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The effecta of 5-bromodeoxyyuridine and 5-fluorodeoxyuridine on differentiation and metamorphosis in xenopus laevis tadpoles

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3.2.7. Detailed analysis of immature hindlimbs

Figure 3.18 *Immature hindlimbs*

3. IMMATURE LIMBS

S shape
T toes

Deformity Index	Start stage 47
24 — 12 = 12	0,2 µg/ml 5-FUdR

S
T — 12 (missing 3)

Left





Deformity Index	Start stage 47
24 — 12 = 12	0,2 µg/ml 5-FUdR

S
T — 12 (missing 3)



Right



Deformity Index	Start stage 46
24 — 12 = 12	10 µg/ml 5-FUdR

S
T — 12 (missing 3)



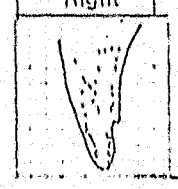
Left



Deformity Index	Start stage 43
24 — 12 = 12	0,2 µg/ml 5-FUdR

S
T — 12 (missing 3)

Right





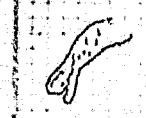
S shape.
T toes

Deformity Index	start stage 43
24 — 14 = 10	0,2 µg/ml 5-FUdR 0,01 mg/ml 5-BUdR

S – 2
T – 12 (missing 3)



Left



Deformity Index	start stage 43
24 — 14 = 10	0,2 µg/ml 5-FUdR 0,01 mg/ml 5-BUdR



S – 2
T – 12 (missing 3)

Right

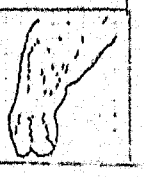


Deformity Index	start stage 47
24 — 12 = 12	0,2 µg/ml 5-FUdR



S
T – 12 (missing 3)

Left



Deformity Index	start stage 47
24 — 12 = 12	0,2 µg/ml 5-FUdR



S
T – 12 (missing 3)

Right



S shape
T toes

Deformity Index	Start stage 46
24 — 8 = 16	10 µg / ml 5-FUdR

S

T - 8 (missing 2)



Right



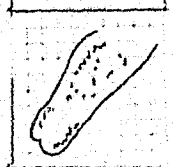
Deformity Index	Start stage 52
24 — 14 = 10	20 µg / ml 5-FUdR

S - 2

T - 12 (missing 3)



Left



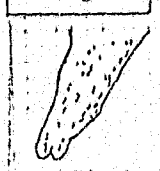
Deformity Index	Start stage 52
24 — 14 = 10	20 µg / ml 5-FUdR

S - 2

T - 12 (missing 3)



Right



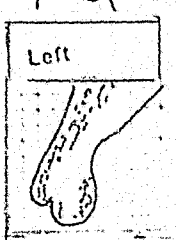
S shape
T toes

Deformity Index	Start stage 43
24 — 10 = 14	0,2 µg/ml 5-FUdR 0,01 mg/ml 5-BUdR

S — 2

T — 8 (missing 2)

Left



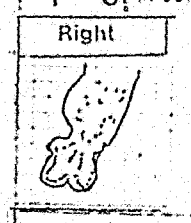


Deformity Index	Start stage 43
24 — 10 = 14	0,2 µg/ml 5-FUdR 0,01 mg/ml 5-BUdR

S —2

T —8 (missing 2)

Right





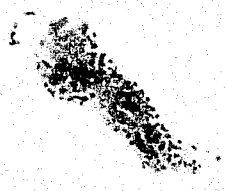
Deformity Index	Start stage 46
24 — 10 = 14	2µg/ml 5-FUdR

S —2

T —8 (missing 2)

Left





Deformity Index	Start stage 46
24 — 10 = 14	2µg/ml 5-FUdR

S —2

T — 8 (missing 2)

Right





S. shape
T. toes

Deformity Index	Start stage 47
24 — 10 = 14	20 μg/ml 5-FUdR 0.01 mg/ml 5-BUdR

S — 2

T — 8 (missing 2)

Left



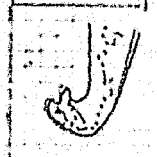


Deformity Index	Start stage 47
24 — 14 = 10	20 μg/ml 5-FUdR 0.01 mg/ml 5-BUdR

S — 2

T — 12 (missing 3)

Right





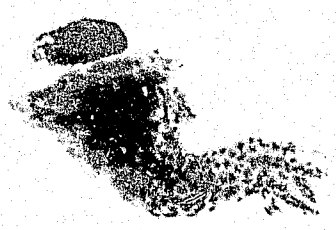
Deformity Index	Start stage 47
24 — 8 = 16	0.2 μg/ml 5-FUdR

S

T — 8 (missing 2)

Left





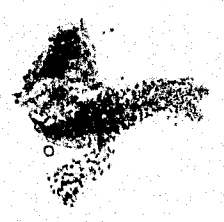
Deformity Index	Start stage 47
24 — 8 = 16	0.2 μg/ml 5-FUdR

S

T — 8 (missing 2)

Right





S shape
T toes

Deformity Index	Start stage 43
24 — 4 = 20	0,2 µg/ml 5-FUdR 0,01 mg/ml 5-BUdR

S

T-4(missing 1)



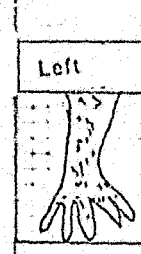


Deformity Index	Start stage 47
24 — 2 = 22	20 µg/ml 5-FUdR 0,01 mg/ml 5-BUdR

S - 2

T

Left





Deformity Index	Start stage 47
24 — 0 = 24	2µg/ml 5-FUdR 0,01 mg/ml 5-BUdR

S

T

Left





S shape L toes	
Deformity Index 24 — 1 = 23	Start stage 47 2μg/ml 5-FUdR 0.01 mg/ml 5-BUdR

S — 1

T

Left	
	
Deformity Index 24 — 1 = 23	Start stage 47 2μg/ml 5-FUdR 0.01 mg/ml 5-BUdR

S — 1

T

Right	
	
Deformity Index 24 — 1 = 23	Start stage 48 10μg/ml 5-FUdR

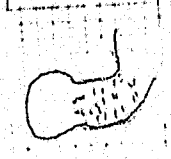
S — 1

T

Left	
	
Deformity Index 24 — 1 = 23	Start stage 48 10μg/ml 5-FUdR

S — 1

T

Right	
	

shape
toes

Deformity Index	Start stage 47
24 — 1 — 23	2μg/ml 5-FUdR 0.01 mg/ml 5-BUdR

S — 1

T



Left



Deformity Index	Start stage 47
24 — 1 = 23	2μg/ml 5-FUdR 0.01 mg/ml 5-BUdR

S — 1

T



Right



Deformity Index	Start stage 50
24 — 2 = 22	20 μg/ml 5-FUdR



S — 2

T

Left



Deformity Index	Start stage 50
24 — 2 = 22	20 μg/ml 5-FUdR



S — 2

T

Right



S shape
T toes

Deformity Index	Start stage 47
24 — 1 = 23	2μg/ml 5-FUdR 0.01 mg/ml 5-BUdR

S – 1
T



Left



Deformity Index	Start stage 47
24 — 1 = 23	2μg/ml 5-FUdR 0.01 mg/ml 5-BUdR

S – 1
T

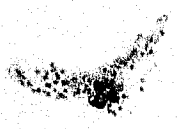


Right

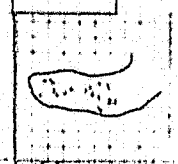


Deformity Index	Start stage 47
24 — 1 = 23	20 μg/ml 5-FUdR

S – 1
T



Left

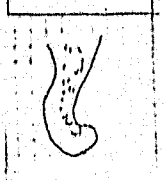


Deformity Index	Start stage 47
24 — 1 = 23	20 μg/ml 5-FUdR

S – 1
T



Right



S. shape
L. toes

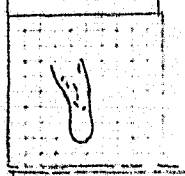
Deformity index	Start stage 48
24 — 1 = 23	10 µg / ml 5-FUdR

S - 1

T



Left



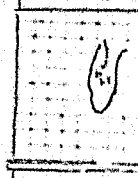
Deformity index	Start stage 48
24 — 2 = 22	10 µg / ml 5-FUdR

S - 2

T



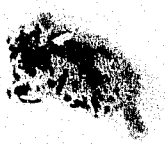
Right



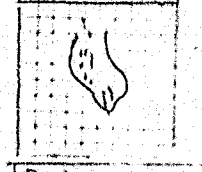
Deformity index	Start stage 53
24 — 0 = 24	2 µg/ml 5-FUdR

S

T



Left



Deformity index	Start stage 53
24 — 1 = 23	2 µg/ml 5-FUdR

S - 1

T



Right



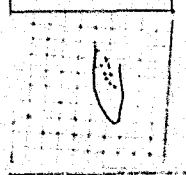
S shape
T toes

Deformity Index	Start stage 50
24 — 0 = 24	20 μg/ml 5-FUdR

S
T



Left



Deformity Index	Start stage 50
24 — 0 = 24	20 μg/ml 5-FUdR



S
T

Right

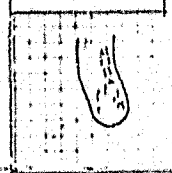


Deformity Index	Start stage 49
24 — 1 = 23	10 μg/ml 5-FUdR

S-1
T

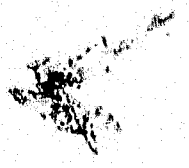


Left



Deformity Index	Start stage 49
24 — 1 = 23	10 μg/ml 5-FUdR

S-1
T



Right



S. shape

T toes

Deformity Index
24 — 13
= 11

Start stage 50
20 μg/ml 5-FUdR



S – 1

T – 12

Left



Deformity Index
24 — 9
— 15

Start stage 50
20 μg/ml 5-FUdR



S – 1

T – 8

Right



Deformity Index
24 — 0
= 24

Start stage 47
20 μg/ml 5-FUdR



S

T

Left



Deformity Index
24 — 0
= 24

Start stage 47
20 μg/ml 5-FUdR

S

T



Right



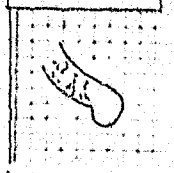
S shape
T toes

Deformity Index	Start stage 46
24 — 1 = 23	10 µg / ml 5- FU dR

S – 1

T

Left



Deformity Index	Start stage 46
24 — 5 = 19	20 µg / ml 5-FUdR

S – 1

T - 4

Left



Deformity Index	Start stage 50
24 — 1 = 23	20 µg / ml 5-FUdR

S – 1

T

Left



Deformity Index	Start stage 49
24 — 5 = 19	10 µg / ml 5- FU dR

S – 1

T – 4

Right

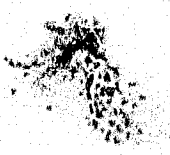


S shape
T toes

Deformity Index	Start stage 53
24 — 1 = 23	10 μg / ml 5- FU dR

S — 1

T



Right



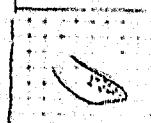
Deformity Index	Start stage 47
24 — 0 = 24	10 μg / ml 5- FU dR

S

T



Left



3.3 Uptake of radioactive thymidine in the presence of 5-FUdR

The results of the experiments in section 2.2 are shown in Table 3.1 below.

Control : Thymidine 5 µCi/ml
Experiment: Thymidine 5 µCi/ml + 20 ug/ml 5-FUdR
6 tadpoles per dish

Control	Counts (50min)	Counts /min	Wt limb (mg)	Counts /min/mg
1	17767	355,34	0,017	20902,4
	67564	1351,28	0,090	15014,2
2	55006	1100,12	0,013	84624,6
	55962	1119,24	0,017	65837,6
3	28272	565,44	0,066	8567,3
	12701	254,02	0,054	4704,1
4	32257	645,14	0,015	43009,3
	144852	2897,04	0,026	111424,6
5	13060	261,2	0,134	1949,3
	29973	599,46	0,009	66606,7
6	11573	231,46	0,031	7466,5
	78800	1576,0	0,017	92705,9
Expt				
1	43816	876,32	0,012	73026,7
	9754	195,08	0,344	567,1
2	6334	126,70	0.092	1377,1
	1962	39,24	0,067	585,7
3	4867	97,34	0,005	19468,0
	6339	126,78	0,099	1280,6
4	19331	386,62	0,015	25774,7
	4873	97,46	0,010	9746,0
5	16693	333,86	0,044	7587,7
	45856	917,12	0,484	1894,9
6	4700	94,00	0,009	10444,4
	5467	109,34	0,015	7289,3

Brackets indicate left and right limbs from the same animal.

Table 3.1 *Results of the experiments showing the uptake of ^{3}H-Thymidine in the presence of 5-FUdR*

The results in Table 3.1 were analysed by an analysis of variance test. First the counts/minutes were analysed of which the results are shown in Table 3.2. Then the counts/minutes/mg were analysed of which the results are shown in Table 3.3. There are three sources of variance

1. Treatments - experiment versus control.
2. Between animals - differences between the 24 tadpoles used.
3. Within animals - differences between the two hindlimbs of the same tadpole.

Source	DF	SS	MS	F
1. Treatments	1	2 377 622	2 377 622	7,67 x
2. Between animals	10	3 100 388	310 039	0,83 NS
3. Within animals	12	4 490 245	374 187	
4. Total	23	9 968 254		

Table 3.2 *Analysis of variance for counts/min*

DF = Degrees of Freedom

SS = Sum of squares

MS = Mean of Squares

F = F Test

x = Significant

NS = Not Significant

The mean count per minute for the experimental animals (913) is significantly higher than that of the control animals (283) at the 5% level i.e. there is a 95% confidence that this difference is genuine.

The variability between counts on the same animal i.e. between the counts in the hindlimbs of the same animal, is of the same order as the variability between animals. This shows the errors of the experimental technique to be of the same order as that of the variability between animals. This is unusual but can be explained by the fact that the hindlimbs of Xenopus laevis are known to develop at different rates and therefore the one limb could be significantly larger than the other hindlimb at the same stage. The standard deviation of the experimental method is : 374187 = 612 counts/minute, i.e. the reading is expected to be within +/- 612 counts 68% of the time, the reading is expected to be within +/- 1224 counts 95% of the time.

Source	DF	SS	MS	F
1. Treatments	1	5 513 660 000	5 513 660 000	4,92 NS
2. Between animals	10	11 205 400 000	1 120 540 000	0,85 NS
3. Within animals	12	15 731 000 000	1 310 917 000	
4. Total	23	26 936 400 000		

Table 3.3 *Analysis of variance for counts/min/mg*
DF = Degrees of Freedom
SS = Sum of Squares
MS = Mean of Squares
F = F Test
x = Significant
NS = Not Significant

The difference between the experimental mean and the control mean (43568 versus 13254) just fails to achieve significance at the 5% level when tested by the F test above. (F = 4.96 is required for significance at the 5% level.)

Again the variability between the hindlimbs of the same animal is of the same order as the variability between the different animals.

The standard deviation of the method is 36206 counts/minute/mg.

The results show, therefore, that thymidine uptake is depressed in the presence of 5-FUdR.

3.4 Buoyant density gradient analysis of DNA substituted with 5-BUdR in place of thymidine

This experiment was set up to test the uptake of 5-BUdR into the tadpole DNA in the absence and presence of 5-FUdR (see section 2.3). In the control shown in Figure 3.19 below the photograph shows one main band which, when analysed by the densitometer, shows up on the trace as peak c.

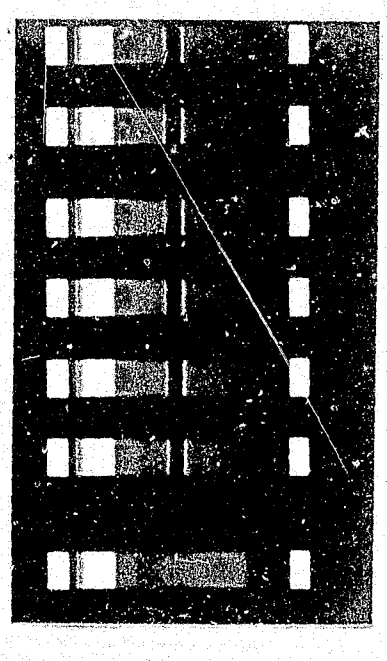


Figure 3.19 *Ultraviolet absorption photograph of DNA from tadpoles swum in wat*

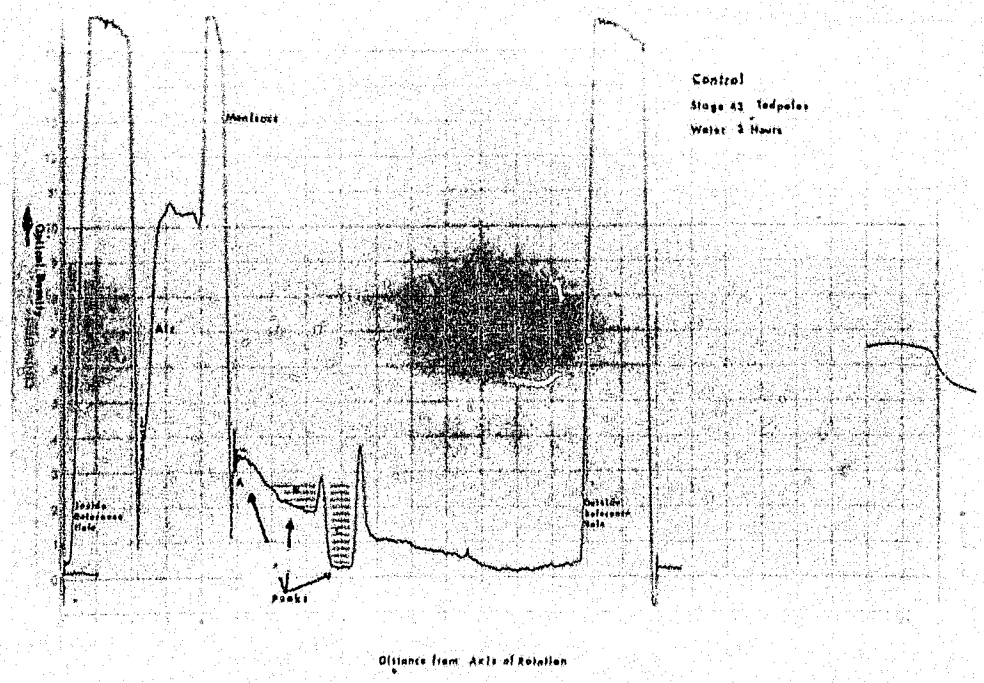


Figure 3.20 *Densitometer trace of the photograph in Figure 3.19, peak c is the main band*

In Figure 3.21 below is the ultraviolet photograph of the DNA from tadpoles swum in 0,1 mg/ml 5-BUdR for five hours. It reveals two bands where there was previously one (in the photograph of the control DNA in Figure 3.19). The densitometer trace in Figure 3.22 below shows these bands as peaks c and d.

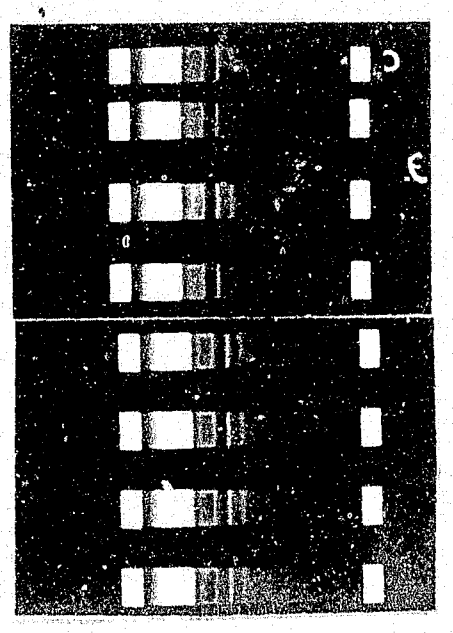


Figure 3.21 *Ultraviolet absorption photograph of the DNA from tadpoles swum in 0,1 mg/ml 5-BUdR for five hours*

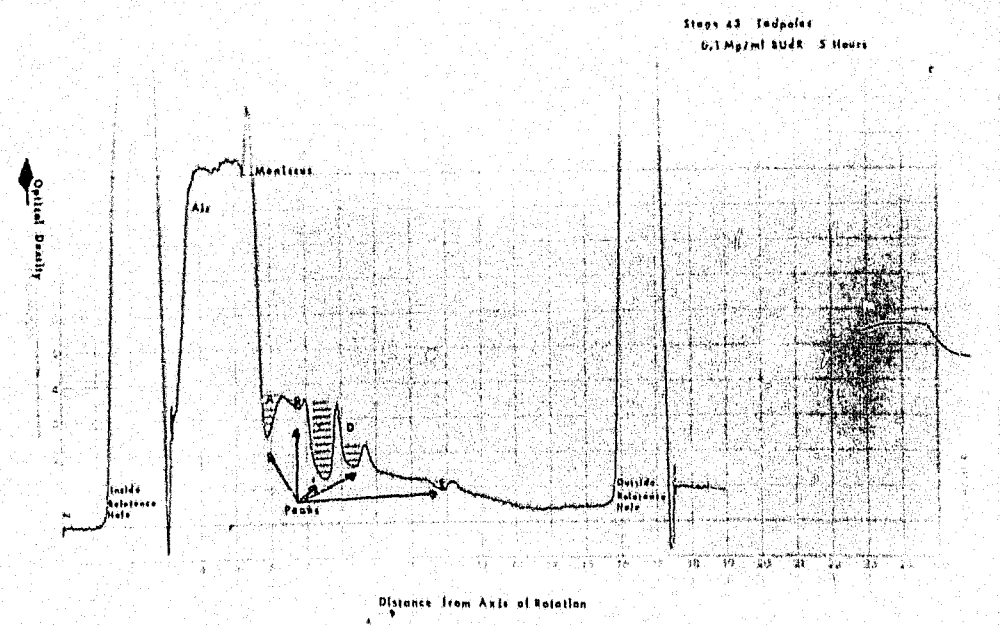


Figure 3.22 *Densitometer trace of the photograph in Figure 3.21, the main peaks are c and d*

In Figure 3.23 below is the ultraviolet absorption photograph of the DNA from tadpoles swum in 0,1 mg/ml 5-BUdR and 0,2 ug/ml 5-FUdR for five hours. Two bands are still visible. In the densitometer trace of the photograph the two main peaks are b and c.

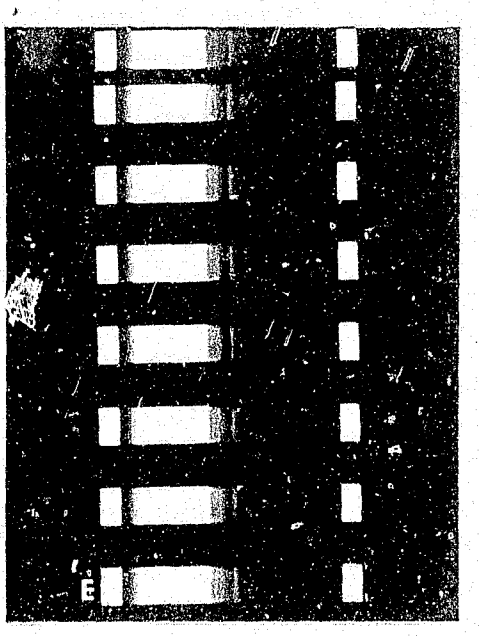


Figure 3.23 *Ultraviolet absorption photograph of the DNA from tadpoles swum in 0,1 mg/ml 5-BUdR and 0,2 ug/ml 5-FUdR for 5 hours*

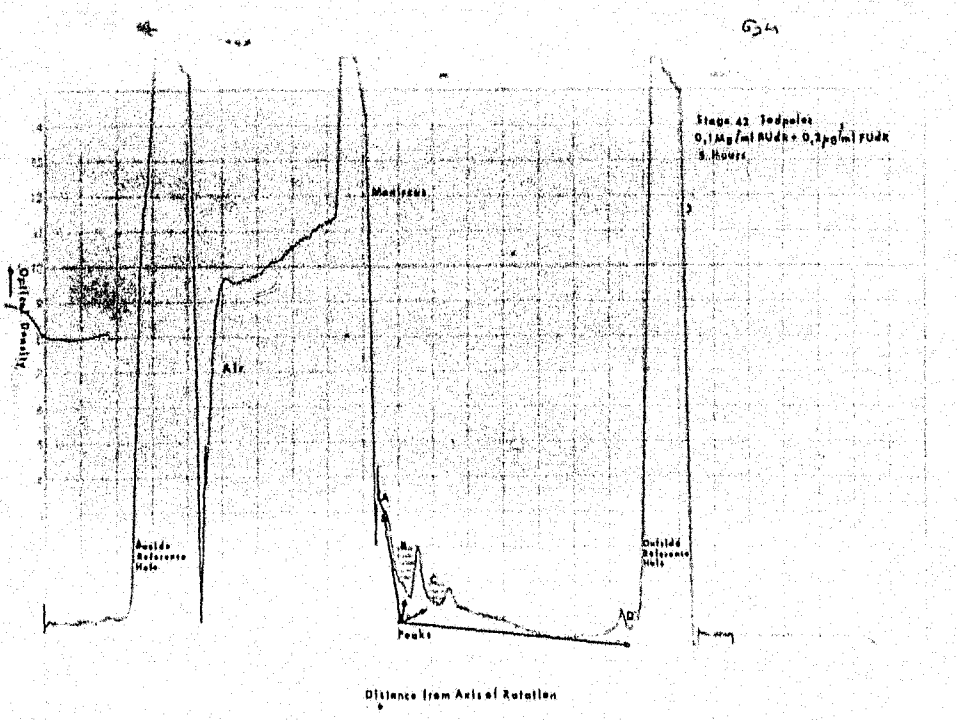


Figure 3.24 *Densitometer trace of the photograph in Figure 3.23 above, the main peaks are b and c*

3.5 Analysis of the effects of different foods on the growth of Xenopus laevis tadpoles

A series of experiments tested the effects of different foods on the growth of tadpoles. The tadpoles were maintained in 2 litre trays and in 200 ml dishes of water, and the food was added in discreet amounts to the water. Financial and experimental constraints required that tadpoles be reared in 200 ml dishes (experiment C). A parallel series of experiments (A and B) was conducted using 2 litre trays. Table 3.4 on page 120 shows that in the small dishes Liquifry was the only suitable food. In the larger trays (Tables 3.5 and 3.6 on pages 123 and 126) optimum growth was obtained using commercial "baby food" and "Complan". In Liquifry, while growth was slower, the tadpoles were carried through metamorphosis. Histograms in Figures 3.25 and 3.26 summarise the results by comparing the number of tadpoles reaching metamorphosis for each feeding regimen.

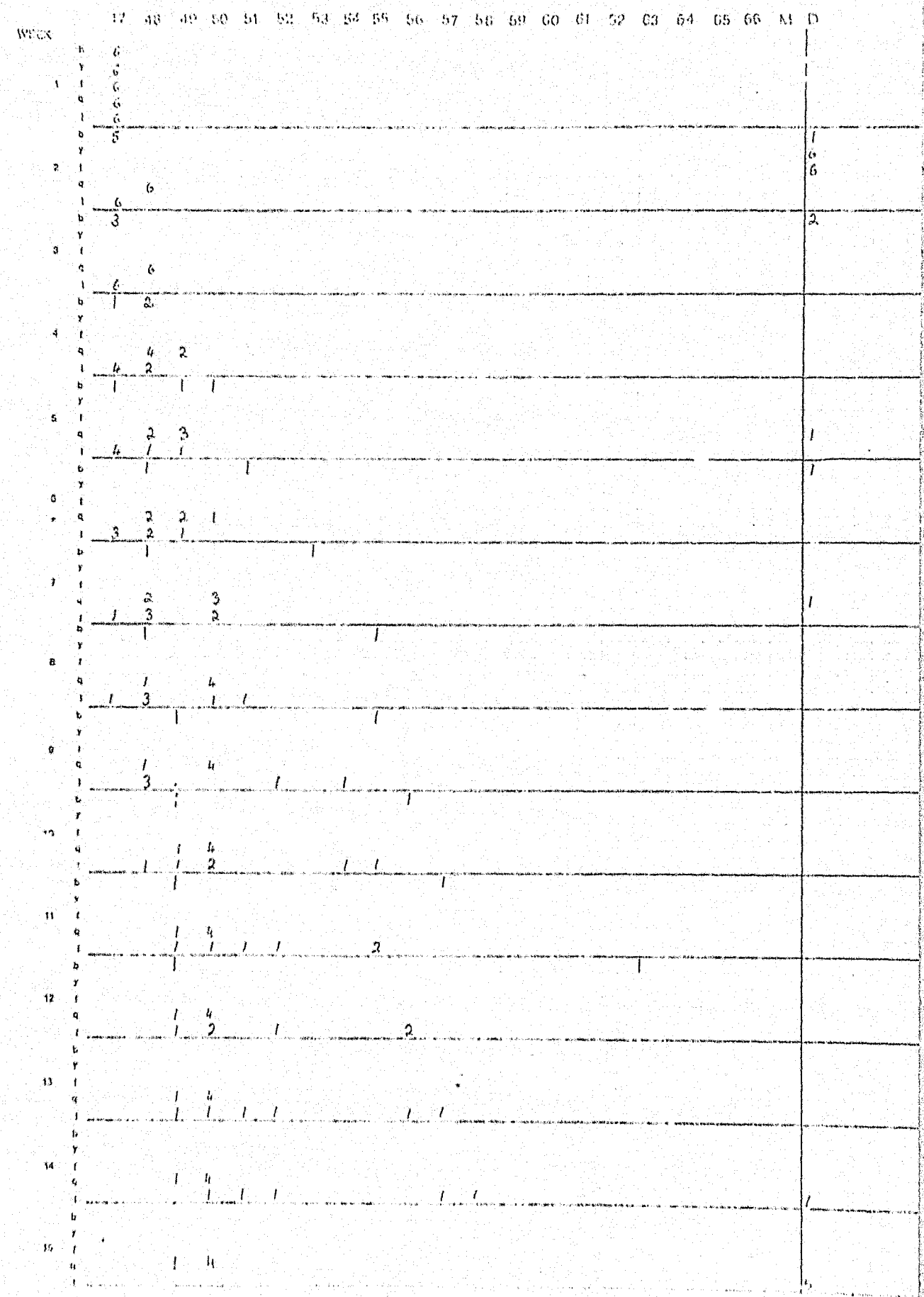
Table 3.4 *Feeding experiment in 200ml dishes*

Experiment C

KEY

b	Commercial baby food
y	Yeast
f	Fish food
q	Liquify
l	Lettuce
c	Complan

Table recording the effect of different foods on tadpoles reared in 200 ml dishes of water. Each food is indicated by a code letter e.g. b = commercial baby food. The figures in the table represent the number of tadpoles living on a particular food regimen at a particular developmental stage per lapsed time. The letter M indicates the number of tadpoles metamorphosed per food per week and letter D indicates the number of dead tadpoles per food per week. For example by week 14 all the tadpoles fed on b, y and f had died before metamorphosis. 5 of the tadpoles fed on liquifry survived, of which 1 had reach stage 49 and 4 had reached stage 50. 6 of the tadpoles fed on lettuce had survived to stage 50, 51, 52, 57 and 58. 1 of the tadpoles fed on lettuce died during week 14. During week 35 only tadpoles fed on liquifry were still growing. Of these 1 had metamorphosed, 1 had reached stage 51 and 2 had reached stage 55. By week 53, 4 out of the initial 6 had metamorphosed. The results are summarised in the histogram in Figure 3.25.


DEVELOPMENTAL STAGE
WEEK
47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 M D
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

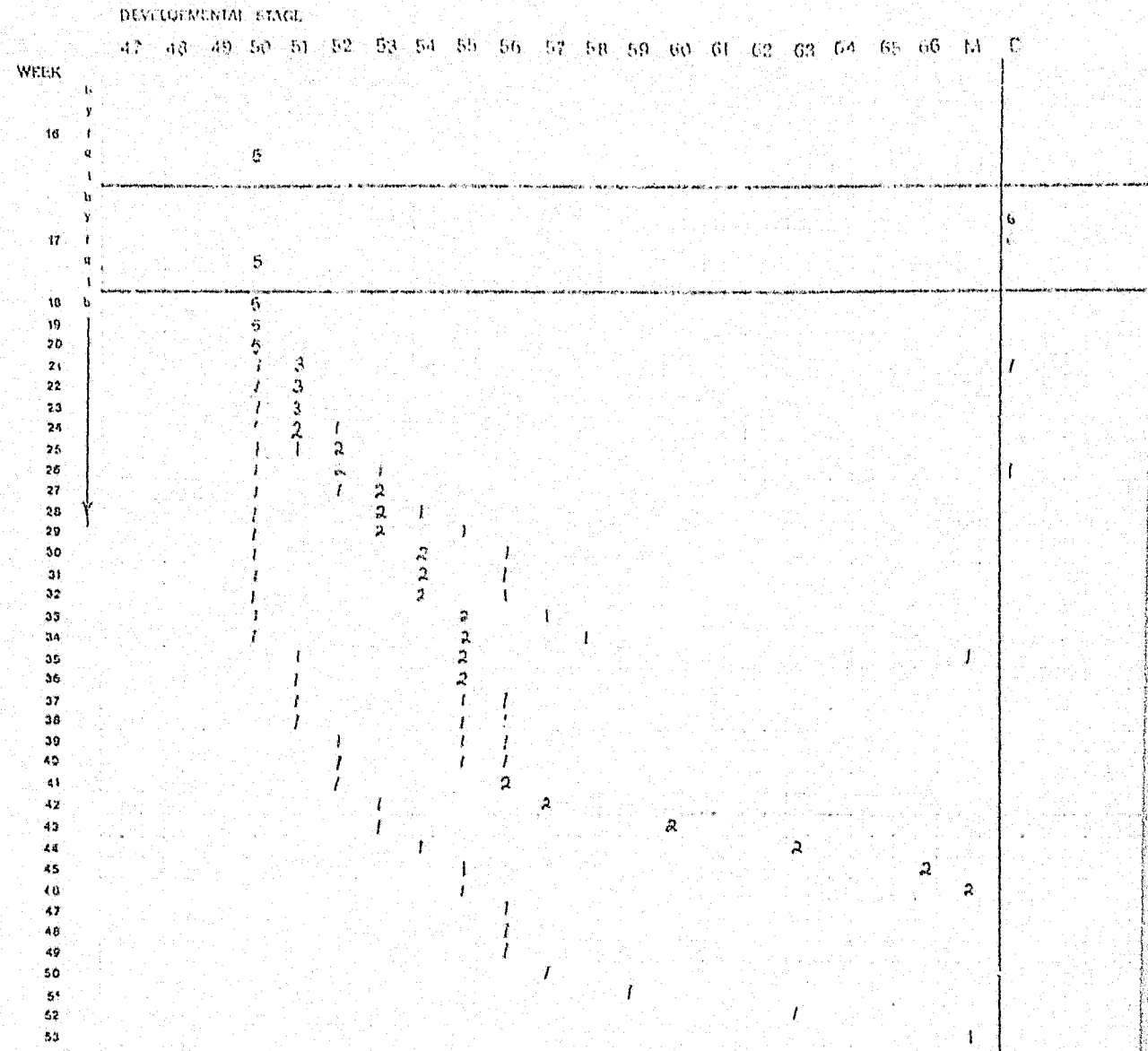

DEVELOPMENTAL STAGE
42 43 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 M C
WEEK
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53

Table 3.5 *Feeding experiment in 2 litre trays*

Experiment A

KEY

b	Commercial baby food
y	Yeast
f	Fish food
q	Liquifry
l	Lettuce
c	Complan

Table showing the development of animals being fed a particular food e.g. indicated by code letter b = commercial baby food, see key below. These tadpoles were reared in 2 litre trays of water. The figures in the table represent the numbers of tadpoles living on a particular regimen at a particular developmental stage per elapsed week. Letter M indicates the number of tadpoles metamorphosed per food per week. For example during week 14 of the tadpoles living on liquifry = q; 2 tadpoles had reached stage 49; 15 had reached stage 50; 2 had reached stage 51 etc. 1 had metamorphosed and 1 had died. By week 38, 1 tadpole was surviving at stage 54. The results are summarised in the histogram in Figure 3.26.

DEVELOPMENTAL STAGE

WEEK		47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	M	D
1	L	60																					
	Y	60																					
	Q	60																					
	C	60																					
	L		2		69	6																	3
2	Y	2	22	29	1																		
	Q	20	32	1																			
	C	1	12	34	1																		6
	L				2	2	5	21	24	2													
3	Y		2	1	36	17	4																
	Q	2	46	8	4																		1
	C		2	3	33	15	1																
	L				1	1	1		15	26	7												2
4	Y			1	14	15	14	8	5														3
	Q	2	34	20	1	1																	1
	C		1	1	4	9	13	12	12														2
	L					1	2			20	39	3											
5	Y				5	4	16	11	10	3													8
	Q		8	17	28		1																4
	C				3	7	3	11	18	8	1												1
	L							3		2	16	29	5										
6	Y				6	3	8	18	8	5													2
	Q		4	9	32	6		1															8
	C				2	3	4	10	11	13	5												
	L							1	3		11	23	4	4		1	1	2			5		
7	Y				2	3		5	12	7	5												6
	Q		7	5	27	11	1		1														
	C				2	1	2		13	16	20	6											
	L								3	1	5	18	1	2	2		5					13	
8	Y					3	2	3	9	12	7	5											
	Q			1	29	11	2		2	1													
	C				1	1	2		7	13	6	13	1		2	1			2			1	1
	L								1	2	8	11	3		2		1	1	1			6	1
9	Y					5	1	12	5	11	2	1	1		2				1				
	Q		1	1	31	9	4		3	2	1												
	C				1		1	1	3	10	10	11		2			1	1				1	7
	L								2	2	11		1	2		4						7	1
10	Y					5		12	6	7	4	1							1			5	
	Q			2	31	4	6	2		5	1	1											
	C				1		1	1	3	12	5	4			1	2	2	2					7
	L										3	7	1		3		1					1	6
11	Y					1	3	2	4	9	4	9					1	1				1	1
	Q			2	30	4	2	3	6		4	1		1									
	C				1		2	1	1	8	7	5		1									8
	L										6	1			2	1	1					4	
12	Y					1	1	3	7	8	3	1	3		1							2	4
	Q			1	18	9	5	4	4	4	2	3						1				1	1
	C					1			4	4	5	8	2				1						1
	L										2	1	1					1				5	
13	Y							2	5	4	3	4		2								5	3
	Q				15	5	5	6	9	3	5	1		1								1	1
	C					1			1	4	6	5		2									6
	L										2							1				3	
14	Y							1	5		3	4						1				2	4
	Q			2	15	2	4	1	10	3	4	4		1	1							1	1
	C					1			1	1	4	4		2	1		1						4
	L										1											2	
15	Y								2		2	1							1				8
	Q				12	5	3		8	6	6	1	3	1								1	
	C											3	1										11
	L																1						
16	Y											1											
	Q				10	7	2		4	4	7	6	1	1								3	
	C																						4
	L																					1	
17	Y																						
	Q				6	6	4	1	2	5		4		2			2						11
	C																						
	L																					1	
18	Y																						
	Q				5	5	3	1	3	1	4	2	1	1				2					3
	C																						
	L																						
19	Y																						
	Q				3	3	1	1	2	2	1			1			1					3	1
	C																						

DEVELOPMENTAL STAGE

WEEK	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	M	D
20				1	3	3	2	3	1	6	4											2
21				1	3	3	2	2	2	1	4		4									
22				1	2	4	1	3	1	2	2		2				1					3
23				1	3	2	2	3	1		2		2				1				2	
24				1	1	3	2	2	1	2	1		1				1				2	
25				1	1	1	3	3	1	3			1				1					
26						1		4	2	3	1	1									3	
27							1	1	1	4	1	1	1			1					1	
28							1	1	1	1	5				1		1					
29							1		2	1			3			2					2	
30								1		1	2						1				4	
31							1				2					1					1	
32								1				1	1								1	
33								1										1			1	
34								1													1	
35								1														
36								1														
37								1														
38								1														

Table 3.6 *Feeding experiment in 2 litre trays*

Experiment B

KEY

b	Commercial baby food
y	Yeast
f	Fish food
q	Liquifry
l	Lettuce
c	Complan

Table showing the development of tadpoles being fed a particular food e.g. indicated by code letter b = commercial baby food, see key below. These tadpoles were reared in 2 litre trays of water. The figures in the table represent the numbers of tadpoles living on a particular food regimen at a particular developmental stage per elapsed week. Letter M indicates the number of tadpoles metamorphosed per food per week. Letter D indicates the number of dead tadpoles for each feeding regimen per week. For example during week 13 (which was measured at week 14) on food q = liquifry, 2 tadpoles had reached stage 48; 1 had reached stage 49; 3 had metamorphosed etc. By week 44 the last 3 tadpoles grown on liquifry had metamorphosed. The results are summarised in the histogram in Figure 3.26.

DEVELOPMENTAL STAGE

WEEK		47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	M	D
1	b	60																					
	y	60																					
	t	60																					
	q	60																					
	l	60																					
2	b	4	51	1																			4
	y	21	30																				3
	t	57																					3
	q	17	36	1																			6
	l	55	2																				3
3	b		2	3	50	1																	
	y	4	14	16	18																		5
	t	43																					14
	q	6	28	6	11																		3
	l	44	6																				7
4	b		8	1	34	6	5	2															3
	y	3	9	2	23	6	5	1															21
	t	26																					1
	q	2	10	14	17	5	2																
	l	44	6																				
5	b		1		9	8	12	1	4														21
	y	3	2	4	24	4	6	1	4	1													5
	t	16	1																				1
	q	1	1	8	27	4	3	2	2														5
	l	42	3																				
6	b				3	3	8	6	5	13	4	2											
	y	2		4	4	12	3	5	4	4	1												3
	t	7	7																				
	q	1	3	11	19	4	2	6		2													
	l	31	9	1	3	1																	
7	b				3	1	4	8	6	8	5	1											
	y		4	3	8	7	8	3	2	2	4												2
	t		1	3	7	1																	2
	q		2	5	19	9	2	3	5		2												1
	l	8	14	7	1		2		2														8
8	b				2	3	3	3	7	5	6	1		2				1	1			1	
	y		1	2	8	2	7	8	5	9	1	2	1										1
	t				7		2		1	1													1
	q		3	4	18	3	7	6	1	1		2											2
	l	1	19	6	6			2	1														5
9	b				1		4	3	7	5	3	6										1	4
	y			2	7	4	7	6	6	4	4	2	1				1			1			1
	t				1	1	3	2	2		1	1											
	q		3	7	15	4	10	4	2	3		2											
	l	3	12	1	9	3	3	1	2														1
10	b				1				11	6	5	4		3									
	y			3	5	2	2	10	3	6	4	5								1		2	2
	t				1			2	2	2	2				1			1					
	q		1	3	16	6	4	7	2	4		1								1			
	l	1	4	3	5	3	3	6	4	1	2												1
11	b								3	8	7	1	2					1	3			2	1
	y			2	3	1	4	2	6	7	2	3		2				1				1	7
	t					1				4	2	1			1							1	1
	q		1	3	14	6	4	6	5	4		1								1			1
	l		6	2	3	2	4	5	2	4	2				1								1
12	b								1	3	9	2	1	2								3	5
	y				2	1	1	3	5	5	4	1	1					3	2				5
	t					1				3	3							1	1				
	q		1	4	12	6	4	5	4	4												1	3
	l		4	3	4	2	4	5	4	2	1	1								1			
13	b								5	4	6		1	1	1								
	y			1		1	2	1	3	3	4	4	1									6	2
	t						1			1	2	2					1	1				1
	q		1	1	6	12	6	3	4	6													3
	l		3	1	6	1	2	3	9	3	1	1			1								1
14	b								4	2	1	2		2				1				5	1
	y					1	2	1	2	3	3	3						1	2			2	
	t						1			1	1	2							1			2	
	q		2		14	4	5	1	3	5	1											1	
	l		1	1	4	1	5		5	5	1											3	
15	b								1	2	1	2										6	
	y						2	1	1	2	4		1						1			2	
	t						1			3								1				1	
	q				11	4	2	3	4	4	2												5
	l				1	2			1	4	3												2

DEVELOPMENTAL STAGE

WEEK		47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	M	D
18	boy						1		3	2	2	1		3				2				4	
	girl							1				3										3	
	liq				10	2	5	3	3	7	2											1	
	ill							1	1	3	4											1	
17	boy								1	3	2	1			1							5	
	girl							1				1					1	1					
	liq				9	1	3	2	5	6	2	2										1	1
	ill							1		4	3												1
18	boy									1	1	4						1				1	
	girl								1										1			2	
	liq				8	2	1	1	6	6	3		2				1						1
	ill							1		2	4												1
19	boy										1	2		1		1			1			1	
	girl										1											1	
	liq				6	2	2	1	6	8	2												
	ill							1		2	4												
20	boy											1		1					1			4	
	girl													1									
	liq				5	4	2	1	2	8	3	2										1	
	ill						1	1	2		2												1
21	boy													1								1	
	girl																		1				
	liq				4	2	3		3	6	6	2											
	ill									3	3												
22	boy																					1	
	girl																					1	
	liq				3	3	2	2	2	5	6	1		1				1					
	ill									1	4												1
23	boy																						
	girl																						
	liq				4	2	3		2	7	5	1						1				1	
	ill									1	4												
24	boy																						
	girl																						
	liq				4	1	2	2	1	6	7	1										1	
	ill									1	3												1
25	boy																						
	girl																						
	liq				4	2	1	1	2		6	4	3	1									
	ill										1												3
26	boy																						
	girl																						
	liq				4	2	1	1	2	6	5			1	1		1					1	
	ill										1												
27	boy																						
	girl																						
	liq				4	1	2	2	2	6	2	2										1	
	ill										1												
28	boy																						
	girl																						
	liq				3	3	1	2	2	4	2	4										2	
	ill										1												
29	boy																						
	girl																						
	liq				3	2		2	2	6	2	1		2			1						
	ill										1												
30	boy																						
	girl																						
	liq				4	1	1		3		2											3	
	ill										1												

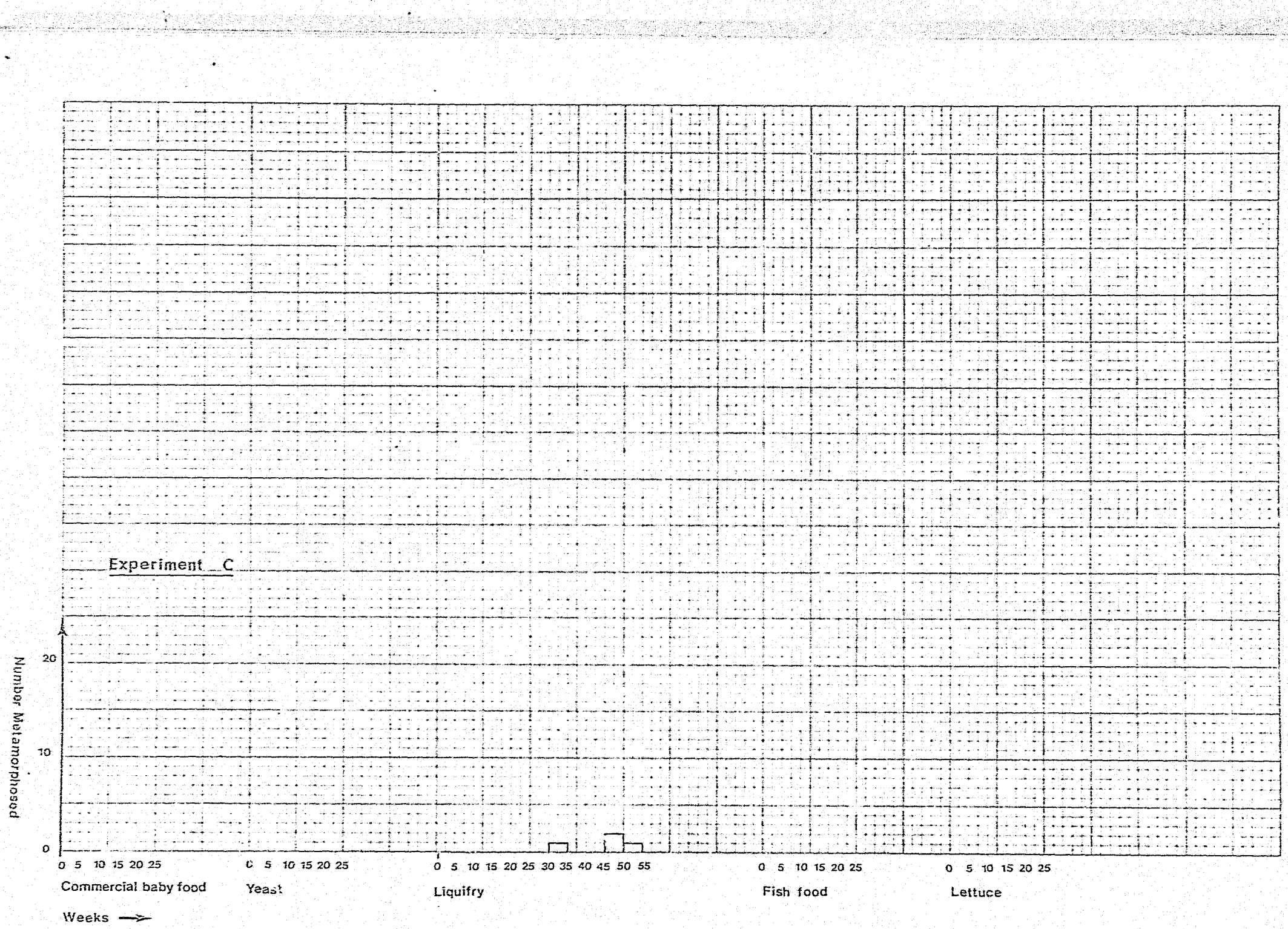
DEVELOPMENTAL STAGE

WEEK	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	M	D
31				2	4		2	1	1	5	2											
32				3		2	1	1	4	1	4			1								
33				2	1		2		6		4		1									
34				2	1		2		4	1	3		3								1	
35					1	1	1	1	2	2	4		1				1				2	
36						1	2		2	2	4		1								2	
37							1	1	1	5	1	2						1				
38									2	1	6										3	
39									2		3		3		1							
40										2	3	1				1					2	
41											3	1				2					1	1
42											3					1					1	
43													1		2						1	
44																					3	
45																						

Figure 3.25 *Graph of the results from experiment C. Number of tadpoles metamorphosed versus weeks for each food*

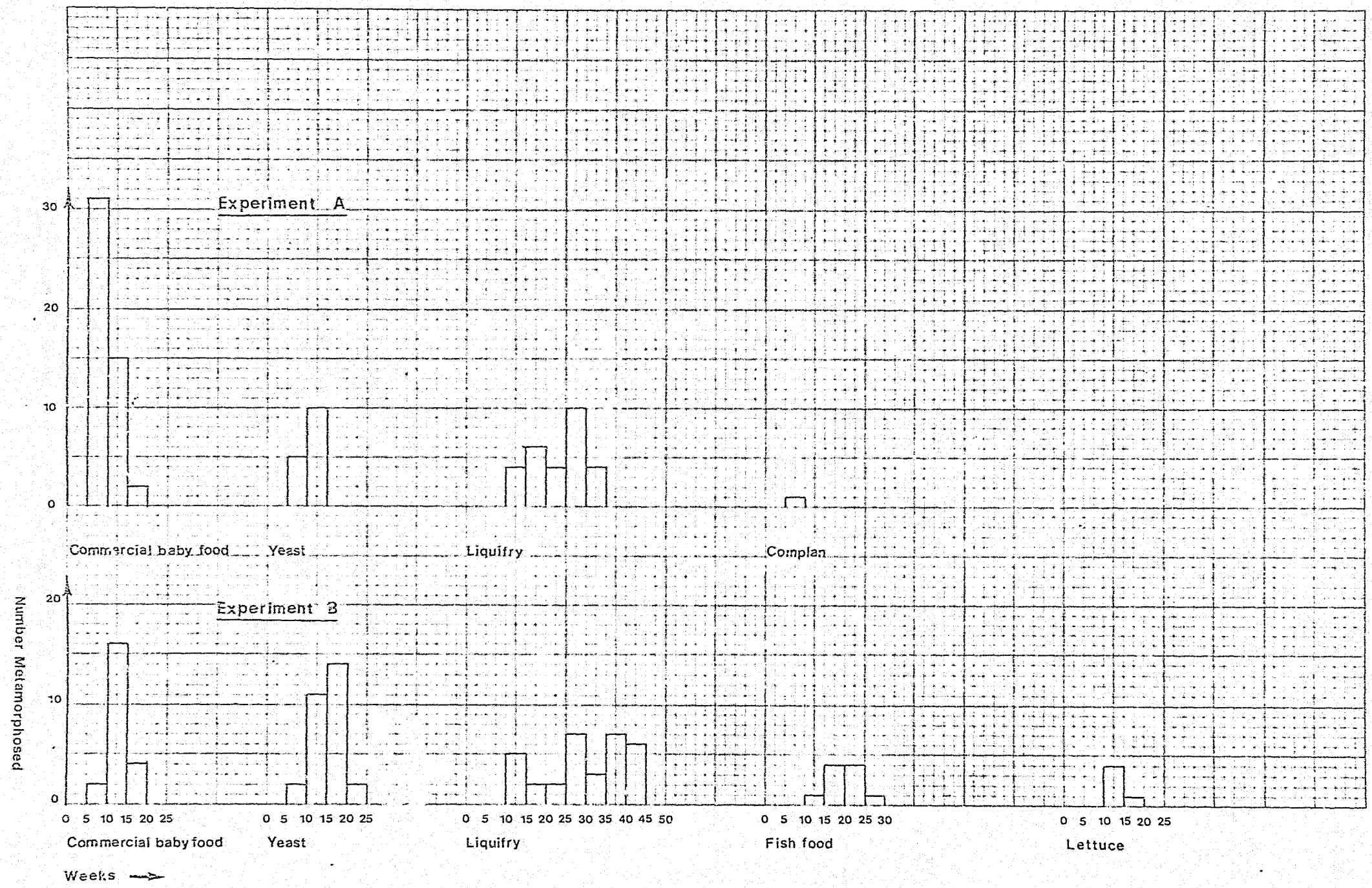
Graph showing the results for experiment C (Table 3.4). The histogram shows the number of tadpoles which metamorphosed under a particular food regimen over 5 week intervals as a function of time. For example 4 tadpoles which were fed on liquifry, metamorphosed during weeks 30 to 35.

Experiment C



Graph showing the results for experiment C (Table 3.4). The histogram shows the number of tadpoles which metamorphosed under a particular food regimen over 5 week intervals as a function of time. For example 4 tadpoles which were fed on liquifry, metamorphosed during weeks 30 to 35.

Figure 3.26 *Graph of results from experiments A and B. Number of tadpoles metamorphosed versus weeks for each food*


Experiment A
30
20
10
0
Commercial baby food
Yeast
Liquifry
Complan
Experiment B
Number Metamorphosed
20
10
0
0 5 10 15 20 25
0 5 10 15 20 25
0 5 10 15 20 25 30 35 40 45 50
0 5 10 15 20 25 30
0 5 10 15 20 25
Commercial baby food
Yeast
Liquifry
Fish food
Lettuce
Weeks

Graph showing the number of tadpoles which metamorphosed under a particular food regimen in experiment A (Table 3.5). The histogram shows the number of tadpoles which metamorphosed as a function of time. For example between weeks 26 to 30, 10 tadpoles which were fed on liquifry, metamorphosed.

Graph showing the number of tadpoles which metamorphosed under a particular food regimen in experiment B (Table 3.6). The histogram shows the number of tadpoles which metamorphosed as a function of time. For example between weeks 16 to 20, 14 tadpoles, which were fed on yeast, metamorphosed.

3.6 Conclusion

In this dissertation a method was devised (the deformity index method) in which each deformity, irrespective of its position along the limb, was given the same weighting. An attempt was made to see whether there was any correlation between the deformity index and the stage of the tadpole, and the concentration of the solution.

A uniform weighting was given to a deformity at any position in the limb as discussed. Thus a deformity could receive a particular rating if two elements were missing (one in the proximal and one in the distal part of the limb) or if both elements in the same in the same section of the limb were missing. This deformity index method of analysis offered no new trend to the deformities which were observed. Although visual analysis did reveal bent and missing digits, there was no correlation between the type of deformity, the extent of the deformity, the concentration of the drug and the stage of immersion of the tadpole.

4. DISCUSSION

4.1 The effects of 5-FUdR on the tadpoles in vivo

As has been mentioned (see page 40) 5-FUdR is an inhibitor of cell division. The effect of 5-FUdR on growing tissues would be observed most markedly in rapidly growing tissues. Such a situation is found during the development of the hindlimb of Xenopus laevis, which was the topic of this investigation. In fact, hindlimb development appears to be a sensitive indicator of cell division, as seen by the variety of abnormalities observed in the basic patterning of the limb when cell division is interfered with. The point of view in this discussion assumes that cell division, together with its consequent events, is an essential parameter in the patterning of the limb.

In the particular experiments carried out in this investigation under 5-FUdR treatment, no cases were observed in which there was no limb development. This suggests that the cells of the limb were dividing, although cell division was presumably continuing at a lower rate. The analysis of FUdR effect must take into account the periodicity of the drug administration. The drug was given in weekly doses and therefore it is assumed that the effect of concentration decayed until the next dose was given. At the high concentration point of drug administration those parts of the limb most actively dividing would be most deleteriously affected. According to Wolpert (1981), one such area of cell division is located at the distal end of the growing limb. He calls this area the "progress zone". (See section 1.5.)

In order to understand how hindlimb abnormalities in Xenopus laevis could result from the inhibition of cell division in the "progress zone", two models will be proposed :

1. The first model will try to explain how a constant level of partial inhibition of cell division in the "progress zone" could lead to deformity in shape, which was observed in the early stages of limb development (NF stages 43 - 53) (see pages 97 to 111).
2. The second model will try to explain the reduction in digit number observed in the later stages of limb development. (NF stages 54 onwards.)

1. Model to explain shape deformity

The normal stage 53 hindlimb is observed below in Figure 4.1.

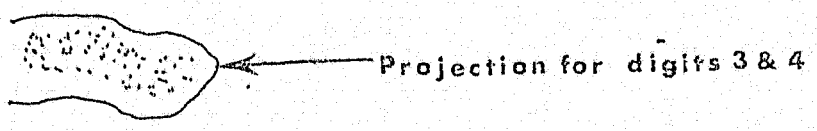


Figure 4.1 *Normal stage 53 hindlimb of Xenopus laevis*

At this stage the first indications of digit formation are seen externally. These are digits 4 and 5, although digit 1 may be the first to actually form. The external observation of digits 4 and 5 is based on the projection of a bulge from the paddle as seen in Figure 4.1.

One of the most commonly observed shape deformities at about stage 53 is illustrated below in Figure 4.2.



Figure 4.2 *Deformed stage 53 hindlimb of Xenopus laevis*

It will be noticed that this deformed limb bends upwards. This particular limb went on to form three digits. In the model to be proposed, use is made of Wolpert's (1981) "progress zone" theory. The "progress zone" comprises the band of rapidly dividing cells near the distal tip of the limb as seen below in Figure 4.3 in Xenopus laevis hindlimb at various stages of development. The "progress zone" theory proposes that all cells spend a period of time in the "progress zone" before being offloaded and differentiating into the forming elements of the limb.

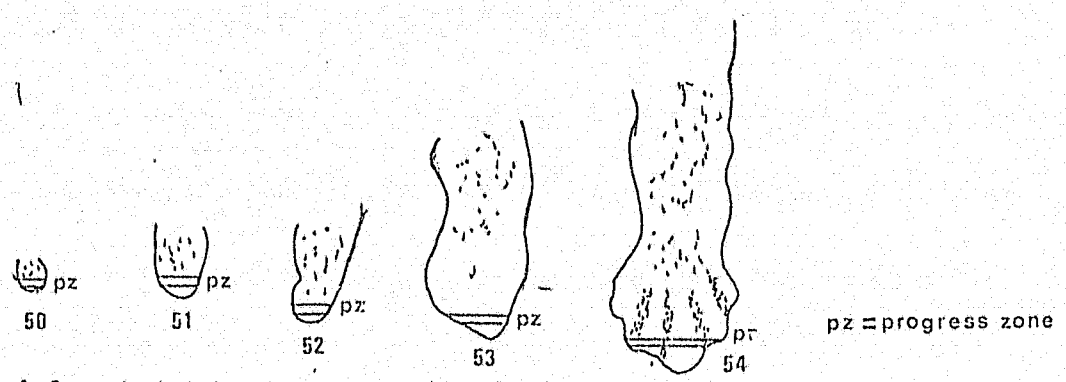


Figure 4.3 *Hindlimb of Xenopus laevis in various stages of development showing the progress zone (Summerbell et al 1973)*

If 5-FUdR is added prior to or during the early stages of "progress zone" development, the number of cells in this zone could be reduced over a period of time and the reduction could be one-sided. If one part of the bud appears to develop ahead of other parts, the conclusion is made that the cells across the antero-posterior axis of the limb are not all at the same stage of development and in fact different regions along the antero-posterior axis (or in the "progress zone") may be dividing at different rates. Perhaps there is a wave of cell division across the antero-posterior axis. This effect could lead to deformities depending on which area is pulsed in high concentration by the applied 5-FUdR. The formation of the deformity in Figure 4.2 is shown progressively in Figure 4.4 below.

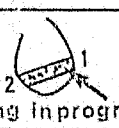
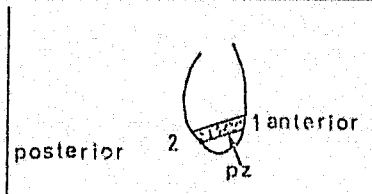
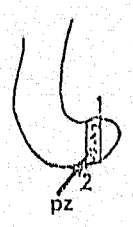
	Stage 50 - appearance normal
	Stage 51 - disruption of cell division on the anterior of the limb marked 1. This side of the limb now has fewer cells than it should have at this stage.
pz::progress zone 	Stage 52 - rapid extension of the side marked 2, due to a larger number of divided cells, causes uneven growth. As a result the limb curves upwards.

Figure 4.4 *Model describing a possible shape deformity in the hindlimb*

By varying the model slightly many other shape deformities could also be explained.

2. Model to explain reduction in the number of digits as a consequence of 5-FUdR treatment

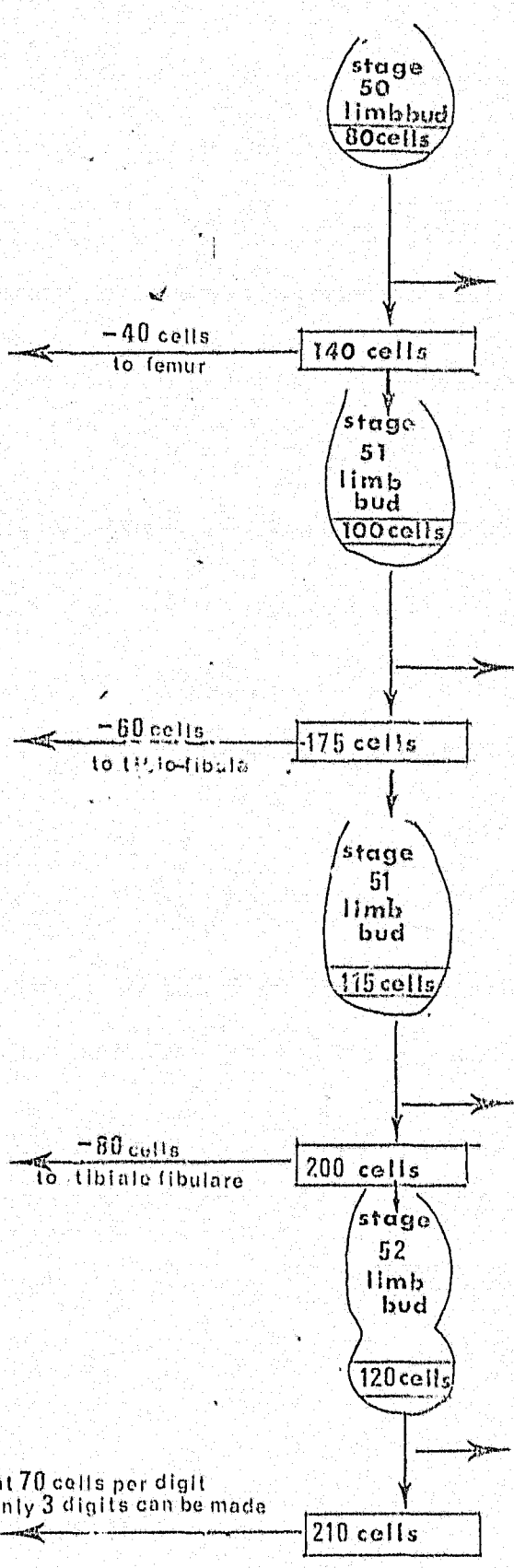
It will be recalled that cells divide in the "progress zone" (Saunders 1977). After a certain period of time, a portion of the cells leave the zone and stop dividing, in order to form the most proximal limb element, the femur. The rest of the cells in the "progress zone" continue dividing and eventually another portion of the cells leave the zone to form the next most distal element, the tibio-fibula. In a similar manner after certain time periods, the tibiale fibulare, metatarsals and phalanges are formed. Now, if a reduction in cell division occurs throughout the process of limb formation then a reduction in the total number of cells in the limb bud should occur. The effect would reach crisis level when the number of cells in the "progress zone" failed to be sufficient to form a particular element. In the model presented below, this crisis stage is reached during the formation of the digits. In this model it is assumed that 5-FUdR inhibits 25% of the dividing cells in the "progress zone". (Continued on page 137a.)

An approximation based on the actual size of the limb elements is made and it is assumed that in the model :

1. The femur has 40 cells.
2. The tibio-fibula has 60 cells.
3. The tibiale fibulare has 80 cells.
4. The digits (metatarsals and phalanges) have 350 cells (70 cells per digit).

A schematic diagram of the model is shown on page 138.

The figure 25% is arrived at in the following way :
It is known from experiments on cells in tissue culture treated with 5-FUdR, that the inhibition of mitosis by the drug can be 100% (Simon 1963). In the experiments in this study, the animals were maintained in 5-FUdR and the drug was renewed once a week, yet the animals continued living. It was clear that 5-FUdR did not inhibit cell division completely, since the animals continued to grow slowly. As a first order approach, it was decided to assume a level of 25% inhibition of mitosis for the model. However, the principle of the model would hold whatever percentage is used, as long as the rate of formation of new cells by mitosis is chosen such that a point is reached when there are insufficient cells to produce a digit of normal size.



These 80 cells should form 160 cells after a division. 20 fail to divide due to 5-FUdR treatment. 140 cells are thus formed of which 40 go off to form the femur.

Loss of 20 cells due to 5-FUdR.

These 100 cells should form 200 cells but 25 fail to divide leaving 175 cells of which 60 go off to form the tibio-fibula.

Loss of 25 cells due to 5-FUdR.

These 115 cells continue to divide but 30 fail to divide leaving 200 cells of which 80 go off to form the tibiale fibulare.

Loss of 30 cells due to 5-FUdR.

These 120 cells continue to divide but 30 cells fail to divide leaving 210 cells.

Loss of 30 cells due to 5-FUdR.

Figure 4.5 *Model to explain the formation of a reduced number of digits*

The crisis now occurs as there are only 210 cells which is enough to form three full digits or perhaps five half-digits. The formation of three full digits or digits with phalanges missing was the normal finding in this research project.

The next question to be dealt with follows from the above model and findings, namely how the cellular material available for digit formation can be partitioned into three elements, as in the above case. To understand the problem, we must introduce the concept of biological inhibition. One type of inhibition has been postulated to occur in biological systems wherever repetitive structures are formed. These repetitive structures may result from a focus of differentiation and an inhibitory influence which spreads out from the focus. The inhibitory influence is assumed to prevent a second structure from forming in the immediate surroundings of the initial focus of differentiation. As the inhibitory influence is thought to decay with distance, a second repeat structure will be able to form at a certain distance from the initial focus, where the initial inhibitory focus fades out. In this way repetitive structures can be formed as is found for example in the distribution of hairs (Balinsky 1981).

Such an analysis could apply to the formation of digits 1, 2, 3, 4 and 5, given that a sufficient mass of cells was present and that all cells are capable of differentiation into digits, and also that certain cells in one region differentiate ahead of others to form digit 1. The result of such a condition could explain the formation of five normal digits as shown in the sketches in Figure 4.6 below.

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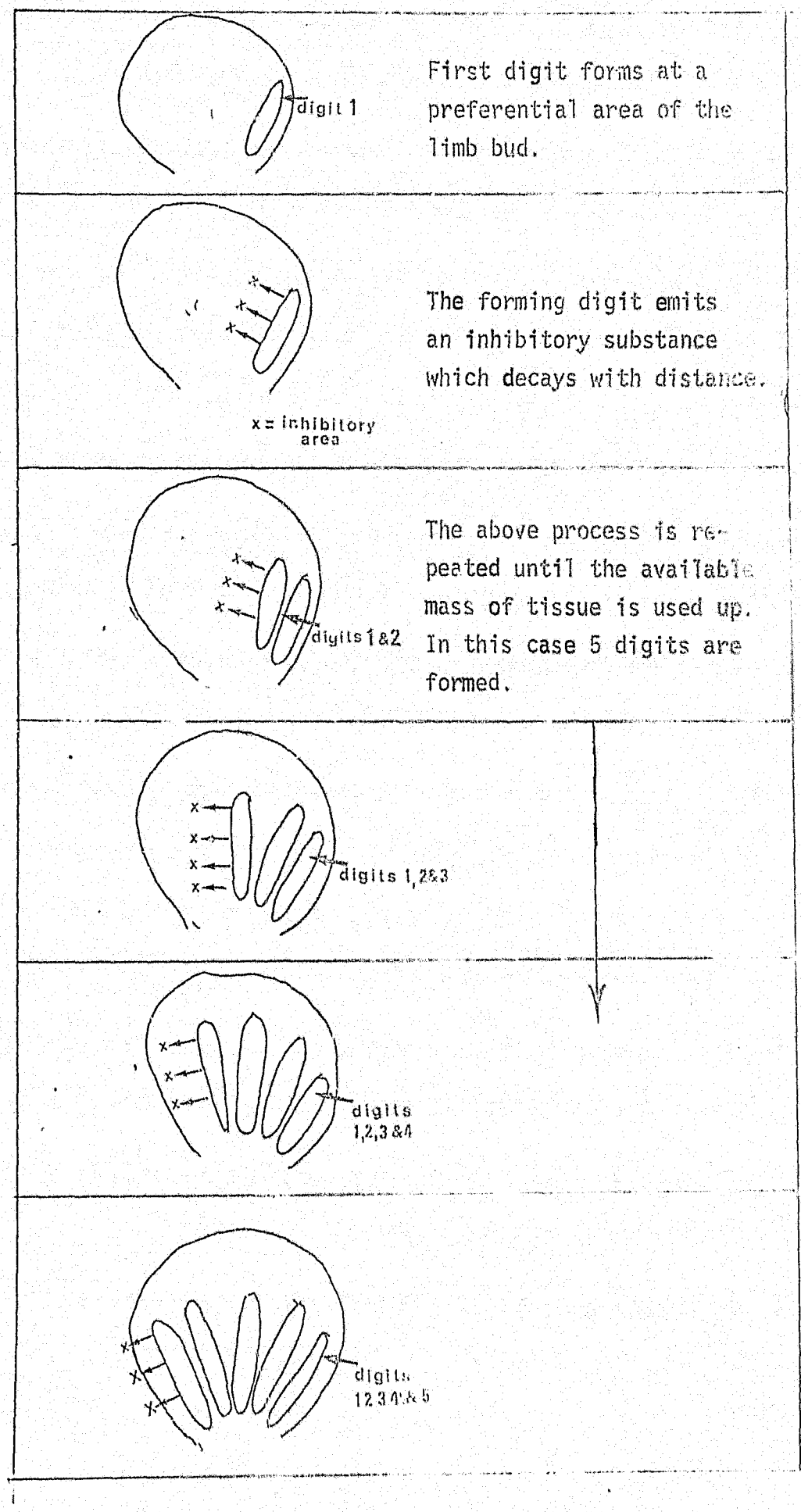

Figure 4.6 *Formation of five digits according to sequential "differentiation inhibition" hypothesis*

It is obvious that if there are a li..ited number of cells available for digit formation, then the sequential differenti tion of digits alternating with inhibition s ggests that elements will be formed in sequential o: der, until there are no more cells to form the last digit. This implies that if there are enough cells to form three digits, then three full digits and not six half-digits will form.

It will be recalled (see section 1.4.2.) that the ZPA (zone of polarising activity) exerts a strong influence on the limb pattern in the A - P direction (antero-posterior). The gradient of influence of this zone has a high point of influence on the limb pattern at the posterior end of the limb and a low point at the anterior end. Balinsky (1981) has found that the digits 1 and 2 appear first followed by 3, 4 and 5. This could mean that the lower gradient of influence anteriorly advances the development of digits 1 and 2 so that they form before 3, 4 and 5.

To sum up, it is seen that, as each digit forms, it inhibits the adjacent cells from forming another digit. This effect is used in the model being proposed to explain how a limited number of cells in the progress zone could be partitioned to form a reduce' number of digits.

In support of the above proposal, in which insufficient cells are available for digit formation, is the observation of Cairns (1977). This worker observed a mutant in the chick, which exhibits the phenomenon of polydactyly. The mutant was called Talpid^2 and the mutant chick had a limb bud of much larger diameter than the normal limb bud. What is most interesting is that the limb bud went on to form more than the usual number of digits. This would be expected according to the argument proposed above as the sequential process of differentiation and inhibition would continue across the limb bud until there was no more available tissue.

The inhibitory model of repeating structures can also be used to explain the formation of a series of bent digits. If the limb bud is initially bent as in the early stages (see pages 97 to 111), differentiation and inhibition would follow the shape of the initial bent bud, as illustrated in Figure 4.7 on page 142, and a series of bent digits would form.

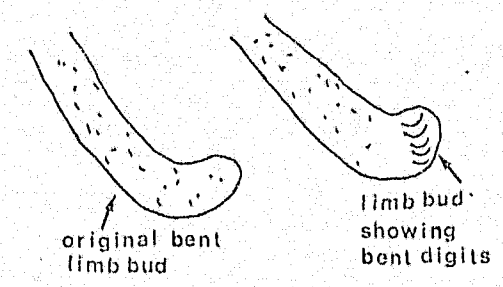

Figure 4.7 *Formation of bent digits*

4.2 Effects of concentration and stage on the severity of the resulting deformities

In the present research, it was necessary to find a favourable concentration of 5-FUdR for the in vivo experiments. Various concentrations ranging from 0,2 µg/ml to 20 µg/ml were tried. All the concentrations used produced similar deformities, as was seen in section 3.2. There does not seem to be a definite relationship between the concentration (at the concentrations used) and the level of deformity, possible due to the periodic nature of the drug treatment. More serious deformities were not produced by the higher concentrations.(N.B. Much higher concentrations than these were not used due to the prohibitive cost of the drug.)This suggests that at the concentrations used, there were always enough cells to form proximal elements of the limbs as mentioned in section 3.2 and that cell depletion only occured during the formation of the digits.

The stage at which the animals were first treated with the 5-FUdR did not appear to affect the severity of the deformity. The animal could be placed into the solution at any stage between 43 and 50 and the same effects were noticed, see section 3.2. This once again suggests that there were enough cells for the proximal elements and the crisis of cell production only occurs during the formation of the distal elements. When treated for the first time beyond stage 50, deformities did not develop, suggesting that the "progress zone" cells required for the formation of all the elements of the limb had already been produced as discussed on pages 13 to 18.

4.3 The use of 5-BUdR to produce deformities

We turn now to some experiments which were carried out to evaluate the effect of a related drug, viz 5-BUdR, which is known to inhibit cell differentiation but not cell division in cells in culture.

(5-BUdR was initially used alone or in combination with 5-FUdR due to its effect in preventing cell differentiation.) Stockdale (1964) prevented the differentiation of muscle cells in culture by using 5-BUdR. In the present research, it was found that 5-BUdR did not appear to cause any deformity as the deformities only appeared when 5-FUdR was present, either in combination with 5-BUdR or on its own. Deformities never appeared when the animals were treated with 5-BUdR only. One explanation for this could be that in the swimming tadpole not enough 5-BUdR was absorbed to have any effect or the giving of periodic doses once weekly allowed the DNA to replicate normally without taking up 5-BUdR between doses and differentiation was not sufficiently affected to be noticed.

①It will be recalled that Agnish and Kochnar (1976) grew isolated limbs in tissue culture with 5-BUdR and found that chondrogenesis was completely suppressed. In the present study whole animals were maintained continuously in water to which the drug was added once a week. During this time the animals would be continuously synthesizing their own supply of 'endogenous' thymidine. It is well known from experiments on the culture of cells in 5-BUdR (Levitt and Dorfman 1972) that the simultaneous addition of thymidine in equimolar amounts to the medium can override the effects of 5-BUdR. It is assumed that 5-BUdR did not affect the experimental animals in this study as endogenous thymidine competed with 5-BUdR, blocking its normally deleterious interference with differentiation as reported in the article by Anderson and Wilt (1972). A number of other tissues also have differentiation blocked reversibly when treated with 5-BUdR. In all these cases, however, isolated cells or tissues were cultured in 5-BUdR continuously in the absence of thymidine.

It could be argued that 5-BUdR did not enter the circulation and therefore present results did not respond to typical 5-BUdR inhibition of differentiation. This is, however, unlikely as tadpoles maintained in water containing ^{3}H-thymidine, take it up, and ^{3}H-thymidine has been demonstrated in the dividing cells of various organs of the tadpole (Goldin 1977). It is extremely likely that 5-BUdR would be taken up in the same way as ^{3}H-thymidine by tadpoles, in line with the way cells in culture take up 5-BUdR. This highly likely assumption could be tested by maintaining tadpoles in ^{3}H-5-BUdR, although the cost of this approach would be prohibitive. A less costly approach would entail injecting small amount of 5-BUdR into the tadpole on a daily basis, although this approach would probably result in a high rate of mortality.*

4.4 The effect of 5-FUdR on the uptake of thymidine

These experiments were set up (see section 2.2) to test the effect of 5-FUdR on thymidine uptake, on the limb in vivo. It will be seen from the results that 5-FUdR inhibited the uptake of ^{3}H-thymidine into the hindlimb buds. There are two possible explanations for this, either so many cells had stopped dividing that less thymidine was required for overall cell division, or (for some reason) 5-FUdR is also able to block the uptake of both enodenous and applied ^{3}H-thymidine (see Figure 1.46). However, the most sensible explanation at this stage would seem to be that the experiment does show that 5-FUdR inhibits the DNA synthesis and thus it blocks and interferes with the metabolism. However, the degree of variance in the method caused this line of experimentation to be abandoned.② This degree of variance could be caused by the fact that tadpoles grown in crowded conditions show varying mitotic rates. This variance can be reduced if they are grown singly in ^{3}H-thymidine as Hurwitz (1979) showed that growing tadpoles show different mitotic rates.

4.5 Buoyant density analysis of DNA by analytical ultracentrifugation

At an early stage of this project, some experiments were carried out, but were discontinued for various reasons. These experiments are briefly summarised.

Experiments were set up to see whether the 5-BUdR was being incorporated into the tadpole DNA during metamorphosis and to see whether the 5-FUdR had any effect on metamorphosis. In the DNA from tadpoles not treated with 5-BUdR (see Figure 3.21) only one main band of DNA was found as should be expected. On the densitometer trace (see Figure 3.23) this is seen as peak c. When 5-BUdR was added, the ultraviolet absorption photograph of the DNA showed two bands, the normal DNA band and the heavy DNA band due to the 5-BUdR. On the densitometer trace this showed up as peak d. When 5-FUdR was added to the solution these same two bands are also seen, peak b and c. However, peak c representing the heavy DNA appears to be wider than peak d the heavy DNA band in the experiment using only 5-BUdR. This suggests that more 5-BUdR was taken up in the presence of 5-FUdR due to the lower concentration of thymidine. Because of the relative insensitivity of the method, these experiments were discontinued.

③ The order of resolution in terms of the analysis of heavy DNA by the analytical ultracentrifuge could be increased by using an alternate strategy. In this alternate approach the DNA could be labelled with an isotope such as ^{3}H and/or ^{14}C (^{14}C-thymidine ^{3}H-5-BUdR) and the heavy and light peaks followed in the preparative ultracentrifuge in a CsCl gradient as in the approach followed by Fabian and Wilt (1973). This would increase the degree of sensitivity of the method allowing one to examine small changes.

4.6 Experiments on growth and feeding of tadpoles

As the chemical 5-FUdR was costly and in short supply, it was necessary to grow the tadpoles in small dishes of 200 ml capacity. The feeding experiments were designed to compare the growth in the normal 2 litre capacity trays with the growth in the 200 ml dishes and to find food that gave optimum growth in the smaller dishes. In the large trays feeding with Complan (commercial product) or feeding with liver and vegetables (commercial baby food) gave the best results as speedy growth was observed.

Liquifry, although not giving such fast growth allowed more of the tadpoles to grow to maturity (Liquifry is a commercial feed for small fish). In the small dishes the best growth was given by Liquifry as in the small quantity of water it did not go bad and cause deaths, which the other two foods did. In the larger trays this was not a problem. As the water could only be changed once weekly owing to the expense of the chemical, it was decided to use the safer and slower Liquifry, which proved to be totally adequate.

4.7 Conclusion

The hindlimb development of Xenopus laevis was affected by 5-FUdR with respect to shape and patterning. This was expected due to the anti-mitotic effect of 5-FUdR. The resulting pattern of the deformed limb was explained in terms of the sequential "differentiation inhibition" model with reference to the "progress zone". 5-BUdR was found to have no effect on the experiments in this study, possibly due to the periodic nature of administration of the drug.① That 5-FUdR was the definite cause of the deformities in the Xenopus laevis hindlimb is borne out by the fact that during the examination of 576 control tadpoles (reared in water during the feeding experiments in section 2.4) not one hindlimb deformity was observed, whereas five out of six tadpoles in the experimental dishes (5-FUdR + water) showed some kind of deformity.

5-FUdR was found to depress external thymidine uptake, confirming the previous finding that 5-FUdR depresses DNA replication, thus having a direct effect on mitosis.② It will be seen from the results that the uptake of thymidine by the left and right hindlimbs varied significantly. It has been observed that in some cases, at least, the hindlimbs of Xenopus laevis start to develop asynchronously. (Personal communication B. Fabian.) For this reason the left and right hindlimbs could differ in weight at the same stage in the animal's growth. A buoyant density analysis confirmed the uptake of 5-BUdR into the DNA of Xenopus laevis tadpoles.

A series of feeding experiments singled out Liquifry as the most suitable nutrient for the experiments carried out in small 200 ml capacity dishes.

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ADDENDUM TO DISSERTATION

Since the time of writing and the preparation of the dissertation for publication, a number of references pertaining to this fast moving field have appeared. Some of the more relevant references are listed below :

Holder N., 1981. Pattern formation and growth in the regenerating limbs of urodelean amphibia.
J.Embryol.Exp.Morph. 65 (suppl.) 19-36

Maden M., 1981a. Experiments on Anuran limb buds and their significance for principles of vertebrate development.
J.Embryol.Exp.Morph. 63, 243-265

Maden M., 1981b. Morphallaxis in an epimorphic system : size, growth control and pattern formation during amphibian limb regeneration.
J.Embryol.Exp.Morph. 65 (suppl.) 151-167

Rowe D. and Fallon J., 1981. The effect of removing posterior apical ectodermal ridge of the chick wing bud and leg on pattern formation.
J.Embryol.Exp.Morph. 65 (suppl.) 309-325

Smith J., 1981. Growth factors and pattern formation.
J.Embryol.Exp.Morph. 65 (suppl.) 187-207

Smith J. and Wolpert L., 1981. Pattern formation along the antero-posterior axis of the chick wing : the increase in width following a polarising region graft and the effect of X-irradiation.
J.Embryol.Exp.Morph. 63, 127-144

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J.Embryol.Exp.Morph. 65 (suppl.) 3-18

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J.Embryol.Exp.Morph. 63, 161-180

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J.Embryol.Exp.Morph. 65 (Suppl.) 129-150

Tickle C., 1981. The number of polarising region cells required to specify additional digits in the developing chick wing. Nature 289, 295-298

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Of these references, the following relate directly to the work described in this dissertation.

Maden (1981a) showed that the frequency of supernumerary hindlimb formation by Xenopus laevis is low. This work is similar to the experiments of Cameron and Fallon (1977) discussed on page 23 of this dissertation.

Maden (1981a) also showed that when a large slice of tissue from the embryonic hindlimb (between the proximal and distal axis) is removed, the expected intercalation of missing tissue does not occur. This is contrary to what happens in the forelimbs of the newts Notopthalmus viridiscens and Amblystoma maculata as discussed on page 31 of this dissertation.

Smith and Wolpert (1981) use x-irradiation to reduce the limb bud width after a graft of the polarising region in the chick wing. This causes a loss in the expected number of duplicate digits. This type of experiment is discussed on pages 22-23 of this dissertation.

A work in the literature that has a distant but interesting bearing on the findings in this dissertation is the work by Tickle (1981) in which she mixed ZPA (polarising) cells with inactive cells from the region along the anterior region of the wing bud of the chick. This mixture was grafted to the anterior region of another wing bud. This grafting of a second ZPA to the limb causes duplicate digits to form (Saunders and Gasseling 1968 on page 22 of this dissertation). Tickle progressively diluted cells from the ZPA (polarising region) with inactive cells, and a definite relationship emerged between the number of ZPA (polarising) cells and the number of duplicate digits formed.

More polarising cells were required to form duplicate digit 4, fewer to form duplicate digit 3 and when there were too few cells no duplicate digits formed.

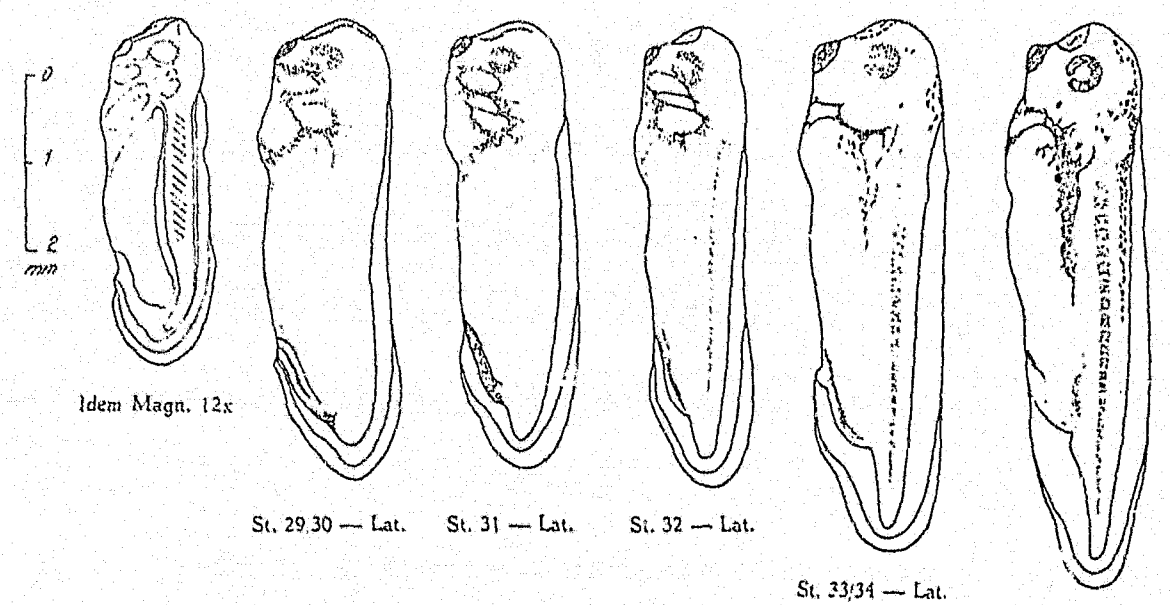
Therefore it appears that the number of polarising cells present can influence morphogenetic development.

These findings by Tickle (1981) concur with the proposal in this dissertation that a particular number of cells is needed for the formation of each element, and that an insufficient number of cells causes absence of digits.

While other recent papers have a bearing on the theoretical aspects of limb development, none concern the experimental findings in this dissertation.

APPENDIX A

A series of normal stages in the development of the Xenopus laevis tadpole (Nieuwkoop and Faber 1967)



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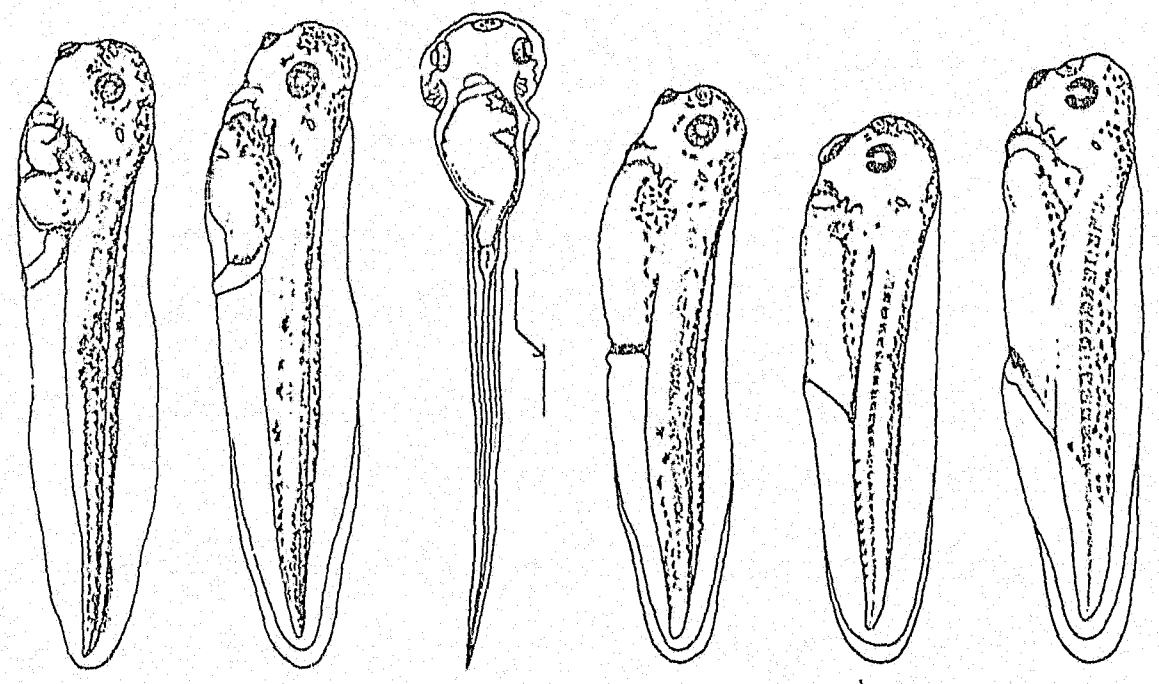
St. 31 — Lat.

St. 32 — Lat.

St. 33/34 — Lat.

St. 35,36 — Lat.



St. 42 — Lat.

St. 41 — Lat.

St. 41 — Ventr.

St. 40 — Lat. (Small ind.)

St. 39 — Lat. (Small ind.)

St. 37/38 — Lat.

Author Christie C A
Name of thesis The effect of 5-bromodeoxyuridine and 5-fluorodeoxyuridine on differentiation and metamorphosis in Xenopus Laevis Tadpoles 1982

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