,0329	0,0359
SE	0,001	0,001	0,001	0,001	0,002
t	7,72	6,610	5,070	8,040	4,460
P	0,001	0,001	0,001	0,001	0,001
Hepatoma	0,0074	0,0078	0,0071	0,0088	0,0091
	0,0064	0,0078	0,0083	0,0071	0,0087
	0,0090	0,0092	0,0079	0,0072	0,0091
	0,0055	0,0084	0,0072	0,0092	0,0091
	0,0076	0,0079	0,0079	0,0089	0,0090
	0,0071	0,0079	0,009	0,0075	0,0091
	0,0076	0,0082	0,0095	0,0078	0,0092
	0,0069	0,0096	0,0071	0,0078	0,0091
Mean	0,0072	0,0084	0,0080	0,0080	0,0090
SE	0,000	0,000	0,000	0,000	0,000
t		2,790	1,700	1,550	5,130
P		0,016	NS	NS	0,001

Table 3.50a A comparison of ARG Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Activity/g Liver

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	1847,569	1859,747	1555,365	1431,201	1442,426
	1878,565	1822,629	1485,165	1897,878	1951,810
	2036,912	2013,471	1437,571	1887,787	2043,780
	1916,974	1990,743	1512,122	1664,685	1821,110
	1903,155	1806,573	1740,670	1859,991	1765,017
	1831,948	1572,082	1740,670	1828,875	1947,108
	1742,650	1650,727	1850,727	1757,631	1649,560
	1674,952	2167,529	1850,727	1780,893	2056,608
Mean	1853,085	1850,436	1646,628	1766,115	1834,675
SE	39,305	68,943	59,347	52,556	74,702
t		0,09	-2,900	-1,330	-0,220
P		NS	0,013	NS	NS
Host	1528,075	2485,772	1982,869	1898,243	1851,865
	1171,128	2055,879	1652,793	1818,276	1641,603
	1518,862	2097,992	1824,770	1792,582	1699,180
	1278,166	1857,191	1700,646	1898,063	1882,376
	1208,673	2079,929	2058,992	1988,403	1667,496
	1355,755	1785,911	1999,889	1881,776	2155,077
	1345,308	1918,200	1851,865	1929,280	2094,715
	1457,819	1677,090	2094,207	1767,053	1918,142
Mean	1357,972	1996,993	1895,752	1871,457	1863,804
SE	47,911	88,179	58,020	26,150	67,695
t	7,990	6,370	7,150	9,410	6,100
P	0,001	0,001	0,001	0,001	0,001
Hepatoma	10,875	10,710	12,424	12,578	11,273
	11,615	10,438	12,542	12,567	12,108
	11,775	10,795	11,920	10,127	11,950
	11,199	10,420	14,667	13,702	12,195
	10,045	13,706	10,515	11,055	11,411
	10,437	12,121	11,785	10,723	10,205
	10,719	11,025	11,978	9,398	10,962
	9,513	10,532	10,050	12,075	10,288
Mean	10,772	11,218	11,98	11,603	11,299
SE	0,272	0,405	0,494	0,469	0,275
t		0,910	2,150	1,530	1,360
P		NS	NS	NS	NS

Table 3.50b A comparison of ARG Activities from untreated vs. hormone treated control animals, hosts and hepatomas. Rats were maintained on a 75% protein diet.

Specific Activity

	Control	β-methasone	Glucagon	β-methasone & Glucagon	dBcAMP
Control	11,920	12,000	11,964	11,193	9,616
	12,120	11,760	11,424	12,553	13,012
	10,721	13,423	11,058	12,586	12,022
	12,367	13,272	11,632	12,805	12,141
	12,280	12,044	11,605	12,400	9,290
	11,703	12,576	11,608	12,193	12,562
	11,243	13,206	12,338	11,718	13,196
	10,805	12,385	10,887	11,872	10,032
Mean	11,645	12,583	11,564	12,165	11,484
SE	0,230	0,238	0,164	0,171	0,560
t		1,900	-0,290	1,520	-0,270
		NS	NS	NS	NS
Host	11,754	17,756	16,524	15,819	17,636
	9,365	16,714	13,218	14,783	13,690
	12,657	15,138	13,034	14,574	14,160
	12,782	14,861	14,172	15,431	17,927
	10,988	15,995	17,158	16,570	15,881
	10,946	14,520	16,251	15,300	17,326
	8,969	14,756	13,375	15,659	17,456
	11,663	12,901	17,452	14,365	15,985
Mean	11,141	15,330	15,136	15,313	16,283
SE	0,492	0,524	0,670	0,257	0,581
t	0,93	5,830	4,810	7,510	6,750
P	NS	0,001	0,001	0,001	0,001
Hepatoma	0,1279	0,1306	0,1553	0,1796	0,1610
	0,1356	0,1305	0,1792	0,1267	0,1659
	0,1280	0,1440	0,1402	0,1141	0,1537
	0,1217	0,1390	0,1727	0,1713	0,1544
	0,1192	0,1671	0,1314	0,1579	0,1630
	0,1228	0,1515	0,1684	0,1073	0,1400
	0,1156	0,1470	0,1409	0,1111	0,1502
	0,1034	0,1404	0,1182	0,1509	0,1302
Mean	0,1217	0,1450	0,1508	0,1399	0,1523
SE	0,003	0,042	0,006	0,010	0,004
t		1,500	3,480	1,700	5,590
P		NS	0,004	NS	0,001

In order to further investigate factors regulating the levels of urea cycle enzymes, a study was made of dietary and hormonal regulation of these enzymes in normal liver, in hepatoma and in the host liver of hepatoma-bearing animals.

The purpose of the first experiment was to study the relationship of urea cycle enzymes in the three liver types in thirty five-day old rats maintained on a 22% protein diet. The rats were trained to an 8-16' feeding regime and were fed normal laboratory food pellets for one week prior to sacrifice. All animals were sacrificed at 10.00 hours . Results are expressed as activity/gram liver, specific activity and percentage of control, and are shown in the tables of results. Also shown are the means of the results of eight individual experiments $\pm$ standard errors. The standard student t-test was performed to determine the significance of differences found. *Probabilities* (P) of less than 5% are considered as significant.

From fig 3.29 it can be seen that all five enzymes are correlated. There is a large significant drop in the activity of these enzymes in the hapatoma as compared with the control livers of nonhepatoma-bearing animals. The results are similar when expressed either as activity/gram liver or as specific activity. This suggests that the drop is specific and not due to some component in the liver such as total liver protein content, carbohydrate or water.

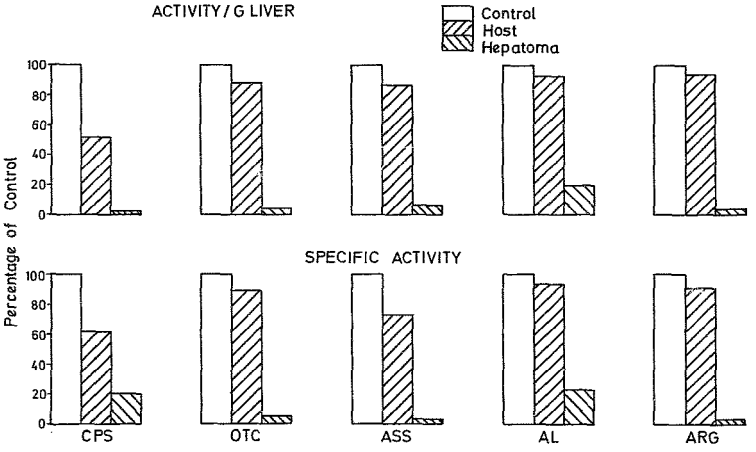


Fig. 3.29 Activities of urea cycle enzymes in control, host and hepatoma. Rats were maintained on a 22% protein diet.

The figures 3.30 co 3.34 show the effect of diet on the activities of urea cycle enzymes in normal livers, hepatomas and the host livers of hepatoma-bearing animals. The experimental conditions were as described previously. The animals were trained to diets containing 0%, 10%, and 75% protein for one week prior to sacrifice.

From these results it can be seen that the decrease in activities in enzyme levels in the host livers and hepatomas occurs on all the diets and that the behaviour of all the urea cycle enzymes is correlated. The fact that the drop occurs on all the diets argues against the possibility of the hepatoma exerting a starvation effect on the host liver through preferential use of metabolites such as amino acids since the drop is seen in rats maintained on a 75% protein diet where amino acid levels are not limiting. These results do suggest the possible existence of a humoral substance which could cause a drop in the activity of the urea cycle enzymes in tumour-bearing animals.

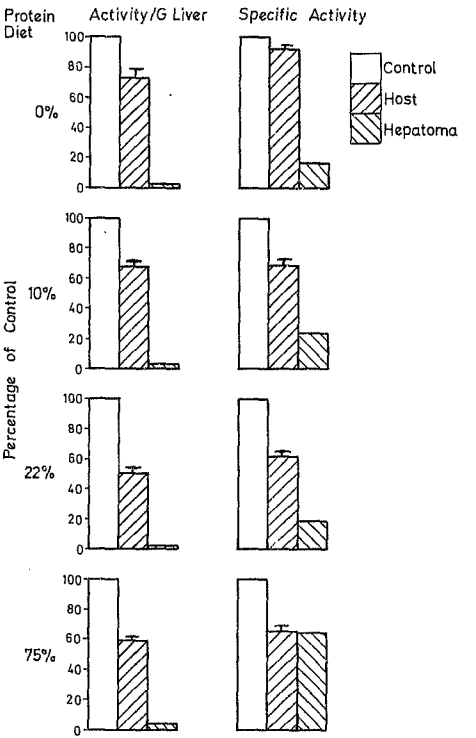


Fig. 3.30 Activity/g liver and specific activity of CPS from untreated host livers and hepatomas expressed as a % of untreated control liver. Rats were maintained on various protein diets.

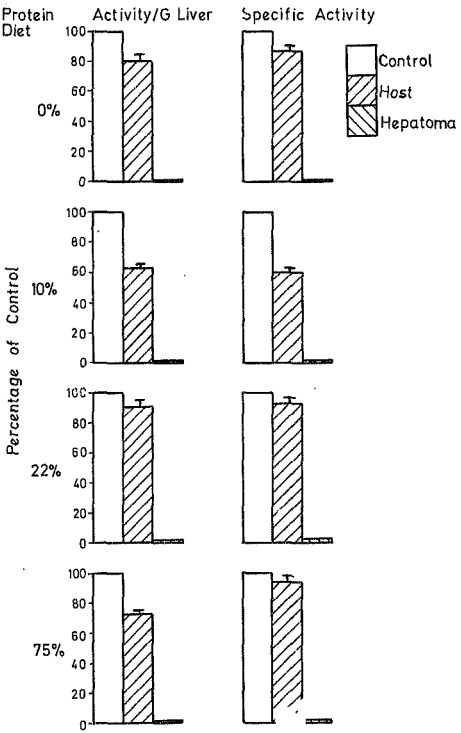


Fig. 3.31 Activity/g liver and specific activity of OTC from untreated host livers and hepatomas expressed as % of untreated control livers. Rats were maintained on various protein diets.

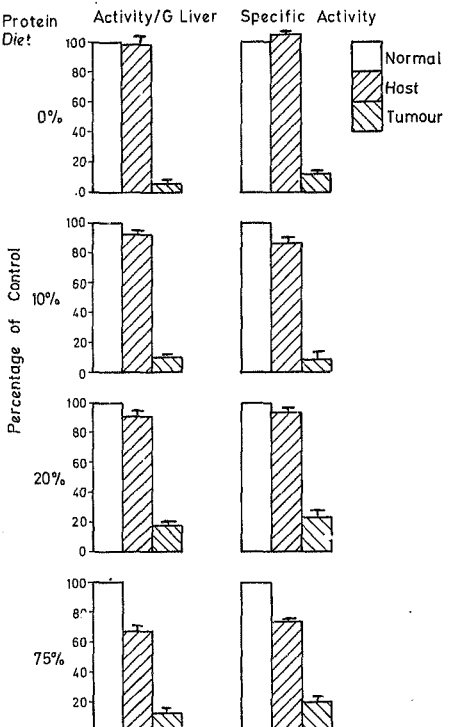


Fig. 3.32 Activity/g liver and specific activity of ASS from untreated host livers and hepatomas expressed as a % of untreated control livers. Rats were maintained on various protein diets.

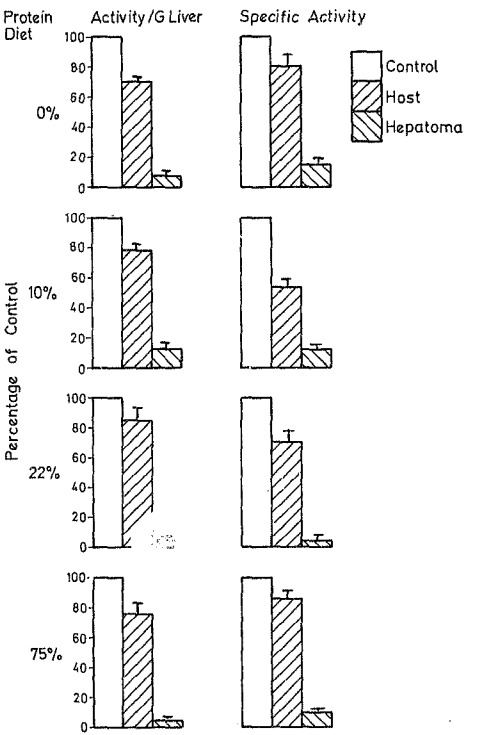


Fig. 3.33 Activity/g liver and specific activity of AL from untreated host livers and hepatomas expressed as a % of *untreated* control livers. Rats were maintained on various protein diets.

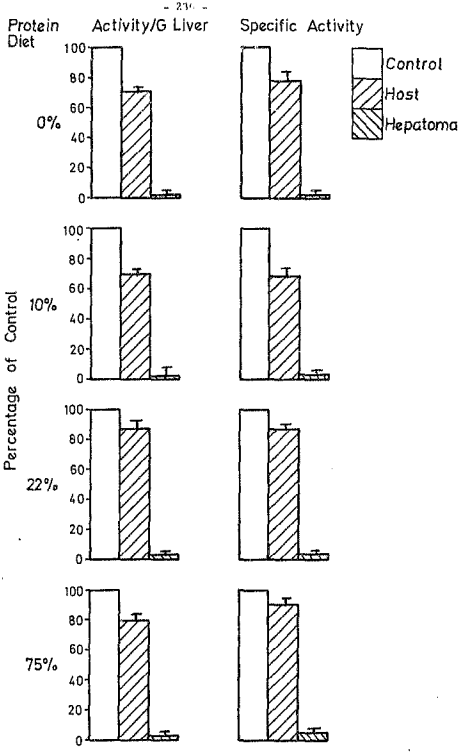


Fig. 3.34 Activity/g liver and specific activity of ARG from untreated host livers and hepatomas expressed as a % of untreated control livers. Rats were maintained on various protein diets.

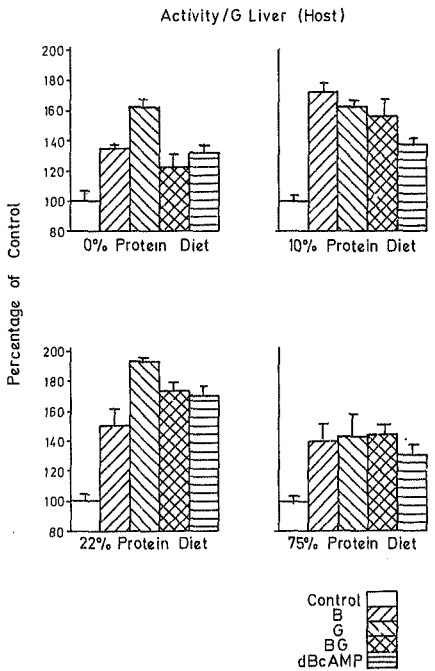


Fig. 3.35 Activity/g liver of CPS from hormone treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets.

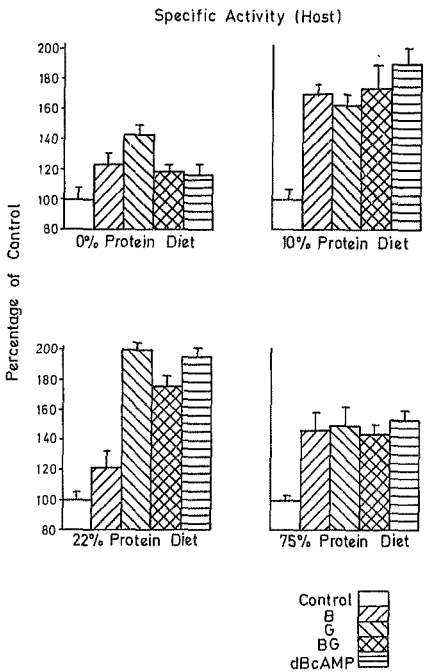


Fig. 3.36 Specific activities of CPS from hormone treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets.

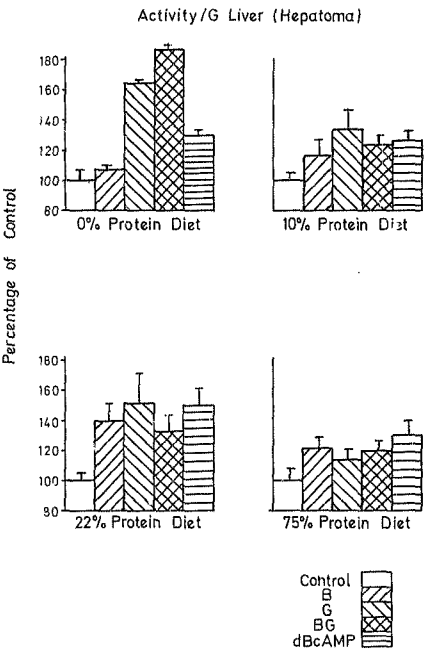


Fig. 3.37 Activity/g liver of CPS from hormone treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets.

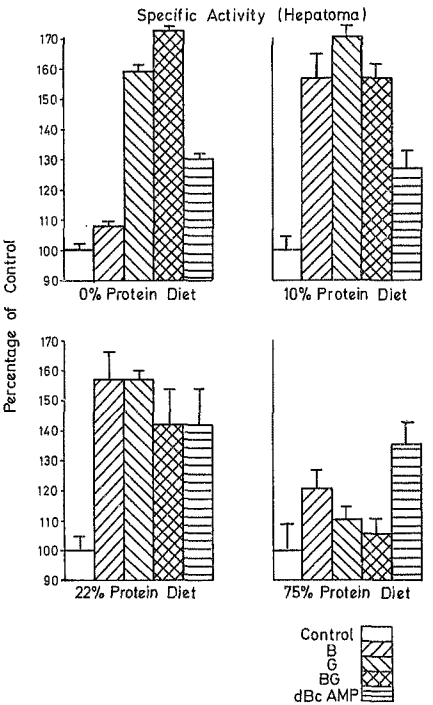


Fig. 3.38 Specific activities of CPS from hormone treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets.

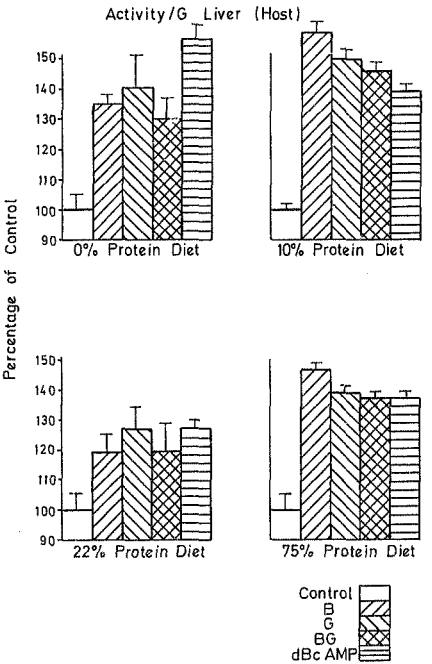


Fig. 3.39 Activity/g liver of OTC from hormone treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets.

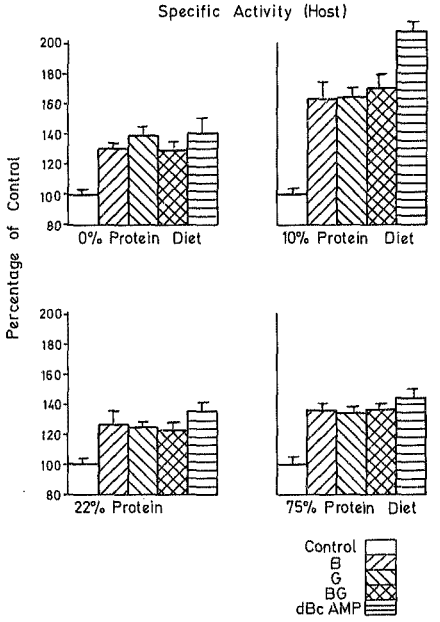


Fig. 3.40 Specific activities of OTC from hormone treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets.

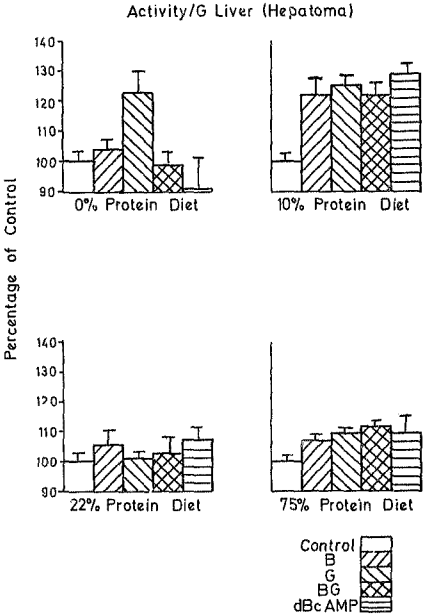


Fig. 3.41 *Activity/g liver of OTC from hormone-treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets.*

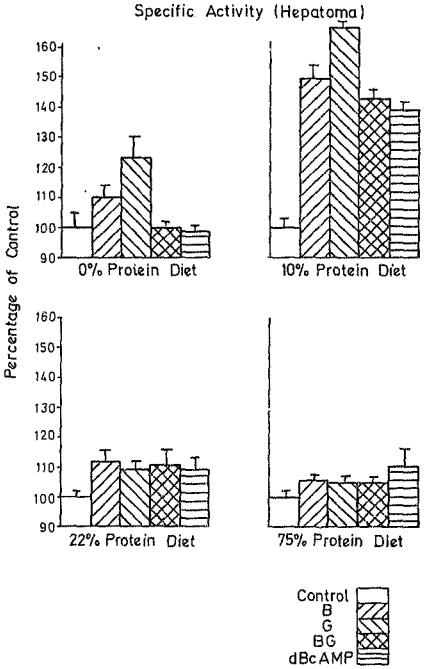


Fig. 3.42 *Specific activity of OTC from hormone-treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets.*

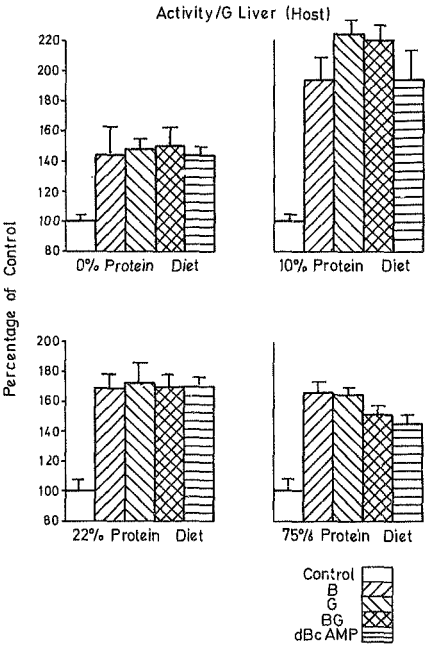


Fig. 3.43 Activity/g liver of ASS from hormone treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets.

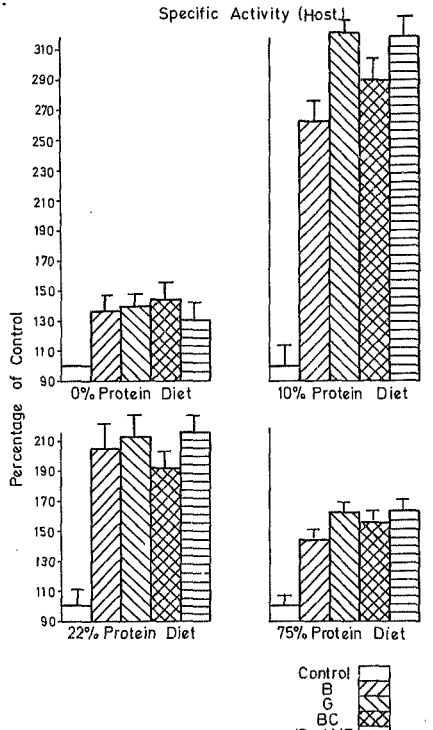


Fig. 3.44 Specific activities of ASS from hormone treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets.

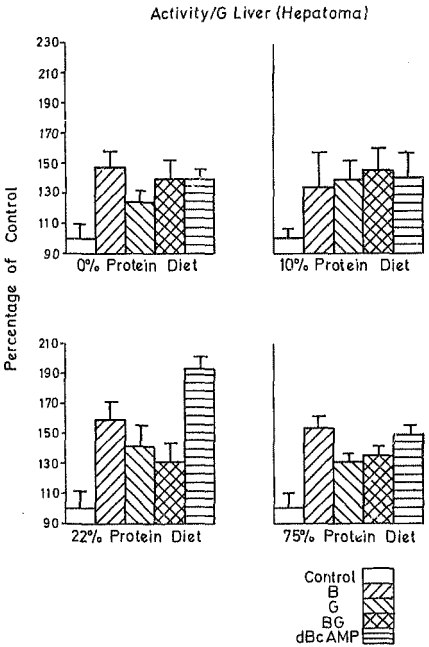


Fig. 3.45 Activity/g liver of ASS from hormone treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets.

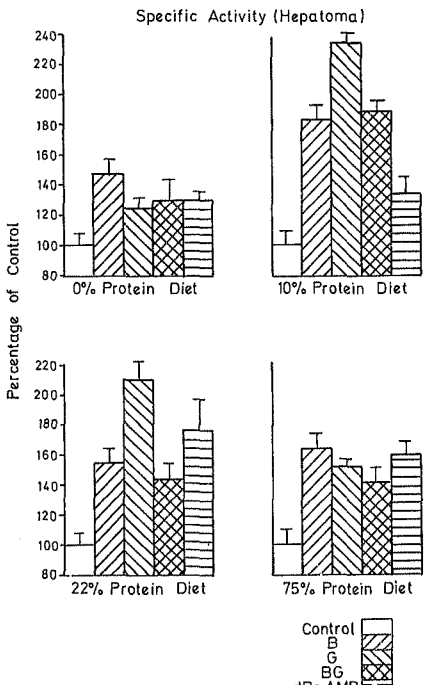


Fig. 3.46 Specific activities of ASS from hormone treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets.

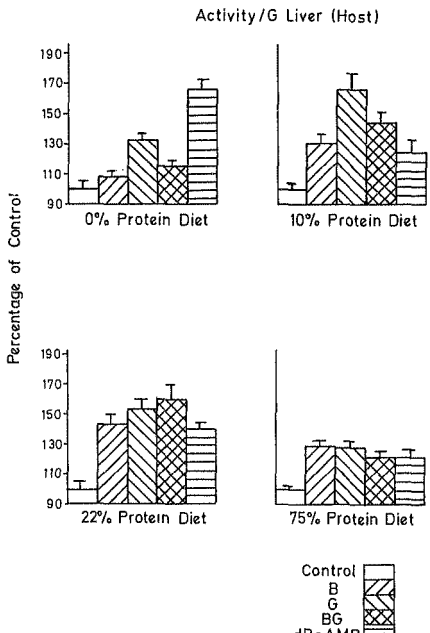


Fig. 3.47 Activity/g liver of AC from hormone-treated host livers expressed as a % of control untreated host livers of rats maintained on various protein diets.

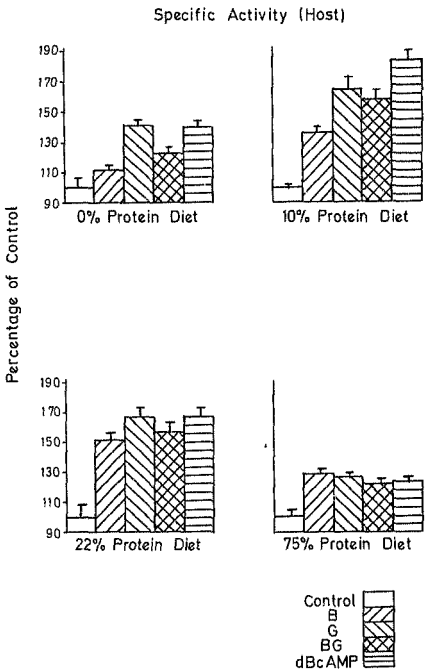


Fig. 3.48 Specific activities of AL from hormone-treated host livers expressed as a % of control *untreated* host livers of rats maintained on various protein diets.

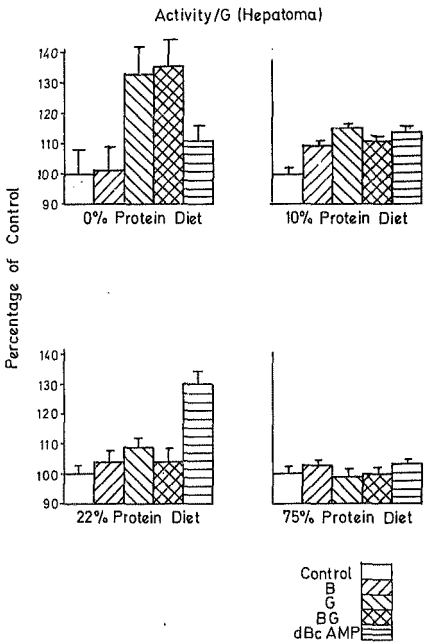


Fig. 3.49 Activity/g liver of AL from hormone-treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets.

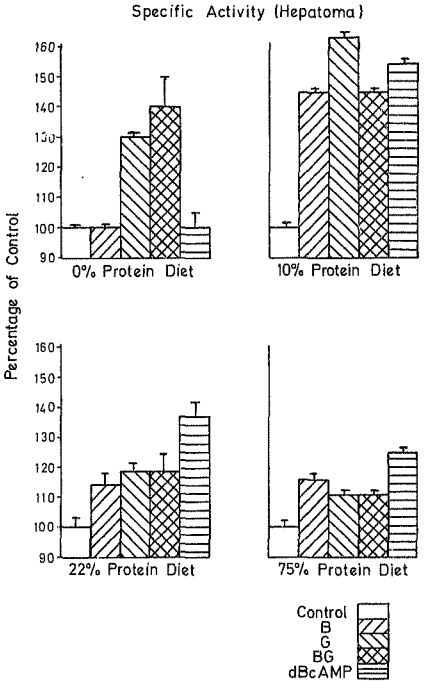


Fig. 3.50 Specific activity of AL from hormone-treated hepatomas expressed as a % of control untreated hepatomas from rats maintained on various protein diets.

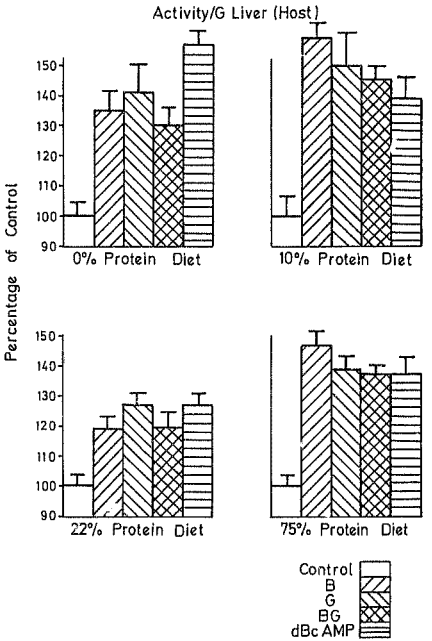


Fig. 3.51 Activity/g liver of arginase from hormone-treated host livers expressed as a % of control untreated host livers. Rats were maintained on various protein diets.

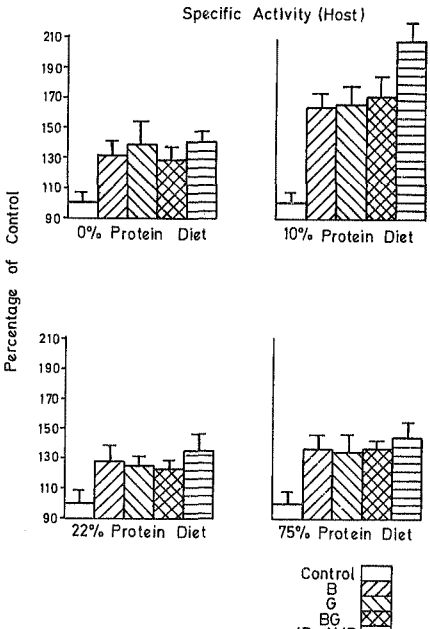


Fig. 3.52 Specific activity of arginase from hormone-treated host livers expressed as a % of control untreated host livers. Rats were maintained on various protein diets.

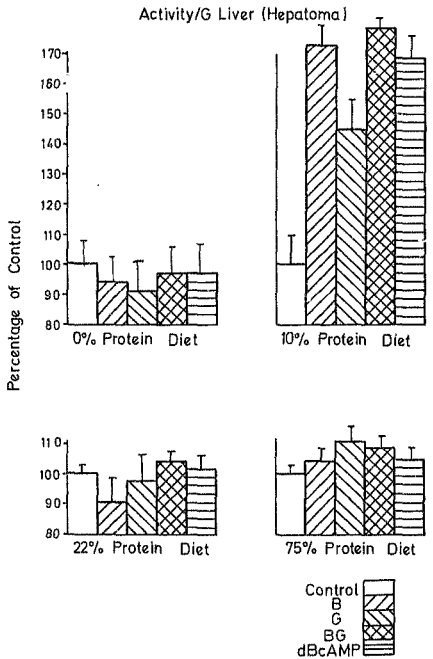


Fig. 3.53 Activity/g liver of arginase from hormone-treated hepatomas expressed as a % of control untreated hepatomas. Rats were maintained on various protein diets.

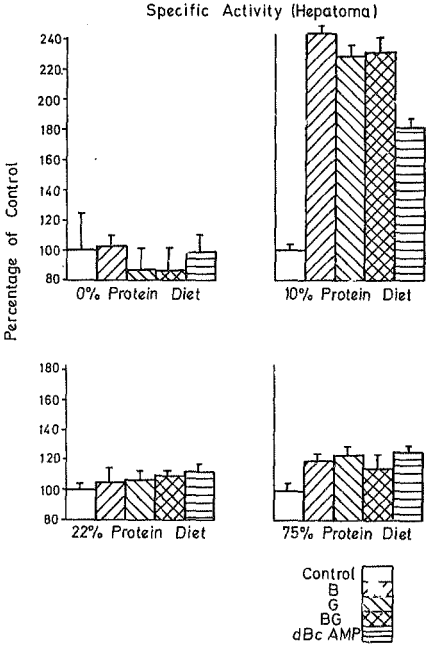


Fig. 3.54 Specific activity of arginase from hormone-treated hepatomas expressed as a % of control untreated hepatomas. Rats were maintained on various protein diets.

Table 3.51 Activities of urea cycle enzymes from hormone-untreated controls, hosts and hepatomas from rats maintained on a 0%, 10% or a 75% protein diet expressed as a % of levels in rats maintained on a 22% protein diet.

Activity/g liver			Specific Activity			% Dietary Protein
Control	Host	Hepatoma	Control	Host	Hepatoma	
CPS						
29,23	38,72	19,52	35,11	52,14	31,43	0
53,98	66,00	98,17	76,70	85,04	33,40	10
100,00	100,00	100,00	100,00	100,00	100,00	22
158,40	167,47	410,12	161,97	172,65	557,14	75
ASS						
77,61	73,32	51,96	83,87	68,10	42,85	0
98,14	82,25	76,53	98,64	85,34	90,48	10
100,00	100,00	100,00	100,00	100,00	100,00	22
153,90	139,66	101,66	130,02	145,69	109,62	75
AL						
66,24	71,61	21,35	73,98	70,01	37,04	0
70,16	73,65	39,00	94,78	88,33	40,70	10
100,00	100,00	100,00	100,00	100,00	100,00	22
304,69	233,79	217,08	303,61	254,70	266,67	75
OTC						
47,48	38,52	18,76	50,54	52,18	33,06	0
77,71	62,69	46,42	86,21	85,42	64,34	10
100,00	100,00	100,00	100,00	100,00	100,00	22
191,31	171,51	134,40	159,40	198,18	196,11	75
ARG						
52,41	45,17	18,12	54,67	52,78	30,60	0
76,31	54,31	28,32	95,41	66,65	37,41	10
100,00	100,00	100,00	100,00	100,00	100,00	22
150,65	120,84	105,89	115,88	129,78	131,58	75

Table 3.52 A Activities as % of control liver for CPS.

% Protein			Control	B	G	BG	cAMP
0	Host	A/g	72,98	100,31	118,60	89,54	96,81
		S.A.	92,42	112,88	132,58	109,09	108,33
	Hep	A/g	0,87	0,94	1,44	1,60	1,10
		S.A.	17,42	18,94	29,55	30,30	22,73
10	Host	A/g	67,56	117,46	110,19	105,37	92,43
		S.A.	69,10	117,71	112,85	120,83	131,94
	Hep.	A/g	2,37	2,78	3,21	2,96	3,03
		S.A.	24,30	38,19	41,67	38,19	30,90
22	Host	A/g	55,13	83,62	105,66	95,58	93,80
		S.A.	62,23	97,07	124,20	109,78	122,07
	Hep.	A/g	1,31	1,81	1,99	1,74	1,97
		S.A.	19,61	29,26	29,26	25,26	25,60
75	Host	A/g	59,28	81,35	83,82	83,97	75,49
		S.A.	65,34	97,87	99,67	95,57	102,29
	Hep.	A/g	3,38	4,15	3,89	4,01	4,45
		S.A.	64,04	77,18	70,61	67,32	87,03

Table 3.52B Host activities as a % of Control Host.
Hepatoma activities as a % of Control Hepatoma.

			Control	B	G	BG	cAMP
0	Host	A/g	100	137,63	162,71	122,90	132,82
		S.A.	100	122,13	143,44	118,03	117,21
	Hep.	A/g	100	107,91	165,51	187,05	129,50
		S.A.	100	108,70	169,57	173,91	130,43
10	Host	A/g	100	173,86	163,10	155,96	136,80
		S.A.	100	170,35	163,32	174,87	190,95
	Hep.	A/g	100	117,02	135,19	124,61	127,47
		S.A.	100	157,14	171,43	157,14	127,14
22	Host	A/g	100	151,70	191,48	173,39	170,16
		S.A.	100	120,75	199,57	174,79	195,16
	Hep.	A/g	100	138,20	152,67	133,43	150,56
		S.A.	100	157,14	157,14	142,86	142,86
75	Host	A/g	100	139,60	143,82	144,08	131,21
		S.A.	100	147,52	150,25	144,06	154,21
	Hep.	A/g	100	122,60	114,73	119,84	131,16
		S.A.	100	120,51	110,26	105,13	135,89

A/g = Activity/g Liver
S.A. = Specific Activity

Table 3.53A Activities as % of control liver for OTC.

% Protein			Control	B	C	BC	cAMP
0	Host	A/g	70,73	89,84	122,60	99,52	95,47
		S.A.	77,57	93,31	125,10	100,53	99,02
	Hep.	A/g	1,35	1,40	1,67	1,32	1,22
		S.A.	2,59	2,85	3,19	2,59	2,56
10	Host	A/g	70,32	91,35	91,75	89,98	85,08
		S.A.	69,26	85,47	89,52	95,23	121,75
	Hep.	A/g	2,01	2,57	2,52	2,47	2,62
		S.A.	2,55	3,82	4,25	3,64	3,50
22	Host	A/g	87,18	119,98	124,48	119,39	112,52
		S.A.	87,28	131,47	139,59	125,37	139,10
	Hep.	A/g	3,41	3,61	3,47	3,52	3,66
		S.A.	4,59	5,19	5,01	5,10	5,03
75	Host	A/g	79,16	102,42	101,70	102,16	95,55
		S.A.	91,19	122,33	120,20	123,06	124,23
	Hep.	A/g	2,39	2,57	2,61	2,67	2,61
		S.A.	4,87	5,15	5,11	5,11	5,37

Table 3.53B Host activities as a % of Control Host.
Hepatoma activities as a % of Control Hepatoma.

			Control	B	C	BC	cAMP
0	Host	A/g	100	127,03	173,35	140,72	134,99
		S.A.	100	120,28	161,27	129,60	127,65
	Hep.	A/g	100	104,09	123,63	99,69	90,68
		S.A.	100	110,29	123,53	100,00	99,00
10	Host	A/g	100	129,88	130,46	127,95	120,97
		S.A.	100	133,00	137,36	149,75	199,46
	Hep.	A/g	100	122,29	125,15	122,44	129,76
		S.A.	100	149,76	167,00	143,07	139,29
22	Host	A/g	100	137,63	142,78	135,95	129,07
		S.A.	100	150,64	160,00	143,64	159,33
	Hep.	A/g	100	106,00	101,93	103,25	107,39
		S.A.	100	112,97	109,24	111,02	109,56
75	Host	A/g	100	131,07	130,14	130,73	122,27
		S.A.	100	134,16	131,82	134,95	135,23
	Hep.	A/g	100	107,62	109,00	111,72	109,01
		S.A.	100	105,79	105,00	105,00	110,25

A/g = Activity/g Liver
S.A. = Specific Activity

Table 3.54 A Activities as % of control liver for ASS.

% Protein			Control	B	C	BG	cAMP
0	Host	A/g	69,87	99,45	101,80	103,17	99,29
		S.A.	79,91	109,84	109,52	114,97	102,72
	Hep.	A/g	7,48	11,03	9,29	10,38	10,38
		S.A.	15,66	23,13	19,73	20,41	20,41
10	Host	A/g	79,10	154,22	179,47	175,02	153,48
		S.A.	54,48	143,45	175,86	159,31	177,93
	Hep.	A/g	12,73	17,10	17,67	19,51	17,95
		S.A.	13,10	24,14	31,01	24,82	17,93
22	Host	A/g	85,31	143,41	147,02	143,96	145,08
		S.A.	71,74	147,10	152,90	139,41	155,80
	Hep.	A/g	5,18	9,24	7,35	5,79	10,02
		S.A.	5,52	10,15	25,81	9,40	11,59
75	Host	A/g	75,98	125,86	125,30	114,74	110,22
		S.A.	85,79	123,86	139,09	133,50	140,10
	Hep.	A/g	5,35	9,79	9,37	9,69	9,40
		S.A.	10,66	17,77	15,24	15,22	17,26

Table 3.54 B Host activities as a % of Control Host.
Hepatoma activities as a % of Control Hepatoma.

			Control	B	C	BG	cAMP
0	Host	A/g	100	144,41	147,81	149,80	144,17
		S.A.	100	137,93	139,79	145,69	130,17
	Hep.	A/g	100	147,45	124,09	139,69	139,69
		S.A.	100	147,83	125,09	130,43	130,43
10	Host	A/g	100	195,75	225,62	221,26	194,04
		S.A.	100	263,29	322,78	292,41	321,58
	Hep.	A/g	100	134,25	139,67	145,30	140,88
		S.A.	100	184,21	235,84	189,47	135,84
22	Host	A/g	100	169,10	172,34	169,75	170,06
		S.A.	100	205,05	213,13	192,93	217,17
	Hep.	A/g	100	159,14	141,96	111,18	193,55
		S.A.	100	155,56	211,11	144,44	177,78
75	Host	A/g	100	165,98	165,23	151,00	145,07
		S.A.	100	144,38	162,13	155,62	163,31
	Hep.	A/g	100	154,19	131,84	135,87	149,05
		S.A.	100	165,67	152,38	142,86	161,90

A/g = Activity/g Liver
A.S. = Specific Activity

Table 3.55A Activities as % of control liver for AL.

% Protein			Control	B	C	BC	cAMP
0	Host	A/g	98,05	106,35	131,67	114,15	164,24
		S.A.	105,88	112,22	141,11	123,33	145,56
	Hep.	A/g	5,94	6,04	7,92	8,12	6,63
		S.A.	11,76	11,76	15,29	16,47	11,76
10	Host	A/g	92,90	122,06	155,23	135,61	116,36
		S.A.	86,09	117,39	142,61	135,65	158,26
	Hep.	A/g	10,24	11,22	11,82	11,41	11,58
		S.A.	9,57	13,91	15,65	13,91	14,78
22	Host	A/g	91,02	134,29	139,27	145,50	123,21
		S.A.	93,57	130,95	155,96	147,70	155,96
	Hep.	A/g	18,48	19,27	20,13	19,34	24,65
		S.A.	24,77	28,44	29,36	29,36	33,94
75	Host	A/g	67,62	87,21	83,06	82,19	82,19
		S.A.	74,71	96,55	95,11	95,54	103,16
	Hep.	A/g	12,71	13,17	12,65	12,72	13,16
		S.A.	20,68	24,14	22,99	22,99	25,86

Table 3.55 B Host activities as a % of Control Host.
Hepatoma activities as a % of Control Hepatoma.

			Control	B	C	BC	cAMP
0	Host	A/g	100	108,02	133,74	115,95	166,83
		S.A.	100	112,22	141,11	123,33	145,56
	Hep.	A/g	100	101,67	133,33	136,69	111,67
		S.A.	100	100,00	130,00	140,00	100,00
10	Host	A/g	100	131,39	167,10	145,98	125,25
		S.A.	100	136,36	165,66	157,58	183,84
	Hep.	A/g	100	109,48	115,42	111,41	114,05
		S.A.	100	145,45	163,64	145,45	154,56
22	Host	A/g	100	143,88	153,03	159,90	139,80
		S.A.	100	151,96	166,67	157,84	166,67
	Hep.	A/g	100	104,63	109,25	104,95	130,60
		S.A.	100	114,81	118,52	118,52	137,04
75	Host	A/g	100	128,97	128,63	122,84	122,33
		S.A.	100	129,23	127,31	126,54	138,08
	Hep.	A/g	100	103,61	99,51	100,00	103,61
		S.A.	100	116,67	111,11	111,11	125,00

A/g = Activity/g liver
S.A. = Specific Activity

Table 3.56 A Activities as % of control liver for ARG.

% Protein			Control	B	G	BG	cAMP
0	Host	A/g	79,75	105,48	111,38	102,56	124,00
		S.A.	87,52	114,57	122,04	112,73	123,25
	Hep.	A/g	0,29	0,27	0,26	0,28	0,27
		S.A.	0,54	0,56	0,47	0,46	0,54
10	Host	A/g	63,68	101,41	95,69	92,15	89,05
		S.A.	59,75	99,25	99,66	102,09	125,16
	Hep.	A/g	0,30	0,52	0,44	0,54	0,51
		S.A.	0,36	0,92	0,75	0,77	0,52
22	Host	A/g	91,36	109,95	115,23	109,53	115,23
		S.A.	93,96	119,80	117,77	115,84	127,27
	Hep.	A/g	0,83	0,75	0,81	0,87	0,85
		S.A.	1,01	1,10	1,10	1,10	1,10
75	Host	A/g	73,28	107,77	102,30	101,21	100,58
		S.A.	95,61	131,55	125,89	131,41	139,73
	Hep.	A/g	0,58	0,61	0,65	0,63	0,61
		S.A.	1,04	1,59	1,29	1,20	1,31

Table 3.56 B Host activities as a % of Control Host.
Hepatoma activities as a % of Control Hepatoma.

			Control	B	G	BG	cAMP
0	Host	A/g	100	135,22	141,44	130,24	157,46
		S.A.	100	130,91	139,44	129,80	140,82
	Hep.	A/g	100	94,25	91,92	97,29	97,15
		S.A.	100	102,12	85,57	84,10	99,59
10	Host	A/g	100	159,29	150,27	146,00	139,26
		S.A.	100	164,45	165,13	170,87	209,49
	Hep.	A/g	100	173,00	145,56	179,70	169,38
		S.A.	100	225,59	209,09	212,72	172,25
22	Host	A/g	100	119,25	127,22	119,79	127,23
		S.A.	100	127,50	125,34	123,29	135,45
	Hep.	A/g	100	90,08	97,32	104,98	102,11
		S.A.	100	104,97	105,51	109,65	112,43
75	Host	A/g	100	147,06	139,60	137,81	137,50
		S.A.	100	137,60	135,86	137,45	145,21
	Hep.	A/g	100	104,63	111,78	109,22	105,38
		S.A.	100	119,15	123,91	114,95	125,14

A/g = Activity/g Liver
S.A. = Specific Activity

The graphs of activities of urea cycle enzymes in control animals, hosts and hepatomas of rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in livers of animals maintained on 22% protein shows that the three liver types are under *similar* types of control with respect to diet since all three types of liver respond to diet, although the host and hepatoma are generally more responsive than control livers, the hepatomas being the most responsive (see figures 3.55 to 3.59).

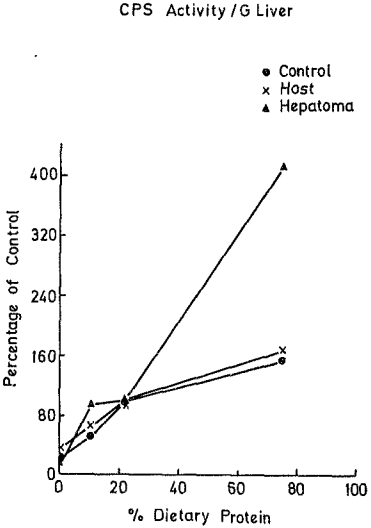


Fig. 3.55a Activity/g liver of CPS in control, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet.

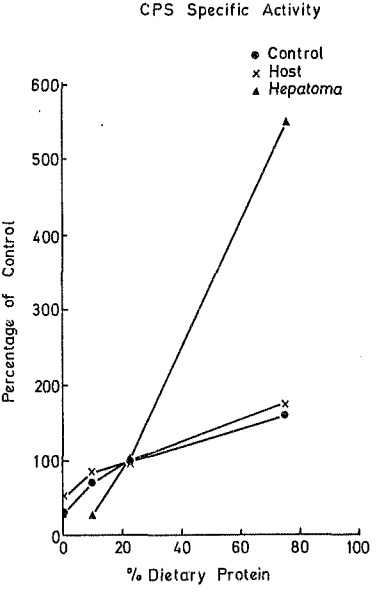


Fig. 3.55b Specific activity of CPS in control, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet.

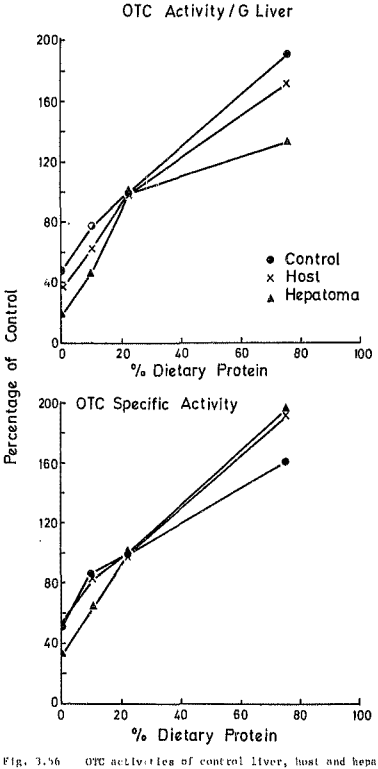
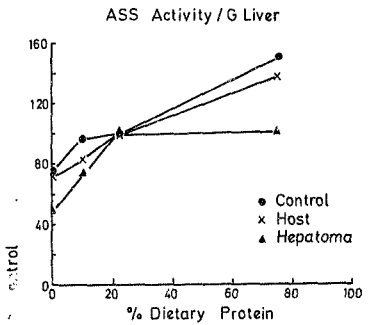


Fig. 3.56 OTC activities of control liver, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet.



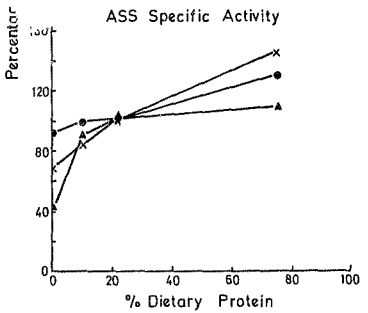


Fig. 3.57 ASS activities of control liver, host and hepatoma from rats maintained on 0%, 10% and 75% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet.

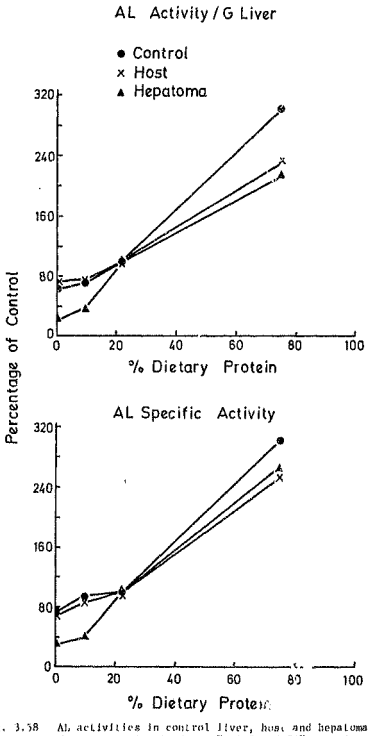


Fig. 3.58 AL activities in control liver, host and hepatoma from rats maintained on 07, 10% and 75% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet.

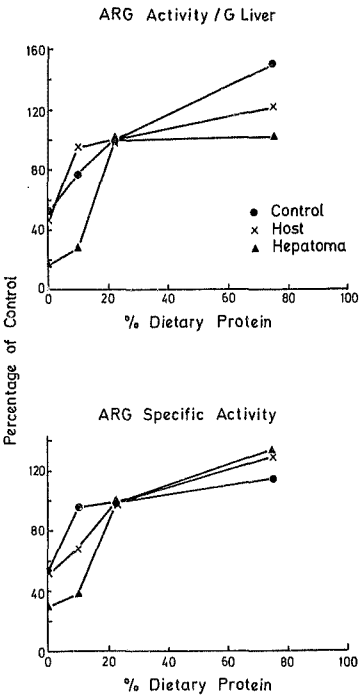


Fig. 3.59 Arginase activities of control, host and hepatoma from rats maintained on 0%, 10% and 22% protein diets expressed as a % of levels in livers of rats maintained on a 22% protein diet.

Table 3.57a

ANALYSIS OF VARIANCE FOR ACTIVITY/G LIVER

Source of Variation			Sum of Squares	DF	Mean Square	F	Significance of F
Main effects			2862000.00	13	220153.812	90.144	0.001
E			2362892.00	4	590723.000	241.878	0.001
Liv			2.219	2	1.109	0.000	0.999
TRT			0.712	4	0.178	0.000	0.999
Diet			73.160	3	24.387	0.010	0.999
2-Way Interactions			15128549.0	62	244008.812	99.912	0.001
E	Liv		1743880.00	8	217985.000	89.256	0.001
E	TRT		43660.215	16	2728.763	1.117	0.332
E	Diet		5024102.00	12	418675.125	171.431	0.001
Liv	TRT		11.095	8	1.387	0.001	0.999
Liv	Diet		43.593	6	7.265	0.003	0.999
TRT	Diet		1.102	12	0.092	0.000	0.999
3-Way Interactions			7164135.00	128	55969.805	22.917	0.001
E	Liv	TRT	187673.562	32	5864.797	2.401	0.001
E	Liv	Diet	3562810.00	24	148450.375	60.785	0.001
E	TRT	Diet	312247.625	48	6505.156	2.664	0.001
Liv	TRT	Diet	13.154	24	0.548	0.000	0.999
4-Way Interactions			622123.625	96	6480.453	2.653	0.001
E	Liv Diet	TRT	622123.625	96	6480.453	2.653	0.001
Explained			433233408.	299	1448941.00	593.283	0.001
Residual			5128704.00	2100	2442.24		
Total			438362112.	2399	182727.000		

By: E Enzyme
Liv Control, Liver or Tumour
TRT Application of E, G, etc
Diet Percent Protein

Table 3.57b ANALYSIS OF VARIANCE FOR SPECIFIC ACTIVITIES

Source of Variation				Sum of Squares	DF	Mean Squares	F	Significance of F
Main effects				162.285	13	12.483	80.197	0.001
E				132.284	4	33.071	212.456	0.001
Liv				0.001	2	0.001	0.004	0.999
TRT				0.001	4	0.000	0.001	0.999
Diet				0.003	3	0.001	0.007	0.999
2-Way Interactions				777.474	62	12.540	80.559	0.001
E	Liv			118.287	8	14.786	94.988	0.001
E	TRT			4.872	16	0.304	1.956	0.013
E	Diet			205.818	12	17.152	110.186	0.001
Liv	TRT			0.001	8	0.000	0.001	0.999
Liv	Diet			0.001	6	0.000	0.002	0.999
TRT	Diet			0.001	12	0.000	0.000	0.999
3-Way Interactions				357.338	128	2.792	17.935	0.001
E	Liv	TRT		10.282	32	0.321	2.064	0.001
E	Liv	Diet		172.737	24	7.197	46.238	0.001
E	TRT	Diet		23.518	48	0.490	3.148	0.001
Liv	TRT	Diet		0.001	24	0.000	0.000	0.999
4-Way Interactions				47.680	96	0.497	3.191	0.001
E	Liv	TRT	Diet	47.680	96	0.497	3.191	0.001
Explained				27717.145	299	92.699	595.524	0.001
Residual				326.887	2100	0.156		
Total				28044.031	2399	11.690		

By: E Enzyme
Liv Control, liver or tumour
TRT Application of B, C, etc
Diet Percent protein

Since the urea cycle enzyme activities were inducible in the host liver irrespective of the diet on which the rats were maintained, it seemed possible that the pattern of inducibility was independent of a nutritional effect of the hepatoma upon the host and suggested the possibility that some humoral factor was responsible.

In order to test the above the following experiment was conducted. All rats were maintained on an '8-16' feeding regime for one week prior to experimentation. Hepatoma-bearing animals and control nontumourous rats (35day old) were exsanguinated, the blood was spun down for 10 minutes at 2000 r p m in the presence of EDTA and the supernatant was preserved for further use.

Normal 35 day old animals were given a single intraperitoneal injection of plasma (2 ml/animal) from tumour or nontumour-bearing animals, or plasma plus glucagon (250 µg/50g) twenty four hours before sacrifice. All animals were then sacrificed at 10:00 hours and the hepatic urea cycle enzyme activities were assayed in the above groups of animals and compared with the activities in control, noninjected nontumour-bearing animals.

From tables 3.58a, b and c it can be seen that a single i.p. injection of plasma from nontumour-bearing animals does not result in a significant alteration in activities of urea cycle enzymes in 35-day old rats, nor are these enzymes inducible by glucagon under these conditions.

In contrast to the above, the enzymes studied all show a significant decrease in specific activity when measured and assayed in animals given a single 24 hour i.p. injection of plasma from tumour-bearing animals.

In animals given a single 24 hour i.p. injection of plasma from tumour-bearing animals plus glucagon there was a significant increase in the specific activities of the urea cycle enzymes as compared to activities in noninjected 35-day old controls (see fig. 3.60).

These results suggest that a humoral factor is present in the plasma of tumour-bearing animals and that this factor is capable of allowing induction by glucagon in animals where induction is normally not present.

Table 3.58a A comparison of Activities of urea cycle enzymes for control vs. plasma and plasma + glucagon treatment in non-tumourous rat livers. Rats were maintained on a 22% protein diet. The plasma was obtained from both tumour and nontumour-bearing animals.

	Activity/g Liver					Specific Activity				
	Control	Normal plasma	Hepatoma plasma	Normal plasma+ Gluc.	Hepatoma plasma + Glucagon	Control	Normal plasma	Hepatoma plasma	Normal plasma+ Gluc.	Hepatoma plasma + Glucagon
CPS										
Normal	5,146	5,127	5,804	5,701	5,130	0,0343	0,0393	0,0305	0,0334	0,0446
Protein	5,511	5,519	5,519	5,927	4,775	0,0394	0,0310	0,0335	0,0301	0,0415
	5,024	4,989	4,839	5,312	5,084	0,0345	0,0352	0,0265	0,0397	0,0598
	5,028	6,021	4,107	5,357	5,435	0,0372	0,0375	0,0304	0,0384	0,0453
	5,731	5,537	4,274	6,683	7,772	0,0342	0,0364	0,0342	0,0377	0,0648
	6,134	4,972	5,146	4,829	5,666	0,0396	0,0388	0,0332	0,0353	0,0472
	5,514	5,627	5,131	5,319	5,222	0,0395	0,0327	0,0332	0,0329	0,0746
	6,134	5,020	4,921	5,122	6,132	0,0373	0,0391	0,0303	0,0385	0,0721
Mean	5,453	5,320	4,967	5,281	5,652	0,0370	0,0363	0,0314	0,0358	0,0562
SE	0,163	0,130	0,202	0,146	0,336	0,0008	0,0010	0,0010	0,0010	0,0046
% of Control		97,56-	91,09-	99,27-	103,65-		98,11-	84,86-	96,80-	151,89-
		2,38	3,70	6,79	6,17		2,70	2,70	2,70	12,43
OTC										
	185,638	197,600	173,884	185,101	175,503	1,428	1,482	0,915	1,041	1,522
	196,025	152,001	174,670	130,114	199,880	1,508	2,125	1,059	1,804	1,738
	191,099	217,250	174,670	192,615	189,276	1,470	1,695	0,919	1,231	2,226
	194,214	225,710	158,856	199,890	187,320	1,494	1,568	1,176	1,683	1,561
	189,300	203,053	170,530	151,943	190,393	1,449	1,249	1,003	1,483	1,587
	188,259	187,292	177,239	224,076	191,038	1,457	1,369	1,043	1,379	1,592
	191,441	175,157	170,232	183,007	223,368	1,357	1,460	1,001	1,774	2,032
	190,676	180,079	173,246	197,033	190,842	1,223	1,421	1,020	1,370	2,245
Mean	191,463	189,710	171,666	182,982	193,728	1,423	1,518	1,017	1,471	1,812
SE	1,304	9,29	2,020	10,376	5,126	0,033	0,0920	0,002	0,096	0,108
% of Control		99,08-	89,66-	95,57-	100,94-		106,68-	71,47-	103,37-	127,37-
		4,85	1,04	5,42	2,68		6,47	1,00	6,75	7,59

Table 3.58bA comparison of Activities of urea cycle enzymes for control vs. plasma and plasma + glucagon treatment in non-tumourous rat livers. Rats were maintained on a 22% protein diet. The plasma was obtained from both tumour and nontumour-bearing animals.

	Activity/g Liver					Specific Activity				
	Control	Normal plasma	Hepatoma plasma	Normal plasma-Gluc.	Hepatoma plasma + Glucagon	Control	Normal plasma	Hepatoma plasma	Normal plasma-Gluc.	Hepatoma plasma + Glucagon
ASS										
	2,125	2,338	1,532	1,878	2,..38	0,0153	0,0160	0,0081	0,0163	0,0196
	1,987	2,078	1,079	2,091	?,166	0,0166	0,0143	0,0056	0,0141	0,0198
	1,518	1,683	1,231	1,344	,9	0,0116	0,0159	0,0065	0,0134	0,0205
	1,966	1,750	2,063	1,752	!	0,0160	0,0161	0,0109	0,0105	0,0258
	1,925	1,873	1,576	2,180	;	0,0149	0,0140	0,0093	0,0177	0,0183
	1,811	1,646	1,573	2,264	;	0,0140	0,0130	0,0094	0,0110	0,0172
	1,870	1,701	1,483	1,527	,9	0,0144	0,0120	0,0087	0,0153	0,0300
	2,208	1,797	1,646	1,984	1,790	0,0170	0,0157	0,0097	0,0156	0,0234
Mean	1,916	1,838	1,522	1,878	2,124	0,0150	0,0148	0,0085	0,0143	0,0218
SE	0,068	0,08-	0,103	0,113	0,151	0,0006	0, 1	0,0006	0,0009	0,0015
% of Control		96,9?-	79,44-	98,02-	115,55-		98,96-	56,67-	93,33-	145,33-
		4,334	5,38	5,90	7,88		6,67	4,00	6,00	10,00
AL										
	2,20-	2,143	2,477	2,013	2,768	0,0170	0,0148	0,0130	0,0230	0,0241
	2,497	2,029	2,560	2,051	3,294	0,0201	0,0159	0,0155	0,0190	0,0296
	2,603	2,627	2,514	2,331	2,477	0,0200	0,0176	0,0132	0,0161	0,0291
	2,286	2,500	2,114	2,029	2,596	0,0176	0,0164	0,0128	0,0166	0,0216
	2,346	2,261	2,097	2,071	2,431	0,0191	0,0190	0,0110	0,0184	0,0203
	2,119	2,135	2,265	2,847	2,718	0,0177	0,0185	0,013?	0,0188	0,0227
	2,323	2,258	2,315	2,736	2,649	0,0179	0,0179	0,0122	0,0151	0,0265
	2,305	2,485	2,119	2,468	2,551	0,0193	0,0200	0,0128	0,0171	0,0300
Mean	2,350	2,305	2,323	2,343	2,686	0,0185	0,0175	0,0130	0,0180	0,0236
SE	0,352	0,074	0,281	0,121	0,100	0,0005	0,0006	0,0005	0,0009	0,0020
% of Control		98,09-	98,85-	99,70-	114,29-		94,59-	70,27-	97,30-	127,57-
		3,15	11,96	5,10	4,25		3,24	2,70	4,86	1,08

Table 3.58c A comparison of Activities of urea cycle enzymes for control vs. plasma and plasma + glucagon treatment in non-tumourous rat livers. Rats were maintained on a 22% protein diet. The plasma was obtained from both tumour and nontumour-bearing animals.

	Activity/g Liver					Specific Activity				
	Control	Normal plasma	Hepatoma plasma	Normal plasma + Gluc.	Hepatoma plasma + Glucagon	Control	Normal plasma	Hepatoma plasma	Normal plasma+ Gluc.	Hepatoma plasma + Glucagon
ARG										
	1094 771	1027,889	1139,723	1198,243	1238,694	9,418	9,897	6,283	9,003	10,771
	1345,210	1058,891	1121,873	1146,142	1732,314	10,348	9,253	6,800	9,792	15,066
	1245,029	1215,840	1032,245	1321,690	1173,559	9,577	9,756	5,433	8,524	13,806
	1241,821	1299,539	1134,233	1171,355	1356,240	9,553	9,439	6,874	9,165	11,302
	1005,194	1110,018	1121,873	1237,713	1333,404	7,732	9,670	5,905	9,792	11,112
	1268,552	1150,014	1324,830	1238,588	1458,642	10,571	9,125	8,029	9,368	12,156
	1222,482	1067,196	1239,692	1110,359	1399,042	9,608	9,823	6,525	9,957	13,990
	1110,042	1192,242	1173,595	1297,066	1453,001	9,539	9,566	7,113	9,548	17,094
Mean	1177,676	1140,201	1161,008	1215,140	1393,112	9,543	9,566	6,620	9,394	13,162
SE	130,053	32,563	31,053	25,727	39,753	0,299	0,973	0,279	0,170	0,785
% of Control		96,82-	98,58-	103,18-	118,29-		100,24-	69,37-	98,44-	137,92-
			3,17	2,15	3,07		10,20	2,92	1,78	8,23

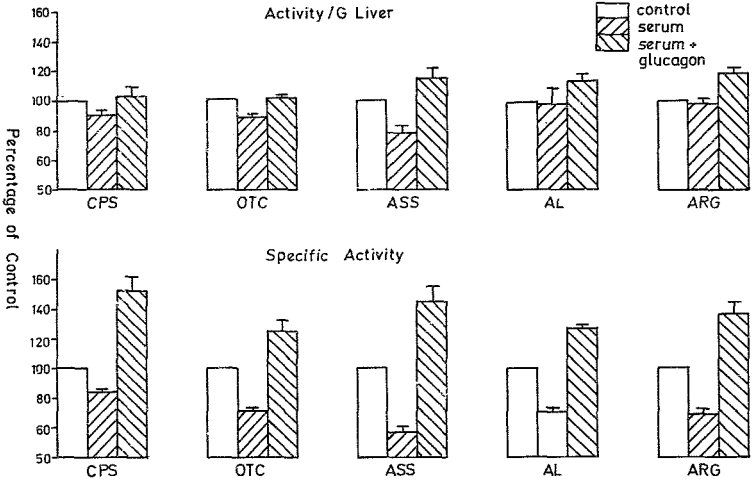


Fig. 3.60 A comparison of activities of urea cycle enzymes from control, serum and serum plus glucagon-treated animals. Rats were maintained on a 22% protein diet.

SECTION 4 - DISCUSSION

4.1 ASSAY SYSTEMS

Before attempting to draw any conclusions from the results it must be stressed that the results obtained do not reflect true activities of the urea cycle enzymes in vivo. Assay conditions are ideal and do not take into account the possibility that inhibitors and other factors influencing enzyme activities may be present in the cells but absent from the assay system and that dissociation and association of the enzyme subunits may be different in vivo as compared to these test tube assay conditions.

The urea cycle enzyme activities as determined by the methods of Balinsky et al. (1972) and Wixom et al. (1972) compare well with those of most other workers (Ratner, 1953; Schimke, 1964; Balinsky, 1969) but are lower than those determined by Nuzum and Snodgrass, (1971) with the exception of OTC which is higher in our determinations.

The fact that OTC activity is lower in Nuzum and Snodgrass's assay method can be explained by the fact that they assayed at pH 7,7 (unpublished) to reduce the background colour in the citrulline determination. We have overcome this problem by including reagent blanks, as described, to correct for the background absorbance.

It is well accepted that the active species of ornithine in the OTC reaction is the zwitterion which constitutes about 10% of total ornithine at neutral pH (Ratner, 1973). Snodgrass (see Ratner, 1973) showed that the Km for ornithine decreased with decreasing pH. Since the present assay was performed at pH 8.3 (the pH optimum) there may have been a greater proportion of ornithine available as the active zwitterion thereby accounting for the higher activity observed.

The assay method of Wixom et al. (1972) for ASS suffers from a drawback since it does not measure directly the amount of product formed but rather the amount of substrate used up in the formation of product. On the other hand, it does not depend on supplementation in ASL. Even though argininesuccinate may accumulate, its concentration is not sufficient to inhibit the reaction (Ratner, 1955). The reaction measured is linear with time (Matthewse,1977). The analytical errors due to subtraction and redrawing of calibration

curves have been minimized by the use of the computer program for drawing the calibration curve and determining enzyme activities.

Activities for all enzymes computed by hand compare very well with those computed by the program with a 5% error margin. The computerized method is probably more accurate since the graph drawn is the best possible fit to the points plotted whilst those drawn by hand are more of an estimation.

The interference of urea present in the citrulline determination of the ASS assay procedure has been minimized by the addition of urease to the assay mixture. This degrades all urea present and formed from zero time until the assay is terminated.

Arginase is assayed at its pH optimum of 9,5. This is far from the physiological pH where its activity is greatly decreased but still greater than that of the rate limiting enzyme ASS (Ratner, 1955).

The other differences observed may be due to specific differences in animals used and perhaps to dietary protein content since urea cycle enzyme activities are very sensitive to dietary protein levels.

4.2 TIME-DEPENDENCE OF UREA CYCLE ENZYME ACTIVITIES

The activities of ASS, AL and arginase were measured at three hourly intervals around the clock and it was found that the activities do not vary significantly with the time of day (see tables 3.1a - 3.2c, fig 3.1)

The results suggest that ASS, AL and arginase do not display a circadian rhythm similar to that of other hepatic enzymes such as tyrosine aminotransferase (Wurtman, 1969) when maintained on an 8-16 feeding regime. These results are consistant with results for CPS found by Shephard (1975).

There does, however, appear to be a time-dependence in inducibility by glucagon in rats trained to the feeding regit When given a single injection of glucagon (250 mg/ 50g rat) 24 hours before sacrifice, inducibility was apparent, reaching a maximum two hours after the onset of darkness, when rats have been feeding for two hours (see fig 3.1). These findings are consistant with those of Shephard (1975) who measured time-dependence of CPS inducibility by glucagon.

Several permissive effects of glucocorticoids for the action of glucagon and cAMP are known and are reviewed by Wicks (1974) and Thomson and Lippman (1974). For example it is believed that glucocorticoids may inhibit phos diesterase and in this way spare cAMP since in cultured ' itoma cel. dexamethasone (1mM) has been shown to inhibit phosphodiesterase with a concomitant increase in cAMP levels being measurable (Manganiello and Vaughn, 1972). Also, Moshino et al. (1975) found that injection of a physiological dose of cortisone acetate restores the reduced response of TAT to dBcAMP in adrenalectomized rats.

Given et al. (1967) found that r ts adapted to controlled feeding regimes show marked cyclic variations in the circulating corticosterone levels. Wurtman and Axelrod (1967) found that glucocorticoids peak at about two hours before the onset of feeding. It is therefore possible that the time-dependence of inducibility by glucagon is actually related to the levels of circulating glucocorticoids which may be permissive for glucagon in this instance.

Wicks (1974b) has suggested that in certain cases of enzyme induction, cAMP and the steroids may influence the same process but that in the absence of steroid, cAMP would exert a reduced effect.

The rhythm seen in inducibility of urea cycle enzymes by glucagon is probably related to feeding as is the circadian rhythm of TAT (Wurtman, 1969) and especially to tryptophan concentrations. The rate of amino acid uptake is influenced by the adrenocortical steroids and since these show daily rhythms they may generate rhythms in tryptophan concentrations.

Tryptophan has a special significance in control of hepatic protein synthesis and if it is omitted from the diet there is a reversible disaggregation of hepatic polysomes as well as the loss of circadian rhythm normally seen in TAT activities (Pronczuk et al, 1968).

Tryptophan also plays a special role in the induction of urea cycle enzymes. Forced feeding of tryptophan has the same effect on increasing urea cycle enzyme activities as does feeding a high protein diet (Muramatsu and Nakagawa, 1971).

If polysome aggregation stimulates protein synthesis of messengers already available and tryptophan stimulates polysome aggregation, then increasing tryptophan concentrations (due to increasing glucocorticoid levels) may induce the synthesis of urea cycle enzymes. These nascent polypeptide chains could be released more rapidly from the polysomes by the presence of cAMP which would act as an inducer by allowing more messenger to become available for translation.

It would be interesting to see if this time-dependence of inducibility is abolished with adrenalectomy. Glucagon increased the activity of CPS irrespective of the protein content of the diet and it seems therefore, that effects of diet and glucagon are independent of each other (Shephard, 1975).

4.3 AGE DEPENDENCE OF INDUCIBILITY

As can be seen from tables 3.3 and 3.4 all five of the urea cycle enzymes are inducible in 28 but not in 35-day old rats (see figs 3.2 and 3.3). If glucocorticoids are permissive for induction of the urea cycle enzymes by glucagon at 28 days, it is conceivable that lack of sensitivity of urea cycle enzymes to glucocorticoids at 35 days would result in a lack of sensitivity to cAMP at the same age.

Several workers have found that after day 29 postnatally, there is no significant increase in arginase activity with a single injection of hydrocortisone (Schimke 1963, McLean and Gurney, 1963). Schimke (1963) found that in adult rats three daily injections of large doses of cortisol (25 mg/100 g) were required to cause an increase in the activities of urea cycle enzymes in proportion to an increase in urea excretion. He suggested that the slow response may be due to the relatively long half life of these enzymes.

Shephard (1975) found that in 28-day old rats a single i.p. injection of glucagon (250µg / 50g) caused a 40-60% increase in CPS activity within twenty four hours in animals maintained on a controlled feeding regime. McLean and Novello 0965)observed a 30% increase in CPS activity but only when rats were given three daily doses of glucagon (100µg/dose) before sacrifice but these rats were fed ad libitum and the lighting was not controlled. The induction of CPS by glucagon in young rats was independent of diet since the increase was seen in the rats maintained on a 10%, 22% or 75% protein diet (Shephard, 1975).

Christowitz (1975) also found that in adult rats daily injections of cortisol were required to demonstrate a significant increase in activity of the urea cycle enzymes and that in adults rats the change was no longer coordinated for all of the enzymes

since AL behaved in an anomolous way to the other enzymes.

Hart (1977) found a significant age-dependence of cortisol inducibility of arginase and ASS in three to nineteen day old rats From days three to fifteen there is a significant increase in the activities of these two enzymes with a single twenty four hour injection of hydrocortisone hemisuccinate (20µg/100g). At days 17 and 19 there is no inducibility and the periods beyond this age were not studied.

It has been suggested that several critical periods of development occur and if the environment is interfered with at these critical stages it is possible to alter the characteristics of the individual in such a way that further development is altered (Scott, 1962). The first critical period occurs in the first postnatal week (Denenberg, 1965) and the weaning period which occurs between the second and fourth weeks also belong among the critical periods (Krček and Palatý, 1967). Premature weaning of the rats results in altered characteristics of the adult, the alterations including changed reproductive function, impairment of fertility, altered regulation of the oestral cycle and an inhibition of the adrenal glands. The adrenals of prematurely weaned rats produce less corticosterone than normally weaned rats and the effect is mediated by the pituitary gland (ibid). If these critical periods do exist, then the sensitivity of urea cycle enzymes to glucocorticoids could alter after day 28 postnatally when weaning is completed and the urea cycle enzymes have reached adult levels.

In adult rats glucocorticoids are required for the maintenance of the basal level of urea cycle enzymes since in adrenalectomized adult rats there is an 80% drop in arginase activity and a 30% drop in ASS, CPS, AL and OTC compared with controls (Shimke, 1963; McLean and Gurney, 1963).

Hence in adult rats the urea cycle enzymes must be responding to glucocorticoids in a way different to that in young rats. After entry into the cell the glucocorticoid interacts with a specific receptor, the resulting complex is then transported to the nucleus, where the steroid-receptor interacts with the chromatin at a site called the nuclear-acceptor. Tsai and Hnilica (1971) found that a

small amount of cortisol binds to a trypsin-resistant fraction of the arginine-rich histones. They therefore suggest that cortisol probably binds to a non-histone protein associated with the arginine-rich histones. The possibility exists that in adult rats the nuclear-acceptor has such a strong affinity for the steroid-receptor complex that under normal conditions the urea cycle enzymes are maximally induced. If however, the basal steroid level drops, as would occur in adrenalectomy then a) not all receptors would be bound to steroid and b) the nuclear-acceptor sites could not be occupied by the steroid-receptor complex and synthesis of new messenger RNA for the urea cycle enzymes would be decreased.

It is well known that dietary induction of urea cycle enzymes is independent of intact adrenals (Shimke, 1963), so the fact that the enzymes are in a state of maximal induction by glucocorticoids need not affect their inducibility by dietary protein as these effects are independent of each other.

The significant increase in arginase activity in adults following three daily injections of hydrocortisone could be due to increased protein catabolism with the release of free ammonia for detoxification which could in turn induce the urea cycle enzyme levels to increase, namely, mediated though a dietary effect. The reverse postulate, that protein diet acts through affecting glucocorticoid levels is not true since the dietary affect is not dependent on the presence of cortisol.

It is conceivable that the nuclear-acceptor site of the steroid-receptor complex at particular loci in the liver cell nuclei can alter with age since this receptor is a nonhistone protein, found in the AP_3 fraction (O'Malley and Schrader, 1976), and nonhistone protein patterns do change with differentiation. Also, not all the acceptor sites are identical since particular steroid-receptor complexes bind preferentially to chromatin from target tissue and not to other chromatin (ibid).

It is possible that the altered response to cAMP in adult rats is due to an alteration in the polysomes in the adult. A similar phenomenon is seen for TAT inducibility by cAMP in vitro (Donovan and Oliver, 1972).

These workers have isolated the microsomal factor responsible for the release of newly synthesized polypeptides from polysomes and found it to be a protein of molecular weight 40 000, which binds cAMP with an association constant of $4 \times 10^9 M^{-1}$ and which possesses cAMP-dependent protein kinase activity.

They have found this release factor to be present in adult liver, but it does not release TAT protein from adult polysomes. Therefore, they predict that some alteration occurs in the polysomes during development.

It seems likely this change must be in the messenger RNA since ribosomes are non-specific and do not alter with age. At least two possible models can explain this phenomenon and are depicted in the following two models (see pages 285 and 286).

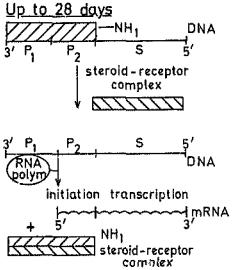
The mechanism depicted in the first model is not inconsistent with the fact that in phage ØX174 two pairs of genes are coded for by the same region of DNA by using different reading frames. Hence this is a known example of a promoter region being transcribed although it is in a prokaryotic and not a eukaryotic organism (Sanger et al, 1977).

Examples similar to that shown in the second model are known. For example, the σ factor of E. coli alters the site of initiation of transcription by RNA polymerase (Burgess et al., 1969) and different T_4 factors are known to alter the site of transcription by E. coli RNA polymerase (Politzer et al., 1970).

The possible existance of either of the mechanisms depicted could be tested in a number of ways, one of which is as follows:- Several of the urea cycle enzymes have been highly purified including CPS (Shephard 1975) and arginase (Peiser, personal communications). By injecting highly purified enzyme into rabbits, specific antibodies can be synthesized. These antibodies could then be used in an affinity chromatography column to isolate from total liver polysomes nascent growing enzyme chains attached to the polysome. Once eluted from the column the mRNA could be separated from the polysome by disaggregation followed by purification. Messenger RNA for a particular enzyme could be isolated in this way from both adult and juvenile rats and compared for base composition and to see if the primary sequences do in fact differ, especially at the $5'$ end. These types of experiments are being done (Sala, personal communications) and although difficult, the above models should be testable up to this point.

MODEL 1

Up to 28 days



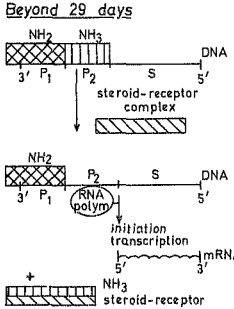
NH_1 = non histone chromasomal accentor protein for steroid-receptor complex present up to 28 days

P_1 = RNA polymerase recognition and promotor site with greater binding affinity for RNA polymerase than P_2 a second promotor site.

S = structural gene

When the nuclear acceptor and steroid-receptor complex interact, the Chromatin becomes available for transcription. RNA polymerase attaches preferentially to P_1 and transcription is initiated at the 3' end of P_1, the transcript formed including P_2 which could then be the 5' end of the messenger specific for cAMP induction

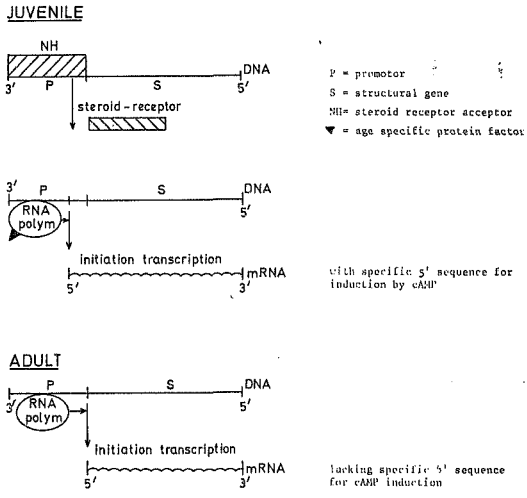
Beyond 29 days



NH_2, NH_3 = new non histone species with NH_3 being the new nuclear-acceptor for steroid-receptor binding.

The mRNA produced beyond 29 days lacks the specific 5' sequence and is no longer inducible by cAMP.

MODEL 2



MODEL 2

Model 2 predicts only one promotor region but an age specific factor is present which attaches to the RNA polymerase and alters the point of initiation on the DNA template such that up to 28 days the mRNA has a unique 5′ terminus, absent [illegible] of age when this protein factor is absent.

Several other possibilities do exist to explain the lack of inducibility by glucagon in older rats, an example of which is that an age-dependent regulatory protein which binds cAMP and controls the rate of translation may be absent in adult rats but present in juvenile rats. Therefore adult rats are non-inducible by glucagon but juvenile rats are.

4.4 REGENERATION AFTER PARTIAL HEPATECTOMY

The pattern of change seen in the urea cycle enzymes after partial hepatectomy of rats maintained on a 22% protein diet is not inconsistant with the molecular correlation theory. The urea cycle enzyme activities vary in a concerted way, showing an initial significant nadir at four hours postoperatively but have returned to control levels by 24 hours (see figures 3.12 to 3.16).

These results do not agree with those of other workers, most of whom have only measured OTC activity after partial hepatectomy. Bhide (1971), Weber et al. (1972), Fausto et al. (1975) and Jänne and Hölttä (1975) have all been unable to measure a significant drop in OTC activity following partial hepatectomy, although they have all predicted that such a drop should occur. Bhide (1971) was able to show a 10% decline in OTC activity by 72 hours and Weber (1972) showed a 17% drop by 24 hours after the operation.

The discrepancy between their results and the present ones may be due to the fact that these rats were maintained on controlled feeding and lighting regimes which may have resulted in the greater synchrony in alterations in activity and so made the drop in activity *apparent* and *significant*.

Schulte-Herman (1975) has stressed the importance of the feeding regime in the appearance of DNA synthetic rhythms in rats maintained on different regimes with ad lib. versus controlled feeding. If the urea cycle intermediates (carbamyl phosphate and aspartate) are being shunted to pyrimidine and purine biosynthesis and finally to nucleic acid synthesis, then it would not be inconsistent with the molecular correlation theory that the urea cycle enzymes show a correlated decrease. This decrease has been observed and may also depend on controlled feeding and lighting regimes for their appearances.

Barbirolli and Potter (1971) found that the pattern of DNA synthesis following partial hepatectomy is dependent on controlled feeding regimes. The first peak which appears at 23 hours is independent of the time of day, but the second peak which appears between 65 and 72 hours is dependent on and follows the stimulus of the controlled feeding schedule.

One of the earliest responses measurable after partial hepatectomy is the rapid increase in activity of ornithine decarboxylase (Hölltä and Jänne, 1972), the enzyme catalyzing the rate limiting step in polyamine biosynthesis from ornithine (Tabor and Tabor, 1972).

Since ornithine is also utilized in urea biosynthesis it is expected that OTC activity would show a correlated decrease with the increase in ODC activity. This does in fact occur, and since it would be uneconomical for just one enzyme in a pathway to drop in activity with a decrease in substrate availability, one would expect that all the urea cycle enzymes decrease in activity in a concerted way, which is in fact the case (*see tables* 3.13a *to* 3.17a).

Teleologically speaking, it is impossible for the cell to do everything that is required for regeneration. One of the earliest requirements is for the synthesis of ODC. The building blocks must be supplied from somewhere and it is possible that early on in regeneration the synthesis of several enzymes, including the urea cycle enzymes are switched off. Later the synthesis would have to be turned on since detoxification of free ammonia is essential for the survival of the animals and so the urea cycle enzyme activities cannot drop below some critical minimum.

Glucocorticoids are well known inhibitors of nuclear DNA synthesis (Kim and Kim, 1970; Pitot and Yatvin, 1973) and DNA polymerase and liver growth are inhibited by glucocorticoids in partially hepatectomized rats (Thomson and Lippman, 1974).

Nesser-Wiest (1975, 1976) showed that immediately after partial hepatectomy there is an increase in corticosterone levels. The peak occurs at four hours postoperatively and is then followed by a drop and a subsequent second increase between 15 and 18 hours. The first rise is apparently due to a decrease affinity of the corticosterone-binding protein in the serum for

Barbirolli and Potter (1971) found that the pattern of DNA synthesis following partial hepatectomy is dependent on controlled feeding regimes. The first peak which appears at 23 hours is independent of the time of day, but the second peak which appears between 65 and 72 hours is dependent on and follows the stimulus of the controlled feeding schedule.

One of the earliest responses measurable after partial hepatectomy is the rapid increase in activity of ornithine decarboxylase (Hölltä and Jänne, 1972), the enzyme catalyzing the rate limiting step in polyamine biosynthesis from ornithine (Tabor and Tabor, 1972).

Since ornithine is also utilized in urea biosynthesis it is expected that OTC activity would show a correlated decrease with the increase in ODC activity. This does in fact occur, and since it would be uneconomical for just one enzyme in a pathway to drop in activity with a decrease in substrate availability, one would expect that all the urea cycle enzymes decrease in activity in a concerted way, which is in fact the case (*see tables 3.13a to 3.17e*).

Teleologically speaking, it is impossible for the cell to do everything that is required for regeneration. One of the earliest requirements is for the synthesis of ODC. The building blocks must be supplied from somewhere and it is possible that early on in regeneration the synthesis of several enzymes, including the urea cycle enzymes are switched off. Later the synthesis would have to be turned on since detoxification of free ammonia is essential for the survival of the animals and so the urea cycle enzyme activities cannot drop below some critical minimum.

Glucocorticoids are well known inhibitors of nuclear DNA synthesis (Kim and Kim, 1970; Pitot and Yatvin, 1973) and DNA polymerase and liver growth are inhibited by glucocorticoids in partially hepatectomized rats (Thomson and Lippman, 1974).

Desser-Wiest (1975, 1976) showed that immediately after partial hepatectomy there is an increase in corticosterone levels. The peak occurs at four hours postoperatively and is then followed by a drop and a subsequent second increase between 15 and 18 hours. The first rise is apparently due to a decrease affinity of the corticosterone-binding protein in the serum for

corticosterone. This results in the release of bound corticosterone into the serum, thereby increasing its activity. It was therefore concluded that during the early hours of regeneration corticosterone blocks the liver cells in G1 of the mitotic cycle and an accumulation of cells at this stage of division occurs. After the early rise in corticosteroids there is an increase in the affinity of the serum corticosterone-binding protein for its substrate and later the cells enter the S-phase synchronously during which time most of the DNA is replicated (ibid.).

Degradative processes may play a role in altering urea cycle enzyme levels during the early hours of regeneration. Examples have been documented where the half-life of the urea cycle enzymes change with changing physiological conditions. For example, changing rats from a high to a low protein diet results in a change from five days to three days in the half-life of arginase. When changed from a 90% protein to a zero protein diet, the half-life of arginase changed to one day (Schimke, 1964; Szepesi and Freedland, 1969). If the half-life of arginase is as low as seven hours, as determined by Das and Waterlow (1974) the order of decrease in urea cycle enzyme activities after partial hepatectomy is easily explained merely by the switching off of synthesis with degradation proceeding as usual. If the half-life of these enzymes is greater than seven hours, then a faster drop could be obtained by a combination of decreased synthesis and increased degradation as occurs in the switch from high to low protein diets (Schimke, 1964).

Since the peak in corticosteroid levels coincides with the minimum in the activity of the urea cycle enzymes at four hours following partial hepatectomy, it could be possible that the increased corticosteroid levels control in some way, the degradative or synthetic processes involved in controlling urea cycle enzyme activities. Following the minimum at four hours there is an increase in the urea cycle enzyme activities, the levels reaching a maximum between 18 and 24 hours post-operatively, this maximum proceeding the second peak in corticosterone activity at between fifteen to eighteen hours by about two hours. It is therefore possible that the rise in activity after the nadir is controlled in some way by the increasing corticosteroid levels.

The drop at four hours is not due to some nonspecific effect caused by shock or stress since the sham operated animals show no significant change in urea cycle enzyme activity (see table 3.22a,b).

4.4.1 Effect of inhibitors of RNA synthesis on urea cycle enzyme activities in regenerating rat liver.

Treatment of hepatectomized rats at zero time with actinomycin D (AMD) and cordecepin resulted in "superinduction" of the urea cycle enzyme activities above control values after ten hours of regeneration (see tables 3.23a to 3.36b and figures 3.21 to 3.24).

Actinomycin D is a potent inhibitor of DNA-directed RNA synthesis and RNA chain elongation (Egyhazi, 1974). With labelling experiments it can be shown that most RNA remains in the nucleus and that no transfer of rapidly labelled RNA to the cytoplasm occurs. It has also been shown that the half-life of messenger RNA from liver cells is prolonged and the decay time of mRNA from Hela cells is considerably shortened in the presence of AMD. With chase experiments it was shown that there is a great decrease in the labelling of heteronuclear RNA (HnRNA) with AMD treatment. There is no increase in either nuclear or cytoplasmic labelling of RNA and practically no labelled, high molecular weight mRNA moves from the nucleus to the cytoplasm in the presence of AMD (ibid).

The phenomenon of superinduction has been observed in a number of inducible systems including the induction of TAT by hydrocortisone (Lee et al., 1970), and has been explained as follows. AMD is believed to block the synthesis of a labile, post transcriptional repressor molecule, a putative molecule preventing maximal expression of potential mRNA of TAT. When the steroid binds with its receptor the synthesis of the repressor is inhibited, following which at four hours after hormone treatment, the repressor is again synthesized resulting in inactivation of TAT-specific mRNA. In the presence of AMD the repressor molecule cannot accumulate, resulting in an increase in functional mRNA for TAT and hence "superinduction" of the enzyme (Tomkins et al., 1969).

This view is not held by everyone however. There is an alternative view, namely, that there is a decreased rate of turnover of proteins in rats treated with AMD due to the rapid rate of turnover of a proteolytic enzyme (Kenney, 1970; Lee et al., 1970). Another possibility exists and that is that there is a decrease in the rate of turnover of mRNA in the presence of AMD. Hence it could be spared for further translation.

A similar explanation could hold for the anomalous effect seen in urea cycle enzymes following regeneration in rats treated with AMD at zero time. If the urea cycle enzyme activities are dropping due to increased degradation by proteolysis, treatment of the rats with AMD could prevent the synthesis or accumulation of proteolytic enzymes and so spare the urea cycle enzyme molecules. If a similar repressor molecule as postulated to be involved in TAT mRNA inactivation is involved in the inactivation of urea cycle enzyme-specific mRNA, then in the presence of AMD, there would be an increased accumulation of functional messenger of urea cycle enzymes and so new urea cycle enzyme molecules could be synthesized. This could result in accumulation of urea cycle enzyme protein above control levels and so account for the anomalous effect seen.

Since in the presence of AMD, there is inhibition of the drop in activity at four hours, this argues in favour of the possibility of increased degradation of the urea cycle enzymes in regenerating liver by a labile proteolytic enzyme of short half-life. The anomalous effect seen could be due to the combined effect of a lack of degradation, thereby sparing the protein molecules, plus the later rise in activity which could possibly be due to the second peak in corticosteroid level namely an induction effect resulting in an overall increase in the activity of these enzymes above control values

In rats treated with cordecepin at the time of partial hepatectomy, there is a significant increase in the activity of urea cycle enzymes above the levels in untreated regenerating liver and in some instance above control nontreated, non-regenerating livers (see tables 3.27a to 3.3ab and figures 3.25 to 3.28).

Cordecepin (3-deoxyadenosine) is believed to inhibit transcription by being incorporated into nascent RNA chains and causing premature termination of these polymers (Siev et al., 1969; Penman et al., 1970). Other workers believe that it blocks mRNA biogenesis by the inhibition of the post-transcriptional addition of the poly-adenosine

tail to HnRNA (Darnell et al., 1971). Cordecepin prevents the formation of complete mRNA molecules, completely inhibits mitochondrial transcription, suppresses the labelling of mRNA but not HnRNA in the nucleus and does not effect transport of either from the nucleus to the cytoplasm. The fact that nucleolar RNA synthesis is more strongly affected than nucleoplasmic RNA argues for the fact that more than one type of RNA polymerase enzyme exists.

Since cordecepin also inhibits RNA synthesis, the same explanation could hold for the anomalous effect seen in the urea cycle enzymes in regenerating rat liver in its presence, as in the presence of AMD.

Cycloheximide inhibition of protein synthesis was attempted in the present study, but the mortality rate in partially hepatectomized rats was very high. Decreasing cycloheximide dosage to the minimum required for inhibition of protein synthesis did not decrease the mortality rate of the rats. Therefore the effect of cycloheximide on urea cycle enzyme activities during regeneration could not be studied.

4.4.2 Dietary effects on activities of urea cycle enzymes in regeneration

The effect of protein deficiency on the regenerative process seems to be one of retardation. Protein deficiency slows down the number of cells moving to the mitotic cycle without affecting RNA synthesis and its movement from the nucleus to the cytoplasm (Stirling et al., 1973). High protein in the diet has the opposite effect, namely, it speeds up the proliferative response. In fact the greater the proportion of protein in the diet, the greater is the induction of hepatic DNA synthesis (Short et al., 1975).

A change from low to high protein diet in rats after partial hepatectomy results in an increase in the number of parenchymal cells in mitosis. Isoleucine, threonine, valine, lysine, methionine and tryptophan are essential for the stimulation of DNA synthesis by protein or amino acids (Short et al., 1975).

The urea cycle enzyme activities show an initial increase in activity after partial hepatectomy in rats maintained on a ten or zero percent protein diet, both of which are protein deficient diets. The enzyme activities begin to drop by 15 hours post operatively,

reaching a minimum by 18 hours. Hence there is a 14 hour lag in the nadir of these enzyme levels in rats maintained on these protein deficient diets as compared with rats fed a normal 22% protein diet. It may be that the turnover rate of these enzymes is slowed down as an adaptation to the lack of amino acids in the diet for the regenerative process (see figs 3.4. to 3.11).

The possibility exists that in the liver after partial hepatectomy starvation activates stress pathways, resulting in the release of cortisol from the adrenal cortex, which may then increase the basal level of urea cycle enzymes as well as inhibiting the mitotic index (Stirling et al., 1973). A lack of amino acids in the diet could retard the synthesis of proteolytic enzymes, with rapid turnover rates, and this could slow down the turnover of the urea cycle enzymes.

A further possibility exists, and that is that when rats are maintained on protein deficient diets, the amino acids required for the regenerative process be supplied by catalysis of non-essential body proteins. The amino acids so supplied would then pass into the liver via the blood stream. An increased serum amino acid level could possibly activate the synthesis of the urea cycle enzymes in a dietary-type response and so account for the initial increase in their activity during the early hours of regeneration in rats maintained on protein deficient diets.

In rats maintained on a 75% protein diet the reverse is true, namely the urea cycle enzyme activities drop rapidly, reaching a minimum by four hours post operatively, but return to control values by between eight and twelve hours, while in rats maintained on a 22% protein diet, control values are only attained between 16 and 24 hours post operatively. Hence it appears that the amount of protein in the diet plays an important role in the rate at which the liver regenerates after partial hepatectomy (Short et al., 1975) and that the urea cycle enzyme activities alter in a concerted manner as do their rates of turnover (see figures 3.17 to 3.20).

The replacement of proteins through continual synthesis and degradation provides a mechanism for changing enzyme levels with alterations in the rate of degradation as well as altered rates of synthesis. The levels of urea cycle enzymes in rat liver may be considered to represent a balance produced by the process of synthesis and degradation, a balance that is consistantly shifting depending on the physiological requirements of the animal.

4.5 UREA CYCLE ENZYME ACTIVITIES IN HEPATOMA

The urea cycle enzymes in DAB induced hepatomas behave in a way that is not inconsistant with the predictions of the molecular correlation theory, viz. they show a decrease in activity correlated with increased activity of related anabolic pathways.

Weber (1966) found that tumour cells can synthesize protein and nucleic acid at increased rates, while amino acid catabolism decreases as compared with that of normal liver. In order to adapt to the requirements for rapid growth there must be an imbalance in the key enzymes of various opposing and competitive metabolic pathways in the tumour.

The relationship between urea, polyamine and pyrimidine biosynthesis has been discussed but it is of importance to point out that ODC activity increases in parallel with an increase in tumour growth rate (Williams-Ashman et al., 1972), as do the activities of aspartate transcarbamylase and dihydroorotase, the first two enzymes involved in orotate utilization to form pyrimidines (Weber, 1972).

Several workers have previously shown that OTC activities decrease in parallel with increased tumour growth rate, in some instances dropping to less than one percent of control levels. Arginase activities are depressed in hepatomas (Fujiwara, 1929; Weil, 1935; Greenstein, 1941) as are ASS and AL activities (Wu et al., 1967). "S also shows an inverse relationship with growth rate (al, 1975).

He control of urea cycle enzyme activities in neoplasia is important as the intermediates of the cycle are required for certain anabolic processes essential to growth. Ornithine may be shunted via ODC to polyamine biosynthesis; carbamyl phosphate may be channelled to pyrimidine biosynthesis and aspartate to both purine and pyrimidine biosynthesis.

Arginase has been implicated as the protein that inhibits cell growth in vitro (Bach and Simon-Reuss, 1953; Sorof et al., 1967; Holley, 1967). If this is true it would be detrimental to the tumour if arginase activity were increased since an increase in arginase activity could cause a drop in the growth rate.

Bach and Swaine (1965) found that intraperitoneal injections of highly purified arginase over a period of four days resulted in a 31-77% drop in growth of Walker carcinomas. These authors suggested that the presence of arginase in the intracellular spaces may set up an "arginine gradient"which could lead to a depletion of arginine from the tumour tissue with a resulting impairment in tissue growth.

Arley et al. (1975) have presented evidence that the growth inhibitor factor from rat liver extracts against H EpZ carcinoma is arginase. It appears that all treatments resulting in altered enzyme activity affect the inhibitory response to tumour growth in a similar way.

A second way in which the urea cycle enzymes have been implicated in carcinogenesis and the control of growth rate is suggested by the fact that urea has been found to protect animals from induction of carcinogenesis by DAB (Lin et al., 1973). Feeding of 5% urea together with 0,06% DAB to rats protected 50% of the animals against hepatocarcinogenesis. Rats fed 0,06% DAB alone showed 100% inducibility of hepatoma by this carcinogen. Proteins from wheat, rye, oats, milk, blood and eggs were all effective in retarding or delaying carcinogenesis by DAB, probably by conversion of waste nitrogen to urea, which would then account for the anticarcinogenic properties of these compounds (ibid). The mechanism of action of urea as an anticarcinogenic agent is not understood.

Urea cycle enzyme activities and hence urea levels can be raised either by hormone treatment (in young rats) or by increasing dietary protein levels.

Several workers have studied the effect of hormone treatment on the urea cycle enzymes in hepatomas and results have been varied. Wu et al. (1967) found that ASS was inducible by cortisol in some Morris hepatomas. Wu et al. (1971) reported that both arginase and ASS activities were inducible by cortisol in fourteen different Morris hepatomas studied in contrast to several other reports (Morris et al., 1964; Potter et al, 1969). Wu et al. (1971) found that although the hepatoma was responsive to cortisol, the host liver was not.

In the present study the effects of both diet and hormone were studied to try to determine whether urea cycle enzyme activities can be induced above normal levels as a possible treatment for cancer.

The first striking finding in these studies was the fact that all five of the urea cycle enzyme activities are considerably and significantly decreased in the host livers of tumour-bearing animals as compared with normal rats maintained on a 22% protein diet. The levels in the hepatoma were extremely decreased, at times reaching levels less than one percent of controls (see fig.3.29) Since the enzymes show a concerted change, these results suggest a common control for regulating the urea cycle enzyme activities.

Other examples of effects of tumours on the host liver have been documented. These include the lowering of catalase activity in tumour-bearing hosts (Adams, 1959; Kampschmidt and Schultz, 1963), increased minor N_2 form of liver pyruvate kinase (Tanaka et al, 1972) increased levels of pyruvate kinase, hexokinase and phosphofructokinase (Suda et al., 1966) and increased ornithine decarboxylase activity (Noguchi et al, 1976). Lawson et al. (1977)have recently measured decreased urea cycle enzyme activities in the host livers of rats bearing large, slow growing tumours as well as decreased urea synthesis as the tumours increased in size.

The hepatoma is extremely responsive to diet (see table 3.51) and in rats maintained on a 75% protein diet, a five-fold increase in CPS specific activity is seen as compared with rats fed on a 22% protein diet. CPS activity is the most responsive and ASS the least. CPS is much more responsive to diet in the hepatoma compared to either the host or control livers but the other enzymes show a similar response to diet in all three locations.

The urea cycle enzyme activities measured in hepatomas drop in rats maintained on either a 10% or a zero % protein diet, the activities being about one third in hepatomas of rats maintained on a zero % protein diet compared with those on 22% protein diets (see table 3.31a to 3.40b).

The host liver responds to diet in a way similar to control liver, being less sensitive to dietary change than the hepatomas (see table 3.51). All five of the urea cycle enzymes respond in a concerted way to the dietary stimulus, showing either an increase or a decrease.

All five of the urea cycle enzymes in hepatoma and host liver were found to be inducible by dBcAMP, glucagon and β-methasone (see tables 3.31a to 3.40b). These results agree with the findings of several other workers who tested the effect of cortisol only (Wu et al., 1967; Wu et al., 1971). The livers of control nontumourous rats of the same age (35-day old) and maintained under similar conditions are non-inducible. However the specific activity of arginase in the hepatoma is increased by about 30% in rats fed a 75% protein diet compared with those fed a 22% protein diet and by a further 15% with hormone treatment (see figs 3.49 and 3.50). In rats fed a 10% protein diet there is also an increase in the activity of arginase by glucagon and β-methasone in the hepatoma tissue.

In general dBcAMP appears to induce the urea cycle enzymes to a greater extent than glucagon and this is in agreement with studies on TAT (Tews et al., 1970). Induction by glucagon, β-methasone and β-methasone plus glucagon together are not significantly different, namely the two are not acting synergistically in this instance. Induction by these hormones is independent of diet since hormone induction is seen in all of the diets tested.

These results support Potter's theory of "oncogeny as blocked ontogeny" since the urea cycle enzymes are inducible by cortisol in juvenile and foetal rat liver but not in adult rat liver (McLean and Gurney, 1963). Also, the level of urea cycle enzymes in the hepatoma is greatly reduced compared to control livers, the level being similar to those of foetal rat livers. The other alternative is that during ontogeny certain cells became locked at a certain stage of development and that oncogeny represents the accumulation of locked in gene configurations of availability that were scheduled to be unlocked as different times in normal ontogeny.

Alternatively during oncogenesis certain regulatory genes are so altered that the cells re-acquire the control mechanisms prevailing in an early period of life. Hence there is a reversion of the regulatory apparatus in cancer cells to that of perinatal liver and this reversion is essential to the mechanism of carcinogenesis (Wu, 1973). The inducibility of arginase by cortisol in perinatal but not adult liver is confirmed by Greengard et al. (1970) and its inducibility in Morris hepatomas is confirmed by Wu et al. (1971).

dBcAMP has been shown to inhibit both DNA synthesis and the growth of H35 cells in culture. This could be due to an induction of arginase which is known to inhibit both DNA synthesis and growth rate of hepatomas in vivo (Holley, 1967). An increased synthesis of urea and depletion of the intermediates for purine, pyrimidine and polyamine could all possibly contribute to the inhibition of growth by dBcAMP.

Glucocorticoids are known to inhibit DNA polymerase, DNA synthesis and cell growth in regenerating rat liver after partial hepatectomy and in malignant cells in culture (Sakuma and Terayama, 1967). This inhibition could possibly be due to increasing activity of urea cycle enzymes in general which could result in the depletion of essential intermediates for anabolic synthesis of DNA, RNA and polyamines and in arginase in particular which could then result in an increased inhibitory effect on growth. An increase in urea cycle enzymes could result in an increased synthesis of urea, which may also inhibit growth in the hepatoma.

The activities of the urea cycle enzymes of host livers of hormone-untreated tumour-bearing animals are significantly lower than those of control non-tumourous animals, but the activities in the hormone-treated host livers are significantly higher (see figs 3.30 to 3.34). The depression of urea cycle enzyme activities in the host livers agrees with the results of Uno et al. (1963) and Lawson et al. (1975) who found that OTC and CPS activities respectively were lower in host livers compared to control livers.

These results suggest one of two things: either the host liver is inducible due to a starvation or some other nutritional effect by the tumour, or the tumour is producing a humoral substance which enables the host liver to respond to these hormones.

The former is apparently untrue since the host liver enzymes are inducible on all diets studied, including a high (75%) protein diet. This is in agreement with the results of Lawson et al. (1975) who found that the activity of CPS in the liver of host animals was depressed and that this drop in activity was independent of some specific nutritional effects of the tumour on the host.

The latter postulate was tested by injecting plasma from tumour-bearing animals intraperitoneally into control nontumourous animals and then testing for inducibility by glucagon (see tables 3.58 a, b, c). It was found that a) the urea cycle enzyme activities dropped in plasma-treated animals compared to control animals and b) the enzymes were inducible by glucagon (24 hour injection of 250 µg / 50 g) in the plasma-injected animals while those of control animals were not.

A control experiment involving the injection of plasma from non-tumourous animals into normal animals showed no change compared to non-injected controls. These results confirm the existence of a humoral substance which is present in the tumour-bearing animals but absent from controls. This suggests that the humoral substance is produced by the tumours themselves and may be the product of tumour-specific unmasked genes and therefore represent an altered state of differentiation.

Other examples are known which suggest the existence of a humoral factor. Tomarek (1969) found that perfused livers of rats bearing Walker 256 carcinoma produced less serum albumin and α and β-globulin than did those from normal rats. Serum factors have been reported for several other effects including DNA and protein synthesis (Dolan et al., 1974). Studies with parabiotic rats also suggest the existence of a circulating factor that effects liver enzyme activities. Johnston(1967) reported increased histidine carboxylase activity in the normal partners where one of the parabiotic pair was tumourous. Similar observations were made by Suda et al. (1966) for the N_2 pyruvate kinase and by Shiba et al. (1964) when measuring tryptophan pyrrollase.

The possibility exists that the humoral substance represents an increased level of glucocorticoid due to stress in the tumour-bearing animals which could then be permissive for induction by

different hormones such as glucagon. This seems to me unlikely, firstly because glucocorticoids are inhibitory to proliferation and should therefore be lower in the tumourous material and secondly, because the effects of β-methasone and glucagon or dBcAMP are nonadditive arguing against a permissive relationship between glucocorticoid and glucagon or dBcAMP in this instance.

It is possible that the tumour has an active system for excretion of steroids into the extracellular fluid and from there into the plasma. In this way there could be a reduced glucocorticoid level in the tumourous tissue to allow for rapid proliferation. This could not however account for the fact that the levels of urea cycle enzymes are reduced in the host livers of tumour-bearing animals compared with controls, nor for the fact that these enzymes areinducible in hepatoma and host livers but not in controls.

I believe that the humoral substance is more likely a substance that is decreasing the overall glucocorticoid basal level and that one possibility is that it is an increased transcortin level. This would not be due to an adaption for hepatoma growth, rather, the hepatoma may be able to grow because of an abnormality in regulation which increases the level of transcortin. If transcortin concentrations increase, the proportion of bound to unbound steroid would increase and this in effect would decrease the amount of glucocorticoid available for physiological interactions since only unbound steroid has physiological consequence. The fact that the activities of urea cycle enzymes are lower in the host argues in favour of this possibility. Desser-Wiest (1975) was able to show alterations in the affinity of serum corticosterone-binding proteins from the early to later hours of regeneration in rat liver after partial hepatectomy.

The present study was unable to test for increased or decreased glucocorticoid levels in tumour-bearing versus control animals as the transplantable tumour altered in such a way that it was no longer transplantable and it was therefore not possible to obtain plasma to measure glucocorticoid levels in host animals.

It would be of interest to try to isolate and characterize this humoral substance as it may shed some light on the factors involved in carcinogenesis.

A number of possibilities exist for the hormonal induction of the urea cycle enzymes in the host liver and hepatoma. Since glucocorticoids are inhibitory to growth, a drop in glucocorticoid levels by increased binding to transcortin, or an increased number of cytoplasmic receptor molecules being in an inactive conformation could result in the basal level of urea cycle enzymes being depressed. Treatment with β-methasone could then result in an increased synthesis of the urea cycle enzymes which were previously not maximally induced, to levels above those of control nontumour-bearing animals.

A second possibility is that there is a reversion to foetal or juvenile type controls where the urea cycle enzymes are inducible by glucocorticoids as depicted in models 1 and 2 under the section on regeneration (see pages 285-6). If there is an alteration in the nonhistone chromosomal proteins, as occurs with differentiation and dedifferentiation, a nuclear-acceptor molecule of the steroid-receptor complex which is sensitive to glucocorticoid may reappear. The transcripts of induction by steroid in this instance would then contain a 5' sequence specific to messengers which are responsive to cAMP and so account for inducibility by β -methasone, glucagon and dBcAMP. Model 2 requires the appearance of a protein factor which alters the site of initiation of transcription *in the DNA template by RNA polymerase*.

The dosage of β-methasone required for induction is one fortieth that of cortisol. This may be due to the fact that β -methasone binds much more strongly to Binder II, a cytoplasmic steroid-receptor molecule and has a strong affinity for this molecule and so is more rapidly involved in activation of the genome (Litwack et al., 1973). It may also be due to the fact that β -methasone does not bind to transcortin (Thomson and Lippman, 1974) and since it is the unbound steroid that is responsible for the physiological response, the greater the amount of unbound steroid, the greater would be the response. The increased effect may be due to a lack of degradation of β-methasone.

4.5.1 Statistical treatment of the data

4.5.1.1 Analysis of variance

An analysis of variance was performed on the data for both activity / g liver and specific activity and both showed the same pattern (see tables 3.61 and 3.62).

The four-way interaction of enzyme, treatment, diet and liver is significant and therefore, an alteration in enzyme activity is dependent on which enzyme is being studied, the diet on which the rats are maintained, the hormone stimulus applied and in which type of liver the enzyme is being assayed.

The analysis of variance explains most of the variability, with little residual variability unexplained, this probably reflecting experimental error. An analysis of variance is statistically invalid in this instance since the enzymes when measured were not independent variables as they were all measured in the same livers. Also, host liver and hepatoma tissue were derived from one animal and are therefore not independent variables. This means that the variability is correllated and does not give a true reflection of what is occurring in the liver. It was useful however since it indicated that a linear additive model could probably not adequately describe the results.

Analysis of variance is restrictive in that it is purely analytical, never predictive and can only interpret results in a linear way. This does not take into account the fact that biological systems do not always behave linearly.

4.5.2 A mathematical model

Dr D. Brade of the C.S.I.R. has designed a semi-predictive and interpretive mathematical model to describe the experimental results of the present study. It is a simple multiplicative model of the form $Y_{ijkl} = K A_i B_j C_k D_l$.

Where Y_{ijkl}	=	predicted enzyme activity
K	=	constant
A_i	=	the coefficient describing the effect of different diets
B_j	=	the coefficient describing the effect different hormone treatment
C_k	=	the coefficient describing the influence of the different urea cycle enzyme being studied
D_l	=	the coefficient for the liver effect and describes the type of liver tissue being studied namely control, host or hepatoma

There are three complete sets of coefficients, both for results expressed as activity/g liver and specific activity and are shown in tables 4A and 4B. There is a separate set of coefficients for each liver type namely control, host and hepatoma. There are four coefficients of diet, namely A_i 1 = 0% protein, 2 = 10% protein, 3 = 22% protein, 4 = 75% protein. There are five coefficients for hormone treatment ; B_j 1 = β-methasone treatment, 2 = glucagon treatment, 3 = β-methasone and glucagon treatment, 4 = dBcAMP treatment, 5 = control untreated. There are five coefficients of enzymes; C_k 1 = AL, 2 = CPS, 3 = OTC, 4 = ARG, 5 = ASS and there are three coefficients for liver; D_l 1 = control liver, 2 = host liver, 3 = hepatoma (see p.303a).

The predicted enzyme activities agree very well with observed values, there being less than 1% error between the two (see tables 4.1 to 4.3 and 4.7 to 4.9). As can be seen from the tables of averages of predicted versus observed values, the values are very similar (Tables 4.4 to 4.6 and 4.10 to 4.12).

In order to predict a particular enzyme activity under given conditions, one would multiply the constant by the appropriate coefficients for given conditions and thereby derive the activity.

It is apparent from the model that livers of control animals are insensitive to hormone treatment but that host liver and hepatoma are responsive, the former being the most responsive (see tables 4A and B column of coefficients for B_j).

A_i = diet		C_k = enzyme	
A_i 1	= 0% protein	C_k 1	= AL
2	= 10%	2	= CPS
3	= 22%	3	= OTC
4	= 75%	4	= Arginase
		5	= ASS

B_j = Hormone stimulus		D_l = liver type	
B_j 1	= β-methasone	D_l 1	= normal liver
2	= glucagon	2	= host liver
3	= β-methasone + glucagon	3	= hepatoma
4	= dBcAMP		
5	= control, nontreated		

The coefficient 1111 would then represent the activity of AL measured in the liver of a normal nontumourous rat maintained on a 0% protein diet and given a single 24 hour i.p. injection of glucagon (250µg/50g).

Table 4.A Model Coefficients for Specific Activity.



K=12,17 (General average=12,1187)

	1	2	3	4	5
A_i	0,6039 0,5727 0,3165	1,0605 0,9306 0,7837	1,0095 1,0393 1,0857	1,3261 1,4574 1,8141	
B_j	1,0454 1,0375 1,1119	0,9826 1,0335 1,0194	1,0277 1,0355 0,9899	0,9662 1,1447 1,0233	0,9832 0,7489 0,8555
C_k	0,0083 0,0079 0,1180	0,0179 0,0153 0,0737	0,7537 0,7381 2,1136	4,2122 4,2307 2,6289	0,0079 0,0080 0,0658
D_l	1,4143 – –	– 1,5646 –	– – 0,0211		

Table 4.B Model Coefficients for Activity/g liver.

K=1472,10 (General Average=1468,62)

A_i	0,5741 0,5771 0,2497	0,7922 0,7282 0,6597	1,0969 1,1399 1,3916	1,5368 1,5548 1,6991	
B_j	1,0253 1,0781 0,9850	0,9553 1,0648 1,0139	1,0169 1,0278 1,0381	0,9972 1,0519 1,0198	1,0052 0,7774 0,9432
C_k	0,0084 0,0079 0,1161	0,0182 0,0149 0,0572	0,7062 0,7424 2,0690	4,2603 4,2267 2,6990	0,0070 0,0081 0,0588
D_l	1,4931 – –	– 1,4930 –	– – 0,0139		

Table 4.1 CONTROL LIVER, SPECIFIC ACTIVITY (observed vs. predicted data).

i = diet
j = hormone
k = enzyme
l = control liver

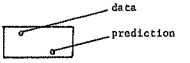


TABLE 4.1 CONTROL LIVER, SPECIFIC ACTIVITY Observed data vs. predicted values.

J	K	IA	1	2	3	4
1	1		0.08	0.11	0.09	0.28
			0.09	0.16	0.15	0.20
1	2		0.12	0.32	0.32	0.53
			0.19	0.24	0.33	0.43
1	3		5.76	14.00	11.41	22.56
			8.19	14.20	13.69	17.98
1	4		43.29	66.02	71.51	108.07
			48.77	80.37	76.50	100.90
1	5		0.11	0.14	0.11	0.36
			0.09	0.15	0.14	0.19
2	1		0.07	0.09	0.08	0.27
			0.08	0.15	0.14	0.19
2	2		0.13	0.24	0.30	0.44
			0.18	0.32	0.31	0.43
2	3		6.95	11.41	10.08	19.60
			7.76	13.52	12.87	16.90
2	4		45.81	71.61	77.16	92.51
			43.02	75.55	71.01	94.47
2	5		0.13	0.16	0.11	0.16
			0.08	0.14	0.13	0.18
3	1		0.08	0.11	0.09	0.29
			0.09	0.15	0.15	0.19
3	2		0.13	0.29	0.31	0.53
			0.19	0.33	0.32	0.42
3	3		8.17	14.19	10.62	21.77
			8.01	14.07	13.39	17.59
3	4		42.84	79.26	73.02	99.32
			44.74	78.63	74.84	98.32
3	5		0.12	0.13	0.12	0.17
			0.08	0.15	0.14	0.18
4	1		0.08	0.09	0.08	0.28
			0.08	0.15	0.14	0.18
4	2		0.13	0.22	0.31	0.51
			0.18	0.32	0.30	0.40
4	3		6.17	10.84	10.14	19.14
			7.57	13.24	12.65	16.62
4	4		40.98	71.31	73.36	91.07
			42.30	74.28	70.71	92.60
4	5		0.12	0.11	0.11	0.15
			0.08	0.14	0.13	0.17
5	1		0.07	0.09	0.09	0.26
			0.08	0.15	0.14	0.19
5	2		0.11	0.23	0.30	0.49
			0.16	0.32	0.31	0.40
5	3		6.31	11.01	10.74	19.88
			7.79	13.52	12.87	16.91
5	4		41.96	76.91	72.37	93.16
			43.05	75.59	71.95	94.52
5	5		0.12	0.12	0.11	0.16
			0.08	0.14	0.13	0.18

Table 4.2 HOST LIVER, SPECIFIC ACTIVITY(observed vs. predicted data).

i = diet
j = hormone
k = enzyme
l = host liver

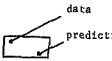
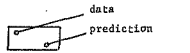


TABLE 4.2 HOST LIVER, SPECIFIC ACTIVITY Observed data vs. predicted values.

j	k	i	1	2	3	4
1	1		0.08	0.11	0.12	0.27
			0.09	0.15	0.11	0.23
	2		0.12	0.27	0.29	0.47
			0.17	0.20	0.31	0.44
	3		5.89	10.66	14.14	24.32
			0.35	13.57	15.15	21.29
	4		47.64	75.57	87.90	122.64
			47.66	77.76	86.86	121.80
	5		0.13	0.17	0.16	0.20
			0.09	0.15	0.16	0.23
2	1		0.10	0.13	0.14	0.27
			0.09	0.15	0.16	0.23
2	2		0.14	0.26	0.37	0.49
			0.17	0.28	0.31	0.44
2	3		7.09	11.04	14.04	23.89
			0.32	13.52	15.19	21.17
2	4		50.74	78.08	86.41	121.86
			47.68	77.48	86.53	121.34
2	5		0.13	0.20	0.17	0.22
			0.09	0.15	0.16	0.23
3	1		0.09	0.13	0.13	0.26
			0.09	0.15	0.16	0.23
3	2		0.12	0.28	0.33	0.47
			0.17	0.28	0.31	0.44
3	3		6.34	12.00	13.48	24.46
			8.33	13.54	15.12	21.21
3	4		46.87	78.52	85.00	122.80
			47.77	77.63	86.69	121.57
3	5		0.14	0.19	0.15	0.21
			0.09	0.15	0.16	0.23
4	1		0.10	0.15	0.14	0.29
			0.10	0.16	0.18	0.25
4	2		0.12	0.30	0.37	0.50
			0.19	0.31	0.35	0.49
4	3		6.25	15.16	14.85	24.69
			9.21	14.97	16.72	23.48
4	4		51.26	96.26	93.39	135.26
			52.81	85.01	93.03	134.39
4	5		0.12	0.21	0.17	0.22
			0.10	0.16	0.18	0.25
5	1		0.07	0.08	0.08	0.21
			0.08	0.17	0.12	0.16
5	2		0.10	0.16	0.19	0.32
			0.13	0.20	0.23	0.32
5	3		4.90	9.02	9.39	18.13
			6.03	9.79	10.94	15.34
5	4		36.39	65.95	68.95	89.12
			34.95	56.14	62.70	87.92
5	5		0.09	0.08	0.08	0.14
			0.07	0.11	0.12	0.17

Table 4.3 HEPATOMA, SPECIFIC ACTIVITY (observed vs. predicted data)

i = diet
j = hormone
k = enzyme
l = hepatoma



j	k	i=	1	2	3	4
1	1		0.01	0.01	0.03	0.07
			0.01	0.03	0.04	0.08
1	2		0.02	0.01	0.03	0.04
			0.01	0.02	0.02	0.04
1	3		0.18	0.48	0.90	1.02
			0.19	0.47	0.66	1.10
1	4		0.23	0.63	0.78	1.48
			0.24	0.59	0.82	1.36
1	5		0.03	0.02	0.01	0.03
			0.01	0.01	0.02	0.03
2	1		0.01	0.02	0.03	0.05
			0.01	0.02	0.03	0.06
2	2		0.02	0.01	0.01	0.03
			0.01	0.02	0.02	0.04
2	3		0.20	0.53	0.84	1.01
			0.19	0.43	0.60	1.01
2	4		0.20	0.66	0.78	1.21
			0.22	0.64	0.75	1.25
2	5		0.02	0.04	0.03	0.03
			0.01	0.01	0.02	0.03
3	1		0.01	0.01	0.02	0.06
			0.01	0.02	0.03	0.05
3	2		0.03	0.01	0.01	0.03
			0.01	0.01	0.02	0.03
3	3		0.15	0.45	0.55	1.02
			0.17	0.42	0.39	0.98
3	4		0.19	0.59	0.80	1.12
			0.21	0.58	0.75	1.21
3	5		0.02	0.03	0.01	0.02
			0.01	0.01	0.02	0.03
4	1		0.03	0.01	0.03	0.07
			0.01	0.02	0.03	0.06
4	2		0.10	0.01	0.01	0.04
			0.01	0.02	0.02	0.04
4	3		0.16	0.44	0.84	1.07
			0.18	0.44	0.60	1.01
4	4		0.22	0.60	0.83	1.22
			0.22	0.54	0.75	1.26
4	5		0.02	0.02	0.01	0.03
			0.01	0.01	0.02	0.03
5	1		0.01	0.01	0.02	0.06
			0.01	0.02	0.02	0.05
5	2		0.02	0.01	0.01	0.03
			0.01	0.01	0.02	0.03
5	3		0.16	0.32	0.49	0.96
			0.19	0.36	0.58	0.84
5	4		0.23	0.28	0.74	0.97
			0.18	0.45	0.63	1.05
5	5		0.02	0.02	0.01	0.02
			0.02	0.01	0.02	0.03

Table 4.4. CONTROL LIVER, SPECIFIC ACTIVITY (observed vs. predicted data)

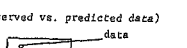


TABLE 4.4	MAIN AVERAGES	CONTROL LIVER SPECIFIC ACTIVITY (Averages of Observed data vs. Predicted values)			
DIET	10.142	10.001	17.084	23.415	
	10.394	10.253	17.375	22.825	
HORMONE	17.889	10.993	17.543	10.571	10.801
	17.992	10.913	17.002	10.629	10.922
ENZYME	0.135	0.294	12.594	72.043	0.138
	0.143	0.309	12.972	72.499	0.136
DIET BY HORMONE	AVGES ARE				
	9.87	10.02	10.09	10.50	9.64
	10.87	10.21	10.63	10.04	10.22
	20.12	10.69	10.04	10.51	17.85
	19.08	17.94	10.67	17.64	17.95
	10.69	17.71	10.93	10.05	10.92
	19.10	17.07	17.77	10.79	17.08
	24.86	22.80	24.42	22.39	22.79
	23.86	22.43	23.34	22.05	22.44
DIET BY ENZYME	AVGES ARE				
	0.07	0.12	6.27	44.12	0.12
	0.09	0.19	7.63	43.78	0.03
	0.19	0.26	12.51	77.02	0.12
	0.15	0.33	13.78	76.89	0.14
	0.09	0.31	10.04	73.08	0.11
	0.14	0.31	13.10	73.15	0.14
	0.28	0.50	20.94	99.91	0.20
	0.19	0.41	17.20	96.14	0.18
HORMONE BY ENZYME	AVGES ARE				
	0.14	0.32	13.42	75.37	0.18
	0.15	0.32	13.56	73.79	0.14
	0.13	0.28	12.22	71.77	0.12
	0.14	0.30	12.75	71.24	0.13
	0.14	0.31	13.24	73.69	0.14
	0.15	0.32	13.27	74.14	0.14
	0.13	0.29	12.07	70.63	0.12
	0.14	0.30	12.53	72.05	0.13
	0.13	0.28	12.21	71.26	0.12
	0.14	0.30	12.75	71.28	0.13

Table 4.5 A comparison of averages of observed vs. predicted data for activities of host livers expressed per mg protein.

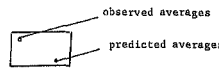


TABLE 4.5 HOST LIVER, SPECIFIC ACTIVITY Observed vs. predicted values.

	MAIN AVERAGES				
DIET	10.633	17.311	19.054	28.225	
	10.985	17.720	19.789	27.750	
HORMONE	19.757	19.725	19.583	21.742	14.170
	19.754	19.679	19.717	21.795	14.260
ENZYME	0.147	243	13.573	80.618	0.157
	0.151	0.292	14.084	80.337	0.132
	AVGES AKG				
DIET BY HORMONE	10.77	11.80	15.71	11.97	8.31
	11.31	11.27	11.29	12.48	8.17
	17.29	17.50	18.22	22.42	11.05
	18.38	18.31	18.35	20.26	13.27
	25.52	20.41	19.82	21.78	15.74
	20.53	20.45	20.49	22.65	14.82
	29.58	29.19	29.58	31.19	21.56
	26.79	28.68	28.73	31.77	20.76
	AVGES AAC				
DIET BY ENZYME	0.09	0.12	6.25	40.58	0.12
	0.09	0.17	6.09	46.14	0.09
	0.12	0.25	11.56	74.44	0.17
	0.14	0.27	13.08	74.97	0.14
	0.12	0.21	13.26	84.23	0.19
	0.16	0.30	14.61	83.72	0.16
	0.26	0.45	23.18	117.12	0.20
	0.22	0.43	20.48	117.40	0.22
	AVGLS HKC				
HORMONE BY ENZYME	0.15	0.29	13.75	82.44	0.16
	0.16	0.30	14.58	83.57	0.18
	0.16	0.32	14.44	83.53	0.18
	0.16	0.30	14.53	83.26	0.16
	0.15	0.26	14.07	83.22	0.17
	0.16	0.30	14.55	83.42	0.16
	0.17	0.32	15.25	92.60	0.18
	0.17	0.33	16.09	92.21	0.17
	0.11	0.19	10.36	60.10	0.09
	0.11	0.22	10.83	60.33	0.11

Table 4.6 A comparison of averages of observed vs. predicted data for activities of hepatoma tissue expressed per mg protein.

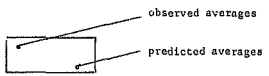
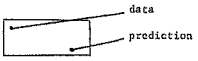


TABLE 4.6 MAIN AVERAGES	HEPATOMA, SPECIFIC ACTIVITY	Observed data vs. predicted values.		
	0.200	0.275	0.408	
DIET	0.261	0.292	0.270	0.467
	0.263	0.268	0.258	0.268	0.219
NORMONE					
	0.286	0.262	0.295	0.263	0.226
	0.028	0.024	0.542	0.677	0.022
ENZYME					
	0.030	0.019	0.544	0.676	0.017
ANGLE AAB					
DIET BY NORMONE	0.09	0.09	0.08	0.10	0.09
	0.09	0.08	0.06	0.06	0.07
	0.23	0.23	0.22	0.19	0.12
	0.22	0.21	0.20	0.21	0.17
	0.28	0.26	0.28	0.28	0.29
	0.31	0.20	0.28	0.29	0.24
	0.53	0.47	0.46	0.49	0.41
	0.52	0.46	0.46	0.48	0.40
ANGLE AAC					
DIET BY ENZYME	0.01	0.01	0.17	0.21	0.02
	0.01	0.01	0.17	0.21	0.01
	0.01	0.01	0.44	0.51	0.03
	0.02	0.01	0.43	0.53	0.01
	0.03	0.01	0.54	0.79	0.01
	0.03	0.02	0.89	0.73	0.02
	0.06	0.04	1.02	1.20	0.02
	0.04	0.03	0.90	1.22	0.03
ANGLE UXC					
NORMONE BY ENZYME	0.03	0.02	0.56	0.78	0.02
	0.03	0.02	0.60	0.75	0.02
	0.03	0.02	0.57	0.69	0.03
	0.03	0.02	0.55	0.69	0.02
	0.03	0.02	0.55	0.64	0.02
	0.03	0.02	0.54	0.67	0.02
	0.03	0.04	0.55	0.69	0.02
	0.03	0.02	0.56	0.69	0.02
	0.02	0.02	0.48	0.65	0.01
	0.03	0.02	0.47	0.58	0.01

Table 4.7 CONTROL LIVER, ACTIVITY/G LIVER (observed vs. predicted data).

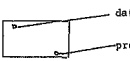
i = diet
j = hormone
k = enzyme
l = control



J	K	L	1	2	3	4
1	1		8.	8.	12.	40.
			11.	15.	21.	59.
1	2		14.	29.	44.	78.
			24.	33.	40.	63.
1	3		894.	1058.	1443.	2824.
			914.	1261.	1746.	2446.
1	4		4987.	7688.	10880.	14493.
			5512.	7608.	10328.	14755.
1	5		12.	12.	15.	22.
			9.	12.	17.	24.
2	1		9.	9.	12.	36.
			10.	14.	19.	27.
2	2		16.	24.	46.	57.
			22.	30.	42.	59.
2	3		847.	1076.	1541.	2612.
			851.	1175.	1626.	2279.
2	4		5693.	6789.	10510.	13173.
			5136.	7086.	9813.	13747.
2	5		15.	11.	14.	23.
			8.	12.	16.	22.
3	1		9.	9.	13.	36.
			11.	15.	21.	29.
3	2		17.	25.	47.	60.
			23.	32.	45.	63.
3	3		853.	1130.	1492.	2616.
			980.	1250.	1731.	2426.
3	4		5748.	7808.	10849.	14128.
			5457.	7544.	10436.	14034.
3	5		15.	13.	16.	23.
			9.	12.	17.	24.
4	1		9.	8.	12.	37.
			11.	15.	20.	28.
4	2		11.	22.	47.	67.
			23.	32.	44.	61.
4	3		898.	1019.	1453.	2391.
			859.	1226.	1648.	2379.
4	4		5287.	7392.	9783.	14577.
			4361.	7398.	10244.	14251.
4	5		14.	11.	14.	20.
			9.	12.	17.	23.
5	1		8.	9.	12.	38.
			11.	15.	20.	28.
5	2		13.	24.	44.	69.
			23.	32.	44.	62.
5	3		787.	1191.	1632.	2931.
			845.	1230.	1711.	2398.
5	4		5157.	7557.	9841.	14829.
			5404.	7457.	10325.	14465.
5	5		15.	11.	14.	23.
			9.	12.	17.	24.

Table 4.8 HOST LIVER, ACTIVITY/G LIVER (observed vs. predicted data).

i = diet
j = hormone
k = enzyme
l = host



J	K	L=	1	2	3	4
1	1		9.	10.	16.	33.
			11.	14.	23.	29.
1	2		13.	26.	36.	56.
			20.	26.	40.	55.
1	3		694.	1881.	1838.	3062.
			1015.	1261.	2005.	2735.
1	4		5491.	7775.	10721.	15976.
			5780.	7293.	11416.	15571.
1	5		15.	18.	31.	29.
			11.	14.	22.	30.
2	1		11.	13.	17.	33.
			11.	13.	21.	29.
2	2		15.	26.	47.	56.
			20.	25.	40.	54.
2	3		692.	1892.	1907.	2981.
			1003.	1265.	1980.	2701.
2	4		5744.	7337.	11438.	15160.
			5780.	7293.	11275.	15279.
2	5		15.	20.	21.	28.
			11.	14.	22.	29.
3	1		9.	12.	18.	32.
			10.	13.	25.	28.
3	2		11.	25.	42.	56.
			19.	26.	38.	52.
3	3		724.	1817.	1829.	2995.
			980.	1221.	1912.	2600.
3	4		5249.	7127.	10680.	14972.
			5511.	6953.	10864.	14946.
3	5		15.	26.	21.	28.
			11.	13.	21.	28.
4	1		13.	19.	18.	32.
			11.	13.	21.	28.
4	2		32.	22.	41.	53.
			20.	25.	39.	54.
4	3		695.	1613.	1724.	2801.
			991.	1250.	1936.	2669.
4	4		6395.	8750.	11438.	14910.
			5640.	7116.	11136.	15103.
4	5		15.	17.	21.	25.
			11.	14.	21.	29.
5	1		8.	8.	11.	26.
			8.	10.	15.	21.
5	2		9.	14.	24.	40.
			18.	19.	26.	40.
5	3		884.	837.	1338.	2291.
			732.	924.	1446.	1972.
5	4		4561.	4882.	8991.	10664.
			4168.	5299.	9232.	11228.
5	5		19.	9.	12.	17.
			8.	10.	16.	21.

Table 4.9 HEPATOMA, ACTIVITY/G LIVER (observed vs.predicted data).

i = diet
j = hormone
k = enzyme
l = hepatoma



j	k	l	1	2	3	4
1	1		0.	1.	2.	5.
			1.	2.	3.	4.
1	2		0.	1.	1.	3.
			0.	1.	2.	2.
1	3		10.	31.	58.	75.
			10.	27.	58.	71.
1	4		14.	40.	73.	90.
			14.	30.	75.	92.
1	5		3.	2.	1.	2.
			0.	1.	2.	2.
2	1		1.	1.	2.	5.
			1.	2.	3.	4.
2	2		0.	1.	1.	3.
			0.	1.	2.	2.
2	3		12.	30.	53.	76.
			11.	28.	60.	73.
2	4		14.	34.	79.	96.
			14.	37.	70.	95.
2	5		1.	2.	1.	2.
			0.	1.	2.	2.
3	1		1.	1.	2.	5.
			1.	2.	3.	4.
3	2		0.	1.	1.	3.
			0.	1.	2.	2.
3	3		10.	29.	54.	78.
			11.	29.	61.	75.
3	4		14.	41.	85.	93.
			14.	38.	80.	97.
3	5		2.	2.	1.	2.
			0.	1.	2.	2.
4	1		1.	1.	3.	5.
			1.	2.	3.	4.
4	2		0.	1.	1.	3.
			0.	1.	2.	2.
4	3		9.	31.	56.	76.
			11.	28.	60.	73.
4	4		14.	36.	83.	98.
			14.	37.	78.	96.
4	5		2.	1.	1.	2.
			0.	1.	2.	2.
5	1		0.	1.	2.	5.
			1.	1.	3.	4.
5	2		0.	1.	1.	2.
			0.	1.	2.	2.
5	3		10.	24.	52.	79.
			10.	26.	56.	80.
5	4		15.	23.	81.	86.
			13.	34.	72.	98.
5	5		1.	1.	1.	1.
			0.	1.	2.	2.

Table 4.10 A comparison of averages of observed vs. predicted data for activities of control livers expressed per gram wet weight of liver.

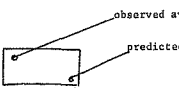


TABLE 4.10 CONTROL LIVER, ACTIVITY PER GRAM LIVER (Observed values vs. Predicted.)

	MAIN AVERAGES				
DIET	1230.956	1715.171	2375.411	3426.604	
	1201.942	1741.198	2411.103	3377.662	
HORMONE	2217.055	2123.779	2235.894	2160.607	2199.524
	2253.726	2099.733	2235.243	2191.953	2209.450
ENZYME	16.806	37.772	1492.015	9375.196	15.963
	18.414	46.857	1.52.158	9364.183	15.206
	AVGES AXB				
DIET BY HORMONE	1123.	1308.	1786.	1251.	1184.
	1294.	1206.	1283.	1258.	1269.
	1750.	1582.	1798.	1640.	1748.
	1765.	1663.	1771.	1736.	1758.
	2419.	2424.	2683.	2262.	2289.
	2472.	2302.	2452.	2404.	2424.
	3572.	3181.	3374.	3449.	3677.
	3463.	3227.	3435.	3369.	3395.
	AVGES AXC				
DIET BY ENZYME	8.	14.	784.	5414.	14.
	11.	22.	891.	5376.	9.
	9.	24.	1095.	7430.	12.
	15.	32.	1230.	7418.	12.
	32.	45.	1492.	12313.	15.
	28.	44.	1703.	13272.	17.
	30.	68.	2677.	14338.	23.
	28.	62.	2305.	14391.	24.
	AVGES BXC				
HORMONE BY ENZYME	17.	40.	1482.	9526.	15.
	19.	41.	1591.	9522.	16.
	17.	36.	1519.	9321.	16.
	18.	38.	1463.	8985.	15.
	17.	39.	1474.	9523.	17.
	19	41.	1578.	9323.	16.
	16.	37.	1389.	9345.	16.
	18.	40.	1548.	9316.	15.
	17.	37.	1595.	9322.	16.
	19.	40.	1566.	9413.	15.

Table 4.11 A comparison of averages of observed vs. predicted data for activities of host livers expressed per gram wet weight of liver.

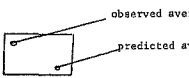


TABLE 4.11 HOST LIVER, ACTIVITY PER GRAM LIVER (Observed values vs Predicted.)

MAIN AVERAGES				
	1727.118	1866.898	2490.596	2460.102
DIET	1288.437	1800.522	2509.298	2417.184
	2241.499	2343.873	2240.649	2388.128
HORMONE				1780.333
	2369.460	2349.250	2259.069	2311.987
ENZYME	18.342	31.898	1863.526	9388.319
				18.688
	17.494	32.787	1731.852	9289.884	17.775
AVGES AND				
DIET BY HORMONE	1230.	1335.	1710.	1426.	928.
	1367.	1351.	1304.	1334.	986.
	1784.	1698.	1840.	1563.	1151
	1725.	1704.	1645.	1684.	1244.
	2527.	2486.	2518.	2649.	2075.
	2701.	2660.	2575.	2635.	1948.
	3819.	3653.	3616.	3564.	2648.
	3564.	3639.	3512.	3595.	2656.
AVGES ARE				
DIET BY ENZYME	10.	12.	704.	5358.	14.
	10.	19.	442.	5361.	10.
	11.	23.	1010.	6774.	17.
	13.	24.	1180.	6785.	13.
	15.	38.	1727.	10853.	19.
	20.	37.	1860.	10589.	20.
	31.	53.	2814.	14376.	25.
	27.	51.	2537.	14443.	28.
AVGES ARE				
HORMONE BY ENZYME	17.	33.	1646.	9991.	20.
	19.	35.	1759.	10013.	19.
	19.	36.	1716.	9921.	21.
	19.	35.	1737.	9892.	19.
	18.	34.	1641.	9317.	20.
	18.	34.	1677.	9548.	18.
	18.	32.	1558.	9873.	19.
	18.	34.	1716.	9772.	19.
	13.	22.	1254.	7199.	12.
	14.	25.	1268.	7221.	14.

Table 4.12 A comparison of averages of observed vs. predicted data for activities of hepatoma tissue expressed per gram wet weight of tissue.

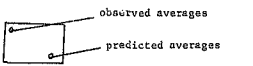


TABLE 4.12 HEPATOMA, ACTIVITY PER GRAM LIVER (Observed values vs. Predicted.)

MAIN AVERAGES	5.275	13.589	27.782	35.194
		13.681	28.436	34.725
DIET	5.102			
HORMONE	20.431	26.685	21.260	21.813	19.912
	20.131	29.720	21.216	20.641	19.277
ENZYME	2.237	1.085	42.158	55.241	1.282
	2.372	1.170	42.283	55.159	1.282
AVGES AXB					
DIET BY HORMONE	5.	6.	5.	5.	5.
	5.	5.	5.	5.	5.
	15.	13.	15.	15.	10.
	13.	14.	14.	14.	13.
	27.	27.	29.	29.	27.
	28.	29.	30.	29.	27.
	39.	36.	36.	35.	33.
	34.	35.	36.	35.	33.
AVGES AXC					
DIET BY ENZYME	1.	0.	10.	14.	1.
	1.	0.	11.	14.	0.
	1.	1.	29.	39.	2.
	2.	1.	28.	36.	1.
	2.	1.	54.	80.	1.
	3.	2.	59.	77.	2.
	9.	3.	75.	91.	2.
	4.	2.	72.	94.	2.
AVGES BXC					
HORMONE BY ENZYME	2.	1.	43.	54.	2.
	2.	1.	42.	54.	1.
	2.	1.	43.	56.	2.
	2.	1.	43.	56.	1.
	2.	1.	43.	59.	2.
	2.	1.	44.	57.	1.
	2.	1.	43.	57.	2.
	2.	1.	43.	56.	1.
	2.	1.	39.	51.	1.
	2.	1.	40.	52.	1.

Also apparent from the model is the fact that in the hepatoma arginase activity drops more than any of the other enzymes which argues in favour of the necessity for *arginase, the inhibitor of proliferation,* to drop in hepatoma, thereby allowing rapid proliferation to proceed (see tables 4A and B column of coefficients for C_k).

The model is multiplicative rather than additive. In other words, instead of adding constant coefficients for given effects, one multiplies by constant coefficients or, in effect, adds on percentage differences.

One would expect a multiplicative type model rather than an additive one to describe a biological system since it takes into account the fact that the regulation of protein levels is regulated by a multiplicity of control points and that changes that occur are seldom additive or independent of other conditions prevailing. For example, the level of a specific protein is controlled both by *its rates of synthesis and degradation.* These rates are controlled by the flux of metabolites through a sequence of reactions and the relative flux and availability of metabolites through these reactions controls the basal level of the protein. The overall flux of metabolites is proportional to the amount of metabolites allowed to flow into the series of reactions at certain controlling or limiting points. Different limiting factors may control the same series of reactions but the controls are enforced at different points in the sequence, the overall *effect of two separate limiting* factors being compounded to give an effective rate of synthesis and degradation and hence the basal level of the protein.

The model demonstrates very clearly certain aspects of *regulation* of the urea cycle enzymes in the three types of liver tissues studied. The first point that is demonstrated by the model is that under the conditions studied, namely urea cycle enzyme activities in normal, host and hepatoma tissue under varying conditions of diet and hormone stimuli, all five of the urea cycle enzymes behave qualitatively in a correlated way within one tissue type suggesting some common regulatory mechanisms. For example, the coefficient for diet and hormone remain constant within a particular liver type, *independent of which* enzyme is being studied, as long as the activities are expressed as a percentage of control values. Therefore, to determine the effect of glucagon on any one of the five enzymes in host liver, one would multiply the enzyme factor by the coefficient for glucagon

(B_j = 2nd column) and the same coefficient is used for all enzymes. In the control liver although the enzymes are correlated, they do not respond to hormone stimuli since the different coefficients for hormone are all very similar. However, all five enzymes are behaving in a correllated way to diet since for a particular diet, the same coefficient is multiplied to determine the effect of that diet on any of the enzymes studied.

The second point demonstrated is that in the host and hepatoma tissue the diets and hormones are independent of each other. Mathematically this is shown by the fact that in order to determine the effect of diet and hormone one can multiply the coefficients in any order without affecting the computed value. This is not true for the liver of thirty five day-old control nontumour-bearing animals.

In contrast to the above, the results of other workers show that in rats beyond 29 days of age the urea cycle enzymes do not behave in a completely correlated way to different dietary and hormonal stimuli and that diet and hormone effects are not independent. For example, in adrenalectomized rats arginase activities drop by 80% while CPS, OTC, ASS and AL drop by only 30% (Schimke, 1963; McLean and Novello, 1963). Christowitz (1975) found that daily injections of cortisol for four days were required to induce urea cycle enzymes significantly in adult rats and that not all the enzymes are correlated. ASS and arginase responses are diet dependent with greater induction on a lower protein diet. The activity of OTC could be lowered by adrenalectomy in rats maintained on a 10% diet and raised by subsequent cortisol treatment. AL activity on the other hand was raised by adrenalectomy and lowered by repeated injections of cortisol (ibid). Inducibility of the urea cycle enzymes is independent of intact adrenals (Schimke, 1963). These results were confirmed by Mattheyse (1977) in his work on liver slices of adrenalectomized rats in vitro. CPS, ASS and arginase responded to cortisol in vitro in these slices in a more positive way than in non-adrenalectomized rats in vivo. AL showed the anomalous drop with cortisol treatment even in vitro.

Shephard (1975) found that in 28 day old rats, hepatic CPS responded to glucagon in rats maintained on 10%, 22% and 75% protein diets and from this concluded that the inducibility by hormone was independent of dietary protein content.

From the above it can be concluded that urea cycle enzymes in host livers and hepatomas are responding to hormone and diet in an independent and correlated way and that the regulation of these enzymes in these tissues is similar to those of juvenile rats up to day 28 postnatally. At day 29 and beyond there is a dramatic change in the regulation of these enzymes such that the response is not always correlated and such that the inducibility by hormone is diet-dependent, but inducibility by diet is independent of hormone.

4.5 REGULATION OF UREA CYCLE ENZYME ACTIVITIES

The urea cycle enzymes have been shown to behave in a concerted co-ordinated way for a number of systems studied (McLean and Gurney, 1963; Schimke, 1962a) including their behaviour in neoplasia and regeneration. This suggests that these enzymes are under similar kinds of controls as those in the model described by Britton and Davidson (1969).

These changes, although qualitatively similar are quantitatively dissimilar and this could be explained by postulating similar but non-identical promotor regions with different affinities for RNA polymerase attachment. During the evolution of the urea cycle the genes coding for the different enzymes may originally have been closely linked to each other and under the control of a single promotor, as in the lac operon of E. coli. This could have been followed by duplications in the promotor region followed by translocations such that these genes, each with their own promotor site could now be dispersed throughout the genome. Slight alterations in the primary sequence of the promotor region could cause changes in the affinity of RNA polymerase for them and so could alter the relative rates of transcription and translation of these enzymes. Evidence exists that loci coding for the urea cycle enzymes are not closely linked in the chromosomes of rats (Soberon, personal communications).

Several isozymic forms of RNA polymerase are known both in prokaryotes and eukaryotes (Travers, 1976). RNA polymerase holoenzymes may exist in functionally distinct forms each of which initiates at a particular class of promotor sites. There may exist a complete spectrum of specificity for different isozymes from binding exclusively to one type of promotor to interacting well with several different promotors (ibid).

Since such isozymes are known to exist it is feasible to postulate that the urea cycle enzymes are controlled by a class of similar but nonidentical promotor sites under the control of a particular isozyme with different affinities for the different promotors. The greater the affinity of the polymerase for the promotor, the more rapid the initiation of transcription, and the greater the amount of mRNA available for translation. This could account for the quantitative differences seen in the urea cycle enzyme activities when induced by particular stimuli.

The above mechanism is just one of several that could explain the coordinated control of urea cycle enzymes. It must be pointed out that the activites are measured under optimal conditions and do not reflect the true activities under physiological conditions. For example the fact that the activity of arginase is very much greater than that of ASS in the assays employed does not necessarily mean that there is more arginase protein present; the arginase reaction may just proceed at a much faster rate than that of ASS and to test whether the absolute amount of enzyme proteins does in fact differ, immunological assays using purified antibodies would have to be performed.

If the absolute amount of enzymes available does actually differ it does not nesessarily mean that the mRNA's are transcribed at different rates. The different amount of proteins may reflect different rates of translation of similar amounts of message, or combinations of rates of synthesis and degradation of mRNA's i.e. differential rates of transcription with differential rates of degradation of messages by ribonucleases, differential rates of translation of proteins with differential rates of proteolysis. Any combinations of the above could result in different amounts of enzyme proteins if these differences do exist.

APPENDIX

```
L.C0C1 C          CALIBRATION & ACTIVITY CALCULATION USING STRAIGHT LINE & EXPONENTIAL
L.0002 C          ***********************************************************
L.0003 C
L.0004 C          FITS TO EQN  CONC = M * ABS + C *   1 - EXP (-ABS/XO ) )
L.0005 C
L.0006            REAL M, MF
L.0007            DIMENSION XX(20), YY(20), HD1(10), HD2(10)
L.0008       21   FORMAT ( 4Y )
L.0009       22   FORMAT ( 10A4 )
L.0010       24   FORMAT ( 'C', 'CALIBRATION VALUES FOR ', 10A4 )
L.0011       24   FORMAT ( 'C', 'CONC = M * ABS + C * ( 1 - EXP (-ABS/XO) )' )
L.0012       25   FORMAT ( 'C', 'M=', 1PE9.2, /, ' C=', E9.2, /, ' XO=', E9.2 )
L.0013       26   FORMAT ( ' ', 'MEASURE', 1PE9.2 / )
L.0014       27   FORMAT ( 'C', ' ACTIVITIES IN MICROMOLS/(PEAK) FOR : ', 10A4 )
L.0015       28   FORMAT ( 'C', '    ABS 1', 4X, 'ABS 2', 4X, 'BACK 1', 3X, 'BACK 2', 6X, 'ACT', / )
L.0016       29   FORMAT ( ' ', 4F9.3, 3X, F9.3 )
L.0017       30   FORMAT ( // )
L.0018       31   FORMAT ( 'C', 6X, 'ABS', 8X, 'CONC', / )
L.0019       32   FORMAT ( 2F10.3 )
L.0020       33   FORMAT ( ' STR LINE FITTED TO LAST', I3, ' POINTS' )
L.0021       34   FORMAT ( 'C', 'FACTOR=', F9.3 )
L.0022       99   FORMAT ( ' NO. ON STR. LINE=', I3, 5X, 'RMS=', 1PE9.2 )
L.0023            SOLVF = 1.0E-6
L.0024 C
L.0025 C          READS CALIBRATION SUBSTANCE
L.0026 C
L.0027            READ ( 5, 22 ) HD1
L.0028            WRITE ( 7, 23 ) HD1
L.0029            WRITE ( 7, 31 )
L.0030 C
L.0031 C          READS NO. OF CALIBRATION POINTS
L.0032 C
L.0033            READ ( 5, 21 ) N
L.0034            DO 1 I = 1, N
L.0035 C
L.0036 C          . READS PAIRS OF ABSORBANCE & CONCENTRATION VALUES FOR CALIBRATION
L.0037 C
L.0038            READ ( 5, 21 ) XX(I), YY(I)
L.0039       1    WRITE ( 7, 32 ) XX(I), YY(I)
L.0040            NM1 = N - 1
L.0041            DO 10 NS = 2, NM1
L.0042            SX = 0
L.0043            SX2 = 0
L.0044            SY = 0
L.0045            SY = 0
L.0046            SXY = 0
L.0047            SX4 = 0
L.0048            SX2Y = 0
L.0049            N1 = N - NS + 1
L.0050            DO 2 I = N1, N
L.0051            X = XX(I)
L.0052            Y = YY(I)
L.0053            SX = SX + X
L.0054            SX2 = SX2 + X * X
```

```
L.0055          SXY = SXY + X * Y
L.0056    2     SY = SY + Y
L.0057          D = SX * SX - NS * SX2
L.0058          M = ( SY * SY - NS * SXY ) / D
L.0059          C = ( SY * SXY - SX2 * SY ) / D
L.005A          SX2 = 0
L.005B          SXY = 0
L.005C          NC = N - NS
L.005D          IF ( NC.GT.1 ) GO TO 51
L.005E          A = YY(1) / XX(1)
L.005F          GO TO 52
L.0060    51    DO 3 I = 1, NC
L.0061          X = XX(I)
L.0062          Y = YY(I)
L.0063          X2 = X * X
L.0064          SX2 = SX2 + X2
L.0065          SX3 = SX3 + X * X2
L.0066          SX4 = SX4 + X2 * X2
L.0067          SXY = SXY + X * Y
L.0068    3     SX2Y = SX2Y + X2 * Y
L.0069          D = SX2 * SX4 - SX3 * SX3
L.006A          A = ( SXY * SX4 - SX2Y * SX3 ) / D
L.006B          B = ( SX2Y * SX2 - SXY * SX3 ) / D
L.006C    52    XC = C / ( A - M )
L.006D          SDEVS = 1.0E50
L.006E          XCI = XC / 2
L.006F          XOF = 2 * XC
L.0070          DXC = XC / 100
L.0071          YC = XCI
L.0072    4     SDEV = 0
L.0073          DO 7 I = 1, N
L.0074          Y = XX(I)
L.0075          IF ( Y.LT.(2C*XC) ) GO TO 14
L.0076          Y = N * Y + C
L.0077          GO TO 15
L.0078    14    Y = N * Y + C * ( 1 - EXP ( -X/XC ) )
L.0079    15    DEV = YY(I) - Y
L.007A          IF ( SDEV.GT.1.0E16 ) GO TO 6
L.007B    7     SDEV = SDEV + DEV * DEV
L.007C          IF ( SDEV.LE.SDEV ) GO TO 8
L.007D          SDEVS = SDEV
L.007E          XCS = XC
L.007F    8     XC = XC + DXC
L.0080          IF ( XC.LT.XOF ) GO TO 4
L.0081          RMS = SQRT ( SDEVS / N )
L.0082          WRITE ( 6, 99 ) NS, RMS
L.0083          IF ( SDEV .LE. SDEVS ) GO TO 10
L.0084          SDEVS = SDEV
L.0085          XCS = XC
L.0086          NF = N
L.0087          CF = C
L.0088          XOFF = XCS
L.0089          NF = NS
L.008A    10    CONTINUE
L.008B C
L.008C C              WRITES CALIBRATION CONSTANTS
L.008D C
L.008E          WRITE ( 7, 24 )
L.008F          WRITE ( 7, 25 ) NF, CF, XOFF
L.0090          WRITE ( 7, 33 ) NF
```

```
L.0114        RMSF = SQRT ( SDEVF / N )
L.0115 C
L.0116 C          WRITES RMS DEVIATION
L.0117 C
L.0118        WRITE ( 7, 26 ) RMSF
L.0119 C
L.0120 C          READS SUBSTANCE FOR WHICH ACTIVITIES ARE TO BE CALCULATED
L.0121 C
L.0122        READ ( 5, 22 ) NO2
L.0123        WRITE ( 7, 27 ) NO2
L.0124 C
L.0125 C          READS NO. OF SETS TO BE ENTERED & FACTOR
L.0126 C
L.0127        READ ( 5, 21 ) NO, FAC
L.0128        WRITE ( 7, 34 ) FAC
L.0129        WRITE ( 7, 28 )
L.0130        DO 12 I = 1, NO
L.0131 C
L.0132 C          READS 2 VALUES OF ABSORBANCE & 2 OF BACKGROUND
L.0133 C
L.0134        READ ( 5, 21 ) ( XX(J), J = 1, 4 )
L.0135        DO 16 J = 1, 4
L.0136          IF ( XX(J).LT.(2C+)OFF1 ) GO TO 17
L.0137          YY(J) = MM * XX(J) + CF
L.0138        GO TO 16
L.0139   17   YY(J) = MM * XX(J) + CF + ( 1 - EXP ( -XX(J)/XOFF ) )
L.0140   16   CONTINUE
L.0141        Y = ( YY(1) + YY(2) ) / 2
L.0142        YO = ( YY(3) + YY(4) ) / 2
L.0143        ACT = ( Y - YO ) * FAC
L.0144 C
L.0145 C          WRITES ABSORBANCE VALUES & ACTIVITY
L.0146 C
L.0147   12   WRITE ( 7, 25 ) XX(1), XX(2), XX(3), XX(4), ACT
L.0148        WRITE ( 7, 30 )
L.0149        END
L.0150 /DATA
```

BIBLIOGRAPHY

ACS, Z., STARK, E. & FOLLY, G., *J. Steroid Biochem.*, 6: 1127–1130 (1975).

ADAMS, D.H., *Brit. J. Cancer*, 13: 704–710 (1959.

ANDERSON, P.M. & MEISTER, A., *Biochemistry*, 4: 2803 (1965).

ARCHIBALD, R.M., *J. Biol. Chem.*, 156: 121–142 (1944)

ARLEY, N.J., CARPENTER, J.W., MORRIS, H.P., *Proc. Soc. Exp. Biol. Med.*, 149: 900–902 (1975).

ASHIDA, K., & HARPER, A.E., *Proc. Soc. Exp. Biol Med.*, 107: 151 (1961).

AYUSO–PARILLA, M., PARRILLA, R., *Biochem. Biophys. Res. Commun.*, 52: 587–589 (1973).

BACH, S.J., & SIMON–REUSS, I., *Biochem. Biophys. Acta*, 11: 396–402 (1957).

BACH, S.J., SWAINE, D., *Brit. J. Cancer*, 19: 379–386 (1965).

BALINSKY, J.B. & BALDWIN, E., *Biochem. J.*, 82: 187–191 (1962).

BALINSKY, J.B., COETZEE, T.L., & MATTHEYSE, F.J., *Comp. Biochem. Physiol.*, 43: 83–95 (1972).

BARBIROLI, B. & POTTER, V.R., *Science*, 172: 738–741 (1971).

BARIL, E.F., JENKINS, M.D., BROWN, O.E., LASZLO, J., MORRIS, H.P., *Cancer Res.*, 33: 1187–1193 (1973).

BARON, D.N., BUCK, C.M., & FOXWELL, C.J., *Adv. in Enzyme Regulation*, 7: 325–336 (1969)

BEATO, M., BIESEWIG, D., BRAENDLE, W., & SEKERIS, C.E., *Biochem. Biophys. Acta.*, 192: 494–507 (1969).

BEATO, M., BIESEWIG, D., BRAENDLE, W., & SEKERIS, C.E., *BBA.*, 208: 125–136 (1970).

BEATO, M., KALIMI, M., KOSTAM, M., FRIGELSON, P., *Biochemistry*, 12 (18): 3372–2273 (1973)

BECK, W., BELLATONE, R.A., & CANELLAKIS, E.S., *Biochem. and Biophys. Research Communications*, 48: 1649–1655 (1972).

BERG, T.L., GOUTIER, R., *Arch. Int. Physiol. Biochem.*, 75: 36–48 (1967).

BIHDE, S.V., *J. Nat. Cancer Inst.*, 47: 707–800 (1971).

BOMBIK, B.M., & BURGER, M.M., *Exp. Cell Research*, 80: 88–94 (1973).

BOURGET, P.A., NATALE, P.J., & TREMBLAY, G.C., *Biochem. Biophys. Res. Commun.*, 45: 1109–1114 (1971).

BRADLEY, M.O., *Dev. Biol.*, 33: 1–17 (1973).

BRITTEN, R.J. & DAVIDSON, E.H., *Science* 165 (3891): 349–357 (1969).

BROWN, G.W. & COHEN, P.P., *J. Biol. Chem.*, 234: 1769–1774 (1959).

JRGESS, R., TRAVERS, A.A., DUNN, J.J., BAUTZ, E.F.K., *Nature*, 222: 537 (1969).

BURKE, W.T., *Cancer Res.*, 22: 10–14 (1962).

BURKE, W.T., MILLER, L., *Cancer Res.*, 16: 330 (1956).

BURNET, F.M., *Aust. N.Z. J. Med.*, 3: 71–77 (1973).

BURNETT, G.H., COHEN, P.P., *J. Biol. Chem.*, 229: 537–543 (1957).

BUTCHER, F.R. & GOLDMAN, R.H., *Biochem. Biophys. Research Commun.*, 48: 23–29 (1972).

BUTCHER, F.R., POTTER, V.R., *Cancer Res.*, 32: 2141–2147 (1972).

CHABAS, A., GRISOLIA, S., & SILVERSTEIN, R., *Eur. J. Biochem.*, 29: 333–342 (1972).

CHUAH, C.C., OLIVER, T.T., *Biochem.*, 10: 2990–3001 (1971).

CIVEN, M., ULRICH, R., TRIMMER, B.M., BROWN, C.B., *Science*, 157: 1563–1567 (1967).

COHEN, P.P., BROWN, G.W., in "*Comparative Biochemistry*" 2: 161–244 (1960) ed. Florkin and Mason, Academic Press, New York.

COHEN, P.P., BROWN, G.W., in *Proceedings of the 5th International Congress of Biochemistry* vol III, 129–137 (1963) Pergamon Press.

DARNELL, J.E., PHILIPSON, L., WALL, R., ADESNIK, M., *Science*, 174: 507–510 (1971).

DAS, T.K., WATERLOW, J.C., *Br. J. Nutr.*, 32: 353–373 (1974).

DE GRONCHY, J., *Biomedicine* (Paris) 18: 6–8 (1973).

DENENBERG, V.H., *Psychol. Rev.*, 71: 335 (1965).

DESSER–WIEST, L., *Cell Tissue Kinet.*, 8: 1–9 (1975).

DESSER–WIEST, L., *Hormone Metab. Res.*, 9: 97–98 (1976).

DOESTHALE, Y.G., & TULPULE, P.G., *Indian J. Biochem.*, 6: 115–120 (1969).

DOLAN, M.L., COETZEE, M.L., SPANGLER, M., & OVE, P., *Cancer Res.*, 34: 3010–3017 (1974).

DONOVAN, G., & OLIVER, I.T., *Biochem.*, 6: 552–557 (1972).

DONOVAN, G., OLIVER, I.T., *Biochem.*, 11: 3904–3909 (1972b).

EGYHAZI, E., *Nature*, 250: 221–223 (1974).

EIL, C., WOOL, I.G., *J. Biol. Chem.*, 248 (14): 5122–5129 (1973b).

EIL, C., WOOL, I.G., *J. Biol. Chem.*, 248 (14): 5130–5136 (1973).

FAUSTO, N., NELSON, T., VAN LANKER, J.L., *Chim. Biophys, Acta.*, 16: 32–40 (1968).

FAUSTO, N., BRANDT, J.T., & KESNER, L., *Cancer Res.*, 35: 397–404 (1975).

FEIGELSON, P., FEIGELSON, M., & GREENGARD, O., *Recent Prog. Horm. Res.*, 18: 491–512 (1962).

FERDINANDUS, J.A., MORRIS, H.P., WEBER, G., *Cancer Res.*, 31: 550–556 (1971).

FREEDLAND, R.A., SZEPESI, B., in *"Enzyme Synthesis and Degradation in Mammalian Systems"* (Karger Basel) pp 103–140 (1971).

FUJIWARA, H., *Physiol. Chem.*, 185: 1 (1929).

GARRARD, W.T., BONNER, J., *Fed. Proc.*, 32: 538 Abs (1973).

GASIOROWSKA, I., *Acta Biochem. Pol.*, 14: 1 (1970).

GASWAMI, M.N.D., & CHATAGNER, F., *Experimentia*, 22: 1–5 (1966).

GERHARD, H., in *"Liver regeneration after experimental injury"*, R. Lesch eds, Intercontinental book publishers pp 340–346 (1975).

GLASER, S.R., *Methods in Enzymology*, 36: 174–481 (1975).

GLASS, K.D., & KNOX, W.E., *J. Biol. Chem.*, 248: 5785 (1973).

GODWIN, E., & RESS, K., *J. Cancer*, 21: 166–173 (1967).

GOLDER, M.P. & BOYNS, A.R., *Biochem. J.*, 129: 79 (1972).

GREENGARD, O., SAHIB, M.K., & KNOX, W.E., *Arch. Biochem. Biophys.*, 137: 447–482 (1970).

GREENSTEIN, J.P., & MIDER, W., *J. Nat. Cancer Inst.*, 1: 687 (1941).

GRISOLIA, S., & COHEN, P.P., *Fed. Proc.*, 11: 222–223 (1952).

GUTTÖRILEIN, G., KNAPPE, J., *Eur. J. Biochem.*, 7: 119–127 (1968).

HAGAR, S.E., JONES, M.E., *J. Biol. Chem.*, 242: 5667–5673 (1967).

HART unpublished (1977).

HENDERSON, I.C., FISCHEL, R.E., LOEB, J.N., *Endocrinol.*, 88: 1471–476 (1971).

HENDERSON, I.C., & LOEB, J.N., *Nature*, 228: 556–557 (1970).

HERZFELD, A., RAPER, S.M., *Biochem. J.*, 153: 469–478 (1976).

HIGGINS, G.M., & ANDERSON, R.M., *Arch. Pathol.*, 12: 86 (1931).

HIGGINS, S.J., ROUSSEAU, G.G., BAXTER, J.D., & TOMKINS, G.M., *J. Biol. Chem.*, 248: 5873–5879 (1973).

HIRSH–KOLB, H., & GREENBERG, D.M., *J. Biol. Chem.*, 243: 6123 (1968).

HOLLEY, R.W., *Biochem. Biophys. Acta.*, 145: 525–527 (1967).

HOLT, P.G., & OLIVER, I.T., *Biochemistry*, 8: 1429–1437.

HÖLTTA, E., *Biochem. Biophys. Acta.*, 399: 420–427 (1975).

HÖLTTA, E., & JÄNNE, J., *Fed. Eur. Biochem. Soc. Le Herrs*, 23: 117–121 (1972).

HOLTZER, H., DENKEN, K., in *"Liver Regeneration after Experimental Injury"* eds. Lesch, R., Reutter, W., Intercontinental Publishers, N.Y. pp.103–118 (1975).

HOPKINS, H.A., CAMPBELL, H.A., BARBIROLI, B., & VAN R. POTTER, *Biochem. J.*, 136: 955–958 (1973).

HOSHINO, J., KÜHNE, U., FILJAK, B., & KRÖGE, H., *FEBS Letters*, 56: 62–65 (1975).

HOSOYANI, C., *Eur. J. Biochem.*, 27(1): 48–52 (1971).

HRADIL, J., MELKA, J., SIMEK, J., LEJSEK, K., *Rev. Int. Hepatol.*, 15: 1119–1130 (1965).

ILLNEROVA, H., *Physiologia Bohemoslaved*, 15: 19–26 (1966).

JÄNNE, J., HÖLTTA, E., & HANNONEN, P., in *"Liver Regeneration after experimental injury"*, R. Lesch eds. Intercontinental Book Publishers 230–240 (1975).

JOHNSON, E.M. & ALLFREY, V.G., *Archives of Biochem. and Biophys.*, 152: 786–794 (1972).

JONES, M.E., *Ann. Rev. Biochem.*, 34: 381–384 (1965).

JONES, M.E., *Adv. Enz. Regul.*, 9: 19–49 (1970).

JONES, M.E., *Current topics in cellular regulation*, 6: 227–265 (1972).

JONES, M.E., ANDERSON, A.D., ANDERSON, C., HODDES, S., *Arch. Biochem. Biophys.*, 95: 499–507 (1961).

JONES, R.B., & LOGAN, I.J., *Archives of Biophys. and Biochem.*, 152: 828 (1972).

JOHNSTONE, M., *Experimentia*, 23: 152–154 (1967).

KALF, G., DAGOUSTINA, M., HUNTER, G., *Cancer Res.*, 31: 2054–2058 (1971).

KALIMI, M., BEATO, M.D., PEIGELSON, P., *Biochem.*, 12 (18): 3365–3371 (1973).

KAMPSCHMIDT, R.F. & SCHULTZ, G.A., *Cancer Res.*, 23: 751 - 755(1963).

KAMURA, A., ASUSHI, T., *Sapporo Med. J.*, 35: 343 - 360 (1969).

KENNEN, A.L., & COHEN, P.P., *Dev. Biol.*, 1: 511 - 525 (1959).

KENNEY, F.T., *Science*, 156: 525 (1967).

KENNEY, F.T., in *Mammalian Protein Metabolism IV* pp 131–176 (1970) eds. H.N. Munro, Academic Press.

KERSON, L.A., & APPEL, J.H., *J. Biol. Chem.*, 243: 4279 - 4285 (1968).

KIM, Y.S., KIM, Y., *J. Biol. Chem.*, 750 (6): 2293 - 2298 (1975).

KIMBERG, D. ., ROEB, J.N., *Biochem. Biophys. Acta*, 246: 412 - 420 (1971).

KOLB, H., KOLB, H.J., & GREENBERG, D.M., *J. Biol. Chem.*, 246: 395 (1971).

KREBS, H.A., HEMS, R., & LUND, P., in *"Inborn errors of metabolism"* Hommes, F.A., & V. Denberg eds. Academic Press New York. 361 - 377, (1973).

KREBS, H.A., HENSELEIT, K., *Z. Physiol. Chem.*, 210: 33 - 66 (1932).

KRČÉK, J., & PALATÝ, V., *Physiologia Bohemoslovaca*, 16: 501 - 507 (1967).

KRUGH, T.R., NEELY, J.W., *Biochemistry*, 12 (22): 4418 - 4425 (1973).

KRUGH, T.R., NEELY, J.W., *Biochemistry*, 12 (9): 1775 - 1782 (1973a).

LA BRECQUE, D.R., PESCH, L.A., BACHNER, N.R., *Fed. Proc.*, 32: 538 Abs. (1973).

LAWSON, D., PAIK, W.K., MORRIS, H.P., WEINHOUSE, S., *Cancer Res.*, 35: 156 - 163 (1975).

LAWSON, D., PAIK, W.K., MORRIS, H.P., WEINHOUSE, S., *Can Research*, 37: 850 - 856 (1977).

LAYNE, E., *Method. in Enzymology*, 3: 448 (1957).

LEA, M.A., SASOVLIZ, D., MUSELLA, A., & MORRIS, H.P., *Cancer Res.*, 30: 1994 - 1999 (1970).

LEE, K.L., REEL, J.R., & KENNEY, F.T., *J. Biol. Chem.*, 245: 5806 - 5812 (1970).

LEPORDA, G., ARATEL, H., HALLER, C., *Rev. Med. Chir. Soc. Med. Nat IASI.*, 76: 147 - 150 (1972).

LEVINE, S., & MULLINS, R.E., *Science*, 152: 1585 - 1592 (1966).

LIN, J. CHEN, V., HORNG, S., TUNG, T., *J. Formosan Med. Assoc.*, 72: 263 - 266 (1973).

LITWACK, G., FILLER, R., ROSENFIELD, S.A., LICHTASH, N., WISHMAN, C.A., SINGER, S., *J. Biol. Chem.*, 248: 7481 - 7486 (1973)

LITWACK, G., & ROSENFIELD, S.A., *J. Biol. Chem.*, 250: 6799–6805 (1975).

LOEB, J.N., TOLENTINO, E.M., *Endocrinol.*, 86: 1033–1038 (1970).

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L., & RANDALL, R.J., *J. Biol. Chem.*, 193: 265 (1951).

LOWENSTEIN, W.R., PENN, R.D., *J. Cell. Biol.*, 33: 235–242 (1967).

LOWENSTEIN, W.R., & KANNO, Y., *J. Cell. Biol.*, 33: 225–234 (1967).

LUSTY, C.J., RATNER, S., *J. Biol. Chem.*, 247: 7010–7022 (1972).

MACMANUS, J.P., BRACELAND, B.M., YOUDALE, T., & WHITFIELD, J.F., *J. Cell Physiol.*, 82: 157–164 (1973).

MANGANIELLO, V. & VAUGHN, M., *J. of Clin. Invest.*, 51: 2763 (1972).

MARLEY, C.G.D., KUKU, S., RUBENSTEIN, A.H. BOYER, H., *Biochem. Biophys. Res. Commun.*, 67: 653–661 (1975).

MARSHALL, M., & COHEN, P.P., *J. Biol. Chem.*, 247: 1641–1669 (1972).

MARSHALL, M., METZENBERG, R.L., & COHEN, P.P., *J. Biol. Chem.*, 233: 102–105 (1958).

MARSHALL, M., METZENBERG, R.L., COHEN, P.P., *J. Biol. Chem.* 236: 2229–2237 (1961).

MATTHEYSE, F.J. Thesis unpublished (1977).

McLEAN, P., *Biochem. J.*, 91: 464–473 (1964).

McLEAN, P., & BROWN, J., *Biochem. J.*, 100: 793–800 (1966).

McLEAN, P., & GURNEY, M.W., *Biochem. J.*, 8: 86–104 (1963).

McLEAN, P., & NOVELLO, F., *Biochem. J.*, 94: 410–422 (1965).

MERCKER, H.J., *Histochemie*, 17: 83 (1969).

MEYER, D.I., & BARBER, A.A., *Chem.-Biol. Interactions*, 7: 231–240 (1973).

METZENBERG, R.L., HALL, L.M., MARSHALL, M., & COHEN, P.P., *J. Biol. Chem.*, 236: 2229–2237 (1957).

MIDER, G.B., *Cancer Res.*, 11: 821 (1951).

MILLER, A.L., & CHU, P., *Enzy. Biol. Clin.*, 11: 497–503 (1970).

MITZUTANI, A.J., *Histochem. Cytochem.*, 16: 172 (1968).

MODJANOVA, E.A., MALENKOV, A.G., *Exp. Cell Research*, 76: 305–314 (1973).

MOORE, P., IORIO, L., McMANUS, J., *J. Pharm. Pharmacol.*, 20: 368–370 (1968).

MORA, J., MARTUSCELLI, J., ORTIZ-PINEDA, J., & SOBERON, G., *Biochem. J.*, 96: 28–35 (1965).

MORRIS, H.P., DYER, H.M., WAGNER, B.P., MIYAJI, H., RECHEIGL, M., *Adv. Enz. Regul.*, 2: 321–333 (1964).

MORTIMORE, G.E., in Handbook of Physiology-Endocrinology, Endocrine Pancreas p 495 (1972) Eds. Steiner, D.F., Freinkel, N., Washington: American Physiological Society.

MOSHER, K.M., YOUNG, D.A., MUNCK, A., *J. Biol. Chem.*, 246: 654–659 (1971).

MOXLEY, M.A., BELL, N.H., WAGLE, S.R., ALLEN, D.O., ASHORMORE, J., *American J. Physiology*, 227: 1058–1061 (1974).

MOYER, G.H., PITOT, H.C., *Cancer Res.*, 33: 1316–1325 (1973).

MURAMATSU, K., & NAKAGAWA, T., *Agric. Biol. Chem.*, 35 (10) : 1594–1602 (1971).

NAKANISHI, S., ITO, K., & TATIBANA, M., *Biochem. Biophys. Res. Commun.*, 33: 774–781 (1968).

NOGUCHI, T., KASHIWAYI, A., & TANAKA, T., *Cancer Res.*, 36: 4015–4022 (1976).

NORDLIE, R.C., BARRICCHIO, F.E., & FOLTEN, D.D., *Biochem. Biophys. Acta.*, 92: 214–221 (1965).

NUDEL, U., LEBLEU, B., & REVEL, M., *Proc. of the National Acad. of Sci.*, 70: 2139–2144 (1973).

NUZUM, G.T., & SNODGRASS, P.J., *Science*, 172: 1042–1044 (1971).

OLSEN, R.E., *Am. J. Clin. Nutr.*, 28 : 262–337 (1975).

O'MALLEY, B.W., & MEANS, A.R., *Science*, 183 (4125) : 610–620 (1974).

O'MALLEY, B.W., & SCHRADER, W.T., *Scientific American*, 234 (2): 32–43 (1976).

O'MALLEY, B.W., SHERMAN, M.R., & TOFT, D.A., *Proc. Natl. Acad. Sci. (U.S.A.)*, 67 : 501 (1970).

ONO, T., BLAIR, G.R., POTTER, V.R., & MORRIS, H.P., *Cancer Res.*, 23: 240–249 (1963).

OUELLETTE, A.J., TAYLOR, M.W., *Biochem.*, 12: 3542–3546 (1973).

PENMAN, S., ROSBACH, M., PENMAN, M., *Proc. Nat. Acad. Sci., (U.S.A.)*, 67: 1878–1885 (1970).

PERAINO, C., & PITOT, H.C., *J. Biol. Chem.*, 240: 3029–3043 (1964).

PETRACK, B., & RATNER, S., *J. Biol. Chem.*, 233 : 1493 (1958).

PITOT, H.C., PERAINO, C., PRIES, N., KENNAN, A.L., in *Adv. in Enzyme Regul.*, 2: 237–247 (1964).

PITOT, H.C., YATVIN, M.B., in *Mammalian Hormones and Enzymes*, 53: 228–325 (1973).

POGO, A.O., *Proc. Natl. Acad. Sci.*, (U.S.A.), 56: 66 (1969).

POLITZER, J.F. GEIDUSCHECK, E.P., *J. Mol. Biol.*, 49: 489–507 (1970).

POTTER, V.R., *Canadian Cancer Conf.*, 8: 9–30 (1969).

POTTER, V.R., WALKER, P.R., GOODMAN, J.I., *Gann Monograph on Cancer Research*, 13: 121–134 (1972).

POTTER, V.R., WATENABE, M., PITOT, H.C., MORRIS, H.P., *Cancer Res.*, 29: 55–78 (1969).

PRONCZUK, A.W., BALIGA, B.S., TRIANT, J.W., & MUNRO, H.N., *Biochem. Biophys. Acta.*, 157: 204–206 (1968).

RALL, T.W., SUTHERLAND, E.W., *J. Biol. Chem.*, 232: 1065–1075 (1958).

RÄIHÄ, N.C.R., SCHWARTZ, A.L., in *"Inborn Errors of Metabolism"* (Homms, F.A. and V. Denberg, C.J. eds) Academic Press London and New York (1973).

RATNER, S., *Methods in Enzymology*, 39: 1 (1955).

RATNER, S., *Adv. Enzymol.*, 39: 1–91 (1973).

RATNER, S., & PETRACK, B., *J. Biol. Chem.*, 191: 693 (1951).

RATNER, S., & PETRACK, B., *J. Biol. Chem.*, 100: 175 (1953).

REICHARD, P., *Acta Chem. Scand.*, 11: 523 (1957).

REYERO, C., HESSER, U., STOCKE, N., *Int. J. Biochem.*, 5: 437–441 (1974).

RIBARAC-STEPIĆ, N., TRAJKOVIĆ, D., & KANAZIR, D., *Steroids*, 22: 155–168 (1973).

RIXON, R.H., WHITFIELD, J.F., *Proc. Soc. Exp. Biol. Med.*, 141: 93–97 (1972).

ROCHOVANSKY, O., RATNER, S., *J. Biol. Chem.* 236: 2254 (1961).

ROCHOVANSKY, O., RATNER, S., *J. Biol. Chem.*, 242: 3839 (1967).

RODBELL, M., BIRNBAUMER, L., POHL, S.L., SUNDBY, S., *Proc. Nat. Acad. Sci. (U.S.A.)*, 68: 909–913 (1971).

RODBELL, M., *Fed. Proc.*, 32 (8): 1854–1858 (1973).

ROURKE, A.W., & HEYWOOD, S.M., *Biochemistry*, 11: 2061–2066 (1972).

ROUSSEAU, G.G., *J. Steroid Biochem.*, 6: 75–89 (1975).

RUDACK, D., DAVIE, B., HOLTEN, D., *J. Biol. Chem.*, 246: 7823–7824 (1971).

RUSSEL, D.H., & LAMBARDINI, J.B., *Biochem. Biophys. Acta.*, 240: 273–286 (1971).

RUSSEL, D.H., & SNYDER, S.H., *Proc. Nat. Acad. Sci (U.S.A.)*, 60: 1420 (1968).

RUTTER, N.J., BLOSTEIN, R.E., WOODFIN, B.M., & WEBER, C.S., *Adv. in Enzyme Regul.*, 1: 39–56 (1963).

SANADA, Y., SUEMORI, I., KATUNUMA, N., *Biochem. Biophys. Acta.*, 220: 42–50 (1970).

SANGDUK, K., PACK, W.K., COHEN, P.P., *Proc. Nat. Acad. Sci. (U.S.A.)*, 69 (12) : 3530–3533 (1972).

SANGER, F., AIR, G.M., BARRELL, B.G., BROWN, L.N., COULSON, A.R., FIDDES, J.C., HUTCHISON III, C.A., SLOCOMBE, P.M., & SMITH, M., *Nature*, 265: (5595) 687–695 (1977).

SAKAI, A., *Nature*, 228: 1186–1187 (1970).

SAKUMA, K., & TERAYAMA, H., *J. Biochem.*, 61: 504–511 (1967).

SAPAG–HAGAR, M.S., MARCO, R., SOLS, A., *FEBS Letters*, 3: 68–71 (1969).

SASAKI, K., DOCKERILL, S., ADAMIAK, D.A., TICKLE, I.J., BLUNDELL, T., *Nature*, 257 (5529) : 751–765 (1975).

SCHIMKE, R.T., *J. Biol. Chem.*, 237: 459–468 (1962).

SCHIMKE, R.T., *J. Biol. Chem.*, 237 (6) : 1921–1924 (1962b).

SCHIMKE, R.T., *J. Biol. Chem.*, 238: 1012–1018 (1963).

SCHIMKE, R.T., *J. Biol. Chem.*, 239: 3808–3817 (1964).

SCHIMKE, R.T., & DOYLE, D., *Am. Rev. of Biochem.*, p 930–976 (1970).

SCHMIDTKE, J., WIENKER, T.H., FLÜGEL, M., ENGEL, W., *Nature*, 262: 593–594 (1976).

SCHULTE–HERMAN, R., in *"Liver regeneration after experimental injury"*, R. Lesch eds, Intercontinental book publishers, pp 315–[illegible] (1975).

SCHULZE, I.T., LOWTY, C.J., RATNER, S., *J. Biol. Chem.*, 245: 4534–4543 (1970).

SCHUIT, K.E., & DICKIE, M.W., *Biol. Neonate*, 23: 171–179 (1973).

SCHWARTZ, A.L., *Biochem. J.*, 126: 89–98 (1972).

SCHWARTZ, R.S., *Nature New Biol.*, 237: 121 (1972).

SCORNIK, O.A., *J. Biol. Chem.*, 249: 3876–3883 (1974).

SCOTT, J.C., *Science*, 138: 949–952 (1962).

SELAWRY, H., GUTMAN, R., FINK, G., RECANT, L., *Biochem. Biophys. Res. Commun.*, 51: 98–203 (1973).

SHEA, M., & KLEINSMITH, L.J., *Biochem. and Biophys. Res. Commun.*, 50: 473–479 (1973).

SHELTON–EARP, H., *Biochem. Biophys. Acta.*, 340: 95–107 (1974).

SHEPHARD, E.A., Thesis "Studies on rat liver carbamyl phosphate synthetase" (1975).

SHIBA, S., MOTSUYASHI, H., & MIYATAKE, M., *Gann*, 56: 121–129 (1964).

SHIGESADA, K., & TATIBANA, M., *Biochem. Biophys. Res. Commun.*, 44: 1117–1124 (1971a).

SHIGESADA, K., TATIBANA, M., *J. Biol. Chem.*, 246: 5588–5595 (1971b).

SHIMBAYASHI, K., Natl. Inst. Anim. Health (Tokyo), 12: 212–213 (1972).

SHORT, J., ARMSTRONG, N.B., GAZA, D.J., LIEBERMAN, I., in *"Liver Regeneration after Experimental Injury"*, Eds Lesch, R., Intercontinental Book Publishers, (1975), 296–308.

SIEV, M., WEINBERG, R., PENMAN, S., *J. Cell. Biol.*, 41: 510–520 (1969).

SNART, R.A., SANYAL, N.N., & AGARWAL, M.K., *J. Endocrinol.*, 47: 149–158 (1970).

SNODGRASS, P.J., *Biochemistry*, 7: 3047 (1968).

SNODGRASS AND LIN, *Federation Proceedings*, 35: 1428 (1976).

STIRLING, G.A., LAUGHLIN, J., WASHINGTON, S.L.A., *Exp. and Molec. Pathol.*, 19: 44–52 (1973).

STRADA, S.J., ROBISON, G.A., in *Current topics in Experimental Endocrinology*, 2: 2–36 (1974). Eds. Jones, V.H.T. and Martini, L., Academic Press incorporated.

SUDA, M., TANAKI, T., SUE, F., KURODA, K., & MORIMURA, H., *Gann*, 1: 127–141 (1966).

SUGIMURA, T., MATSUSHIMA, T., KAWACHI, T., KOGURE, K., RANAKE, N., MIYAKA, S., SATO, H., *Gann Monograph of Cancer Research*, 13: 33–45 (1972).

SUNSHINE, G.H., WILLIAMS, D.S., RABIN, B.R., *Nature New Biol.*, 230: 133–136 (1971).

SZILAGI, A.K., *J. Neurol. Sci.*, 18: 143–154 (1973).

TABOR, H., & TABOR, C.W., *Adv. Enz. Regul.*, 36: 203–268 (1972).

TANAKA, T., HARANO, Y., SUE, F., & MORIMURA, H., *J. Biochem.*, 62: 71–91 (1967).

TANAKA, T., YANAMA, S., MIYAHARA, M., KAKU, R., IMAMURA, K., TANIUCHI, K., & SUDA, M., *Gann*, 63: 555–562 (1972).

TATIBANA, M., SHIGESADA, K., *J. Biochem.*, 72: 113–147 (1972).

TATIBANA, M., SHIGESADA, K., in *"Advances in Enzyme Regulation"*, 10: 249–271 (1972).

TEWS, J.K., WOODCOCK, N.A., & HARPER, A.E., *J. Biol. Chem.*, 245: 3026–3032 (1970).

THOMSON, E.B., LIPPMAN, M.E., *Progress in Endocrinology and Metabolism*, 23 (2): 159–202 (1974).

TOMKINS, G.M., GELEHRTER, T.D., GRANNER, D., MARTIN, D., SAMUELS, H.H., & THOMPSON, E.B., *Science*, 166: 1974–1980 (1969).

TOPOREK, M., *Cancer Res.*, 29: 1267–1271 (1969).

TRAVERS, A., *Nature*, 263: 641–646 (1976).

TSAI, Y.H., & HNILICA, L.S., *Biochem. Biophys. Acta.*, 238: 277–287 (1971).

UTSUNOMIYA, T., TAKESHI, T., *J. Cell. Biol.*, 29: 387–403 (1966).

VANDEN DRIESSCHE, T., *Biosystems*, 6: 188–201 (1975).

VIRAGH, A., *Am. J. Pathol.*, 49: 825–839 (1966).

VIRDEN, R., *Biochem. J.*, 127: 503–508 (1972).

WALKER, P.R., & POTTER, V.R., *Adv. in Enzyme Regulation*, 10: 339–364 (1972).

WANNEMACHER, R.N., WANNEMACHER, C.F., YATVIN, M.B., *Biochem. J.*, 124: 385–392 (1971).

WATERLOW, J.C., STEPHEN, J.M.L., *Clin. Sci.*, 35: 287–305 (1968).

WATSON, G., & LANGAN, T.A., *Federation Proceedings*, 33: 588 Abs (1973).

WEBER, G., *Gann. Monograph.*, 1: 151–178 (1966).

WEBER, G., *Am. Assoc. Cancer Res. Proc.*, 14: 73 (1973).

WEBER, G., QUEENER, S.F., MORRIS, H.P., *Cancer Res.*, 32: 1933–1940 (1972).

WEBER, G., QUEENER, S.F., FERDINANDUS, J.S., *Adv. Enz. Regul.*, 9: 63–95 (1971).

WEBER, G., *Gann Monograph of Cancer Research*, 13: 47–77 (1972).

WEIL, L., *J. Biol. Chem.*, 110: 201 (1935).

WICKS, W.D., *Science*, 160: 997–998 (1968a).

WICKS, W.D., In *"Regulatory Mechanisms for Protein Synthesis in Mammalian Cells"*, edited by M. Lamberg, A. San Pietro, and P.T. Kenney, pp 143–155, 1968b, Academic Press N.Y.

WICKS, W.D., *J. Biol. Chem.*, 246: 217–223 (1971).

WICKS, W.D., LEWIS, W., McKIBBIN, J.B., *Biochem. Biophys. Acta.*, 264: 177–185 (1972).

WICKS, W.D., VAN WIJK, R., & McKIBBIN, J.B., *Adv. in Enz. Regulation*, 11: 117–135 (1973).

WICKS, W.D., in *Advances in Cyclic Nucleotide Research*, 4: 335–348 (1974) ed. P. Greengard and G.A. Robinson, Raven Press, New York.

WICKS, W.D., in *"The Biochemistry of Hormones"*, vol. 8, 211–214, Eds. Rickenberg, H.V., Butterworths, University Park Press. (1974b).

WILLIAMS-ASHMAN, H.G., COPPOC, G.L., WEBER, G., *Can Res.*, 32: 1924–1932 (1972).

WILLIAMSON, D.H., LUND, P., KREBS, H.A., *Biochem. J.*, 103: 514–526 (1967).

WILLIAMSON, J.R., GIMPEL, J.A., DELEEW, G.J., HENSGENS, H.E.S.J., MEYER, A.J., & TAGER, J.M., *Physiol. Chem.*, 355: 1270 (1974).

WIRA, C., & MUNCK, A., *J. Biol. Chem.*, 245: 3436–3438 (1970).

WIXOM, R.C., REDDY, K.M., COHEN, P.P., *J. Biol. Chem.*, 247: 3684 (1972).

WU, C., *Int. J. Cancer*, 4: 438–447 (1973).

WU, C., BAUER, J.M., MORRIS, H.P., *Cancer Res.*, 31: 12–18 (1971).

WU, C., ROBERTS, E.H., BAUER, J.M., *Cancer Res.*, 27: 956–961 (1967).

WUNNER, W.H., BELL, J., & MUNRO, H.N., *Biochem. J.*, 101: 417–428 (1966).

WURTMAN, R.J., *Adv. Enz. Regul.*, 7: 57–67 (1969).

WURTMAN, R.J., AXELROD, J., *Proc. Nat. Acad. Sci. (U.S.A.)*, 57: 1594–1598 (1967).

YANAGI, S., & POTTER, VAN R., in press.

YEOMAN, L.C., TAYLOR, C.W., BUSCH, H., *Biochem. Biophys. Res. Commun.*, 51: 956–966 (1973a).

YEOMAN, L.C., TAYLOR, C.W., JORDAN, J.J., BUSCH, H., *Biochem. Biophys. Res. Commun.*, 53: 1067–1076 (1973b).

YIP, M.C.M., & KNOX, W.E., *J. Biol. Chem.*, 245: 2199–2204 (1970).

YIP, M.C.M., KNOX, W.E., *J. Biochem., Tokyo*, 127: 893–899 (1972).

Author Brebnor Linda Denise
Name of thesis The Activities Of The Urea Cycle Enzymes In Control, Regenerating And Neoplastic Rat Liver. 1977

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.