FIGURE 27

Specific activity of G.P.T. in the livers of metamorphosing Rana tadpoles.

Experimental conditions - see opposite Figure 24.

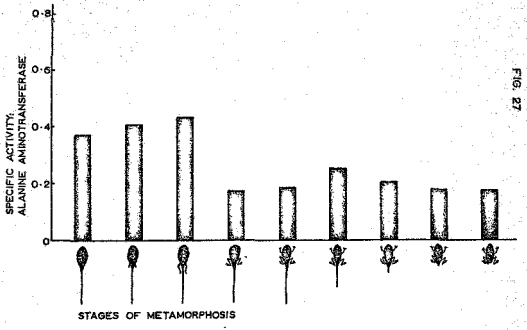
0·8
0·6
0·4
0·2
0
SPECIFIC ACTIVITY:
ALANINE AMINOTRANSFERASE
STAGES OF METAMORPHOSIS

FIG. 27

FIGURE 28

The effect of various hormones on the activity per unit wet weight of G.O.T. in Xenopus liver.

Control:

The control frogs were injected with water instead of a hormone and then treated exactly as the experimental frogs. All the frogs were starved for the duration of the experiment and the maximum period of starvation was 2 weeks. The control figures represent a mean of 2 experiments, each consisting of 4 frogs, carried out at the beginning and end of the 2 weeks starvation period.
The other results are each a mean of 4 frogs.

Experimental:

The hormone solutions were injected into the peritoneum every day for four days and the enzyme activities analysed on the fifth day. Two ml. of the solutions was injected per 100 g. frog.

ACTH:	0·1 mg./ml.
Aldosterone:	0·1 mg./ml.
Corticosterone:	1·25 mg./ml.
Hydrocortisone:	1·25 mg./ml.
Insulin:	0·8 units/ml.
Thyroxine:	1·25 mg./ml.
Triiodothyronine:	1·25 mg./ml.
Vasopressin:	15-20 I.U./ml.

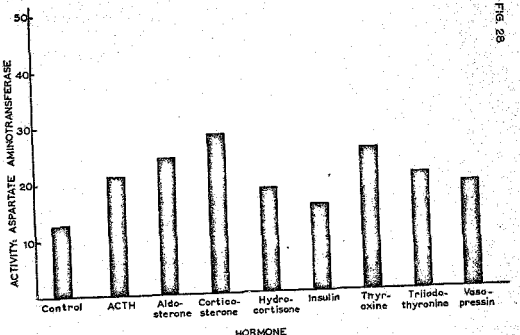
ACTIVITY: ASPARTATE AMINOTRANSFERASE
50
40
30
20
10
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 29

FIGURE 29

The effect of various hormones on the activity per unit wet weight of G.P.T. in Xenopus liver.

Experimental conditions - see opposite Figure 28.

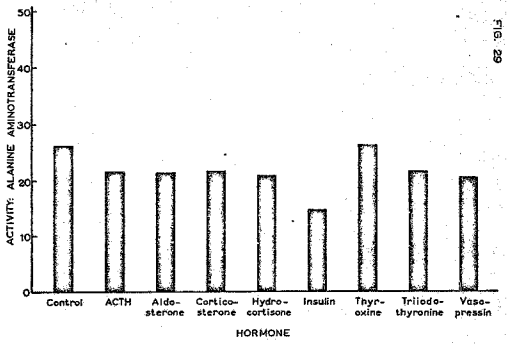
ACTIVITY: ALANINE AMINOTRANSFERASE
50
40
30
20
10
0
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 29

FIGURE 30

The effect of various hormones on the specific activity of C.O.T. in Xenopus liver.

Experimental conditions - see opposite Figure 28.

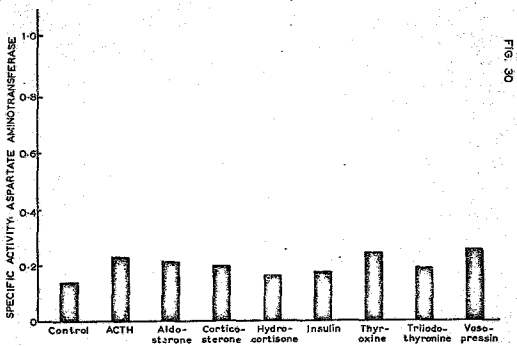
SPECIFIC ACTIVITY: ASPARTATE AMINOTRANSFERASE
1·0
0·8
0·6
0·4
0·2
0
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 30

FIGURE 31

The effect of various hormones on the specific activity of G.P.T. in Xenopus liver.

Experimental conditions - see opposite Figure 28.

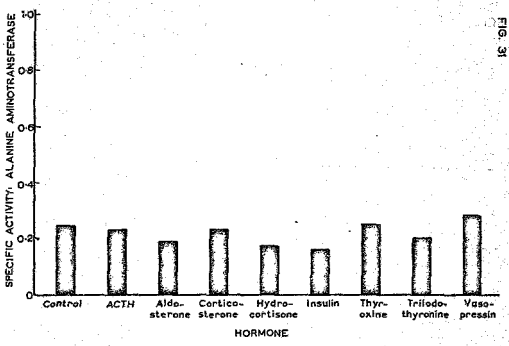
SPECIFIC ACTIVITY, ALANINE AMINOTRANSFERASE
1.0
0.8
0.6
0.4
0.2
0
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 31

FIGURE 32

The effect of various hormones on the ratio o. the specific activities of G.O.T./G.P.T. in Xenopus liver.

Experimental conditions - see opposite Figure 28.

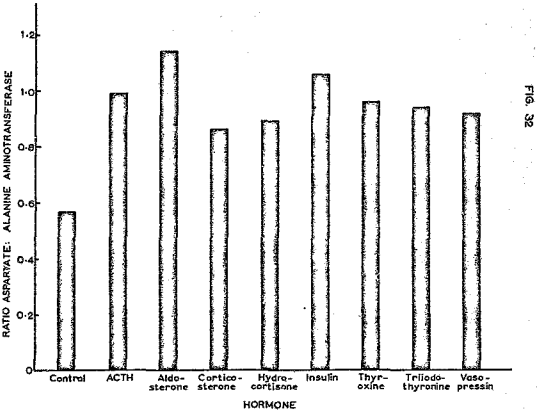
RATIO ASPARYATE: ALANINE AMINOTRANSFERASE
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 32

FIGURE 33

The effect of various hormones on the activity per unit wet weight of G.O.T. in Xenopus kidney.

Experimental conditions - see opposite Figure 28.

ACTIVITY: ASPARTATE AMINOTRANSFERASE
0
10
20
30
40
50
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG 88

FIGURE 34

The effect of various hormones on the activity per unit wet weight of G.P.T. in Xenopus kidney.

Experimental conditions - see opposite Figure 28.

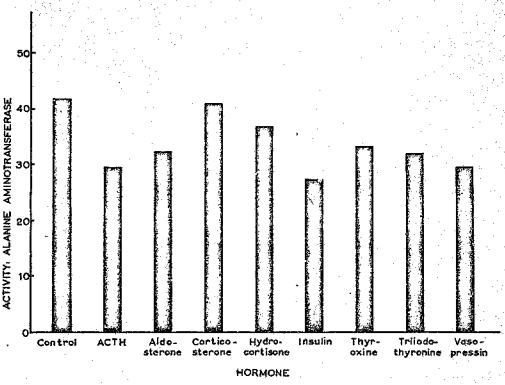
ACTIVITY: ALANINE AMINOTRANSFERASE
0
10
20
30
40
50
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 34

FIGURE 35

The effect of various hormones on the specific activity of G.O.T. in Xenopus kidney.

Experimental conditions - see opposite Figure 28.

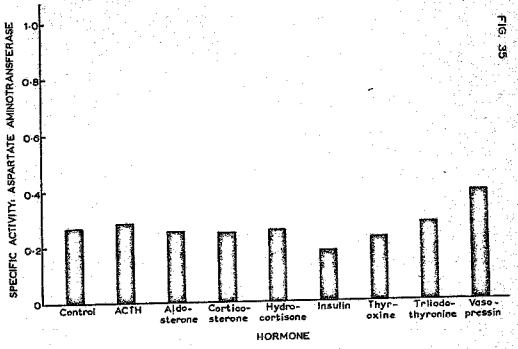
SPECIFIC ACTIVITY, ASPARTATE AMINOTRANSFERASE
1·0
0·8
0·6
0·4
0·2
0
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 35

FIGURE 36

The effect of various hormones on the specific activity of G.P.T. in Xenopus kidney

Experimental conditions - see opposite Figure 28.

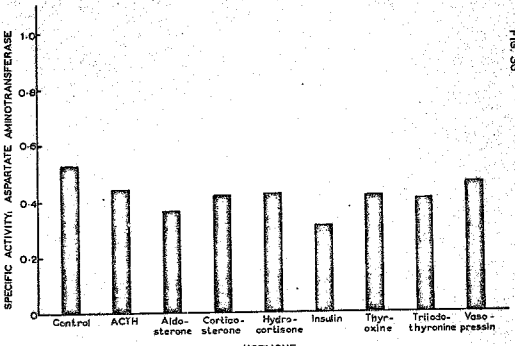
SPECIFIC ACTIVITY: ASPARTATE AMINOTRANSFERASE
1·0
0·8
0·6
0·4
0·2
0
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 36.

FIGURE 37

The effect of various hormones on the ratio of the specific activities of G.O.T./G.P.T. in Xenopus kidney.

Experimental conditions - see opposite Figure 28.

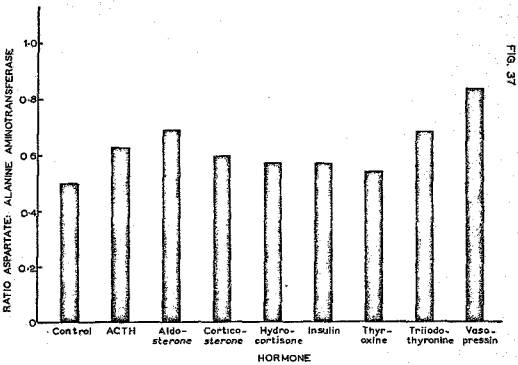
RATIO ASPARTATE: ALANINE AMINOTRANSFERASE
1·0
0·8
0·6
0·4
0·2
0
Control
ACTH
Aldo-sterone
Cortico-sterone
Hydro-cortisone
Insulin
Thyr-oxine
Triiodo-thyronine
Vaso-pressin
HORMONE

FIG. 37

FIGURE 38

The effect of starvation on protein concentration of *Xenopus laevis* liver homogenate.

PROTEIN CONCENTRATION mgs/ml
100
80
60
40
20
0
10
20
30
40
50
60
70
80
90
DAYS STARVED

FIG. 35

DISCUSSION

A. THE EFFECT OF ENVIRONMENT ON G.O.T. AND G.P.T. IN XENOPUS LAEVIS LIVER AND KIDNEY.

Janssens (1964), whose experiments have been discussed on P. 43 first attempted to correlate the level of transaminases with the predominant end-products of nitrogen metabolism. He suggested that the ratio of aspartate to alanine transaminase is specially significant as an indicator of the direction in which waste nitrogen is being channelled. A high ratio of aspartate to alanine transaminase would channel nitrogen from alanine, (a major resevoir of α-amino nitrogen in plasma and body fluids), into aspartate and hence urea nitrogen. A low ratio of the two enzymes would favour the conversion of alanine nitrogen into ammonia by transdeamination. The aim of the present work was to test this hypothesis by comparing the ratio of the two enzymes in a number of species and under different physiological conditions.

When Janssens kept Xenopus adults in saline for five weeks, the ratio of the two enzymes rose significantly for the first two weeks, and then slowly returned to control values. With Janssens' results in mind, the frogs in the present study were returned to fresh water after

16 days in saline. Contrary to expectation, the ratio was similar to that of the control frogs. There was, however, a long term effect of the saline treatment. The ratio of the two enzymes started rising in the 4th week, i.e. 2 weeks after the return to fresh water, and this continued until the end of the experiment, giving an increase of almost 3-fold by the 11th - 4k (Figure 8, P. 79). This increase in the ratio was almost entirely due to an increase in the specific activity of G.O.T., G.P.T. being unaffected by the saline treatment. It seems significant that this effect was only observed in the liver, the kidney enzymes showing no response to salinity.

This long term effect may suggest that adaptation to a hypertonic environment does involve some regulatory control on the ratio of the two transaminases, but the physiological significance of this is not clear in view of the finding of Balinsky et al (1967b) that there is no change in the ratio in naturally aestivating frogs. It must be borne in mind, however, that water restriction by hypertonic medium is not the same as natural aestivation. As McBean & Goldstein (1970) point out, during aestivation, the dehydrated animal channels its waste nitrogen into urea, while there is a general reduction in waste nitrogen excretion. Thus the effect is merely to increase the

proportion of nitrogen channelled into urea. In the saline-adapted toad, however, the synthesis and excretion of urea is increased, this being an important osmoregulatory process.

A similar survey of the long term effects of water restriction was done on the urea cycle enzymes by C. Coe (unpublished). Using the same experimental conditions, he found that the urea cycle enzymes rose to a peak after return to fresh water and that the levels dropped gradually to normal after about 16 days.

B. COMPARATIVE SURVEY OF TRANSAMINASE LEVELS IN VARIOUS AMPHIBIA.

A comparative survey of transaminase levels in different species of Amphibia was next carried out. A high ratio of G.O.T. to G.P.T. was shown to accompany predominance of urea excretion over ammonia. Thus Xenopus, which is ammoniotelic, was shown to have a low ratio, while in the ureotelic species the ratio was high. In Bufo regularis, the ratio was double that of Xenopus, in Rana angolensis it was three times that of Xenopus, while in Rana fuscigula it was 5-6 times higher than in Xenopus. In all cases the difference in the ratio was due to both an increase in the specific activities of G.O.T. and a decrease in those of G.P.T. (Figure 16, P. 87, 17. P. 88 & 18. P. 89).

Rana cancrivora is an unusual frog in that it is one of a few species which can live in both fresh and salt water. It adapts to the latter by raising the osmotic pressure of the blood by increasing its blood urea concentration. No difference in the ratio of the two transaminases was found in the animals kept in different concentrations of sodium chloride. The ratio was much lower, however, in

animals kept in fresh water. There was therefore, no correlation of transamination levels with the osmolarity of the surroundings. Balinsky, Dicker & Elliot (unpublished) found a close correlation between the salinity of the environment and the levels of the urea cycle enzymes, but it should be pointed out that their experiments were very long term ones, involving adaptation to a particular environment for four weeks.

A much lower ratio was found in Rana cancrivora living in fresh water. This result could be explained by the postulate that, in the total absence of sodium chloride in the environment, ammonia excretion becom associated with sodium retention. The altered ratio of the transaminases may channel a larger proportion of nitrogen into ammonia, thus enabling the absorption of sodium ions from the lumen of the kidney tubules in exchange for ammonium ions, or for hydrogen ions, which are then neutralized by the excreted ammonia. This process, which the animal only uses intermittently, might be different from that operating in other species of Rana, which are permanently in fresh water, and this would explain the difference in ratio from the other species.

C. CHANGES IN G.O.T. AND G.P.T. LEVELS DURING THE METAMORPHOSIS OF XENOPUS LAEVIS AND RANA ANGOLENSIS.

As discussed on P. 43 it was expected that the ratio of G.O.T. to G.P.T. should increase during metamorphosis concurrently with the change from ammoniotelism to ureotelism.

The ratio of the two enzymes did not change significantly in Xenopus during metamorphosis, in agreement with the fact that Xenopus remains ammoniotelic (Figure 23, P. 94). In Rana, however, there is a considerable increase in the ratio, due to an increase in the specific activity of G.O.T. as well as a decrease in that of G.P.T. during prometamorphosis. In the young adult, the ratio is raised further to almost double that at the onset of metamorphosis, corresponding to the change from ammoniotelism to ureotelism (Figure 27, P. 98).

D. THE EFFECT OF VARIOUS HORMONES ON TRANSAMINATION IN XENOPUS.

In the liver, all the hormones investigated had the effect of increasing the ratio of G.O.T. to G.P.T., while in the kidney the effect was not as pronounced.

1. Liver

All the hormones increased the activity per unit wet weight of G.O.T. over the control values, the greatest effect being with corticosterone and the smallest with insulin (Figure 28, P. 99). The activity per unit wet weight of G.P.T. was decreased by all the hormones except thyroxine, the greatest effect being that of insulin (Figure 29, P. 100).

If specific activity is considered, that of G.O.T. was raised above the control level by all the hormones, the greatest increase being with vasopressin and the least with hydrocortisone (Figure 30, P. 101). The specific activity of G.P.T. was lowered significantly by aldosterone, hydrocortisone, insulin and triiodothyronine, and was unaffected by the others (Figure 31, P.102).

All the hormones raised the ratio of G.O.T. to G.P.T. Aldosterone had the greatest effect, raising the ratio more than two-fold, while corticosterone had the smallest effect, raising the ratio one and a half times (Figure 32, P. 103).

Thus aldosterone and possibly some of the other hormones may play an important role in the induction or activation of G.O.T. in the liver. It is worth noting that the results of C. Coe (unpublished) suggest a possible induction of urea cycle enzymes by aldosterone as well as by corticosterone and hydrocortisone.

It is also interesting to note that, as mentioned on P. 36, Rutman (1968) found no effect by hydrocortisone on either transaminase in frogs, although it induced enzyme activity in rats and pigeons. He also found that this effect was abolished by injections of insulin, although Rosen (1964) could not show any effect with insulin on the enzymes in rat liver.

2. Kidney

The activity per unit wet weight of G.O.T. appeared to be unaffected by all the hormones except aldosterone

and possibly vasopressin, which raised the activity, and insulin, which lowered it (Figure 33, P.104). The activity per unit wet weight of G.P.T. was lowered considerably by all the hormones except corticosterone which appeared to have no effect. The lowest activity was found with insulin (Figure 34, P.105).

The specific activity of G.O.T. appeared to be unaffected by all the hormones except insulin which lowered it and vasopressin which raised it (Figure 35, P.106). All the hormones lowered the specific activity of G.P.T., the greatest effect being with insulin (Figure 36, P.107).

The ratio of G.O.T. to G.P.T. was raised by all the hormones except possibly thyroxine. The largest increase occured with vasopressin (Figure 37, P.108).

E. CONCLUSION.

From the results of this investigation it would appear that the ratio of the two transaminases does change in different species and under different conditions, and that these changes can frequently be correlated with the predominance of ammonia or urea as the nitrogenous end product of metabolism. Moreover, the ratio of the specific activity of aspartate to alanine transaminase shows a better correlation than the activity per unit wet weight or the specific activity of the individual enzymes, for example in the study on metamorphosing Rana tadpoles (Figures 26-28, P. 97-99) and also on the effects of water restriction in Xenopus (Figures 6-8, P. 77-79). The results therefore support the use of this ratio as suggested by Janssens.

All the hormones used in the present investigation appeared to produce an increase in the ratio in the liver, although for different reasons. Thus for example, ACTH and thyroxine affect this entirely by an increase in liver G.O.T., while insulin increases the ratio mainly by decreasing liver G.P.T., with only a slight increase in G.O.T.

While all the hormones appear to affect the ratio of the two transaminases, one is left with no clear picture of the hormonal basis of the natural regulation of these enzymes. As the effects of the environmental changes on the ratio in adult Xenopus were equivocal, it may be that another model situation, such as a developing Amphibian, would yield a clearer answer to this problem.

SUMMARY

1. The effect of environment on the levels of aspartate and alanine aminotransferase in the liver and kidney of Xenopus laevis were studied. The ratio of aspartate to alanine transaminase activity was measured and its correlation with the predominance of different pathways of nitrogen metabolism was studied.

2. It was found that water restriction causes an increase in the ratio of aspartate to alanine aminotransferase in the liver, which has been seen to accompany the increase in urea production caused by dehydration.

3. Levels of the two enzymes were compared in various species of Amphibia. High ratios of the enzymes were found to be present in ureotelic animals and low ratios in ammoniotelic animals.

4. The effect of metamorphosis on transamination was studied in Xenopus and Rana tadpoles. An increase in the ratio of the two enzymes was seen to accompany a change from ammoniotelism to

ureotelism, such as that found in Rana tadpoles. Xenopus did not show this increase in agreement with the fact that there is no change to ureotelism during metamorphosis.

5. The effect of various hormones on the ratio of transaminase activities was studied in the liver and kidney of Xenopus laevis.

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