ANTERIOR PITUITARY AND GROWTH IN THE AXOLOTL (*AMBLYSTOMA TIGRINUM* (GREEN) NEOTENIC FORM)

II. THE EFFECT OF INJECTION OF GROWTH-PROMOTING EXTRACTS UPON THE UTILIZATION OF FOOD

BY N. H. HOWES

Department of Zoology and Comparative Anatomy, University College, London

(*Received* 25 *January* 1938)

I. INTRODUCTION

THE problem of the mechanism by which growth-promoting extracts of the anterior pituitary cause an increase in the growth rate, is one of extreme importance. This problem has been investigated in mammals chiefly by tissue analyses of control and experimental animals (Bierring & Nielsen, 1932, rats; Wadehn, 1932, mice; Lee & Schaffer, 1934, rats) and by analyses of body fluids (Teel & Watkins, 1929, dogs; Teel & Cushing, 1930, dogs and rats; Gaebler, 1933, dogs; Gaebler & Price, 1937, dogs). The general conclusion to be drawn from the results of these investigators is that growth-promoting extracts cause a retention of nitrogen, a slight retention of water, little change in ash and a loss of fat.

The investigation of the effects of various growth-promoting extracts upon axolotls described in a previous paper (Clements & Howes, 1938) provided an opportunity for obtaining comparative data relating to the ratio between food consumed and increase in body weight. Examination of the records has shown the results to be of some interest and they are therefore discussed below.

II. MATERIALS AND METHODS

In the experiments of Clements & Howes, five tanks each containing ten animals were used. The animals were treated over a period of 12 weeks, as follows:

Tank 1. Injected with saline extract of muscle.
Tank 2. Injected with Schockaert's (1931) extract of anterior pituitary.
Tank 3. Injected with phyone (Van Dyke & Wallen-Lawrence, 1930).
Tank 4. Injected with prolactin (Bates & Riddle, 1935).
Tank 5. Injected with phyone plus prolactin.

Another four tanks of ten animals of the same age and parentage, kept and treated in exactly the same way as those above except that they were not injected, were also available. All the animals were of the same age and the total weights of the animals in tanks 1–5, respectively, were nearly equal at the beginning of the experiment.

The animals were weighed individually each week and from these data the absolute increase in weight of any animal or batch of animals for periods in multiples of a week could be calculated. Results so obtained showed that the animals in tanks 2, 3, 4 and 5 grew very much more rapidly than those in tank 1.

The animals were fed upon lean beef, liver and worm, the last with its gut contents not removed, cut into standard pieces of approximately the same size. All the axolotls had been trained to take food offered to them with forceps and therefore the number of pieces eaten by the inhabitants of each tank could be counted. Upon this basis, if the pieces were shown to be of standard weight, it would be possible to construct a balance sheet for food intake against tissue laid down plus food metabolized or lost as roughage.

To investigate the constancy in weight of the pieces of food, batches of 100 pieces were weighed at intervals throughout the experiment. Results so obtained are recorded in Table I.

Table I

	Weight (g.) of 100 pieces of		
	Liver	Beef	Worm
	30·0	25·0	40·0
	28·0	29·0	40·0
	35·0	23·0	31·3
	29·2	26·0	28·4
	37·0	24·0	34·4
	36·5	33·0	37·5
	31·7	31·0	35·3
	34·0	34·0	45·5
	30·0	23·8	40·6
	33·0	24·2	43·0
Total	324·4	273·0	376·0
Mean	32·4	27·3	37·6
S.D.	±3·01	±4·11	±5·29

The deviation from the mean of an individual weighing never reached 2 × S.D.

From this table it will be seen that estimates of weight of food consumed can be made within an accuracy of approximately ± 15 %. The animals were almost invariably fed by the author.

III. ACCURACY OF THE METHOD

To test the accuracy of the method, two tanks of untreated animals were compared over two consecutive periods of twelve weeks each. The number of pieces eaten of the three foods was counted and the total weight of each kind consumed calculated from the data given in Table I. These weights were then added together and the total food eaten by each group of animals obtained for comparison with the grams increase in weight. The results are expressed in the form $\frac{\text{food intake}}{\text{weight gained}}$ and have been calculated for each tank separately both over the whole experiment and for each of the two periods.

The maximum difference in the ratio $\frac{\text{food intake}}{\text{weight gained}}$ calculated for any two of the periods investigated in these two tanks, whether the same or different tanks be compared, was 0·49 ± 0·31, which is not significant. It may therefore be assumed that under the conditions of this experiment, two tanks each containing ten axolotls of identical age and covering the same ranges of growth rates give concordant results within the limits of the error inherent in the calculation of the weight of the food.

Table II

	Food	Tank 6		Tank 8	
		No. of pieces	Wt. of food in g.	No. of pieces	Wt. of food in g.
Period I	Liver	619	200·5 ± 18·6	616	199·5 ± 18·5
	Beef	297	81·1 ± 11·1	275	75·1 ± 11·3
	Worm	393	147·7 ± 20·7	391	147·0 ± 20·7
	Total		$429·3 \pm \sqrt{\{(18·6)^2 + (11·1)^2 + (20·7)^2\}}$ = 429·3 ± 30·8 g.		$421·6 \pm \sqrt{\{(18·5)^2 + (11·3)^2 + (20·7)^2\}}$ = 421·6 ± 30·8 g.
	Change in weight:		226·1–369·7 g.		213·5–340·8 g.
	∴ Wt. gained by animals:		143·6 g.		127·3 g.
	$\frac{\text{Food intake}}{\text{Wt. gained}}$		= 2·99 ± 0·21		3·31 ± 0·24
Period II	Liver	597	193·4 ± 18·0	620	200·8 ± 18·6
	Beef	270	73·3 ± 11·1	323	88·2 ± 13·3
	Worm	386	145·1 ± 20·4	356	133·9 ± 18·8
	Total		412·2 ± 30·3		422·9 ± 29·7
	Change in weight:		369·7–499·8 g.		340·8–490·6 g.
	∴ Wt. gained by animals:		130·1 g.		149·8 g.
	$\frac{\text{Food intake}}{\text{Wt. gained}}$		= 3·17 ± 0·23		2·82 ± 0·20
Both periods together	Liver	1216	394·1 ± 36·6	1236	400·3 ± 37·2
	Beef	567	154·8 ± 23·3	598	163·2 ± 24·6
	Worm	779	292·2 ± 41·2	749	280·8 ± 39·5
	Total		841·1 ± 59·8		844·3 ± 59·6
	Change in weight:		226·1–499·8 g.		213·5–490·6 g.
	∴ Wt. gained by animals:		273·7 g.		278·1 g.
	$\frac{\text{Food intake}}{\text{Wt. gained}}$		= 3·07 ± 0·22		3·04 ± 0·21

Sources of error arise in that the food in the gut of the animals was not taken into account, but since the animals were kept in a state of repletion throughout, this should not greatly affect the difference in weight which is used in the calculations; nor is any allowance made for the varying amounts of roughage and water in the foods. Observations suggest that axolotls are unable to digest either the cuticle of earthworms or most of the fat present in beef.

The method is not ideal but several other ways of estimating the food intake have been tried without success. First, it might have been possible to weigh the animals immediately before and after feeding, but any method involving the handling of axolotls soon after feeding almost invariably makes them vomit. Second, weighing the animals, feeding them and reweighing 12–24 hr. later is useless. Investigation has shown that axolotls may continue to increase in weight for more than 48 hr. after a feed. It is thought that this is due to water being retained or taken up to aid in the digestion and absorption of food. Third, an axolotl will not, as a rule, eat several pieces of food in rapid succession, so that to feed one to repletion involves the offering of food at intervals of about 5 min. over 20 min. or so. It is therefore impracticable when dealing with large numbers of animals, to weigh the food plate before and after every feed. Fourth, it would probably prove practicable to feed axolotls on capsules containing a known quantity of a semi-synthetic diet, but since data on such a diet are not yet available, the method could not be employed in this instance.

IV. RESULTS

The results obtained with the treated animals are given in Table III. Thus the

Table III. *Comparison of food intake with increase in weight of axolotls injected with growth-promoting hormones over a period of* 12 *weeks. All animals* 23 *weeks old. Dosage in all cases equivalent to* 1 *mg. wet weight of tissue per gram of axolotl per day*

Tank no., treatment, initial and final wts.	Food	No. of pieces	Wt. of food g.	Wt. gained by animals g.	Total wt. of food minus wt. gained = food catabolized or lost as roughage g.	Food intake / Wt. gained (Approx. percentage of food retained, in brackets)
Tank 1: Controls injected with muscle extract, 179·3–367·3 g.	Liver	673	218·0 ± 20·3			
	Beef	238	65·0 ± 9·8			
	Worm	577	217·0 ± 30·5			2·66 ± 0·20
	Total		500·0 ± 37·9	188·0	312	(37·6)
Tank 2: Injected with Schockaert's extract, 185·1–695·1 g.	Liver	1388	449·6 ± 41·8			
	Beef	521	144·2 ± 21·4			
	Worm	693	260·5 ± 36·7			1·47 ± 0·12
	Total		752·3 ± 59·6	510·0	241	(67·8)
Tank 3: Injected with phyone, 176·4–689·1 g.	Liver	1306	423·0 ± 39·3			
	Beef	431	117·7 ± 17·7			
	Worm	746	280·5 ± 39·5			1·60 ± 0·11
	Total		821·2 ± 58·5	512·7	309	(62·4)
Tank 4: Injected with prolactin, 177·2–492·6 g.	Liver	1130	366·1 ± 34·0			
	Beef	310	84·6 ± 12·7			
	Worm	655	246·2 ± 34·7			2·21 ± 0·16
	Total		696·9 ± 50·2	315·4	381	(45·3)
Tank 5: Injected with phyone + prolactin, 169·9–681·4 g.	Liver	1383	448·0 ± 41·6			
	Beef	465	124·1 ± 19·1			
	Worm	767	288·4 ± 40·6			1·68 ± 0·12
	Total		860·5 ± 61·3	511·5	349	(59·4)

differences between the ratios $\frac{\text{food intake}}{\text{weight gained}}$ with their standard errors, of control and hormone-injected animals are:

Control and Schockaert's	1·19 ± 0·23
Control and phyone	1·06 ± 0·23
Control and prolactin	0·45 ± 0·26
Control and phyone plus prolactin	0·98 ± 0·23

These results are to be taken as characteristic only of single tanks of ten axolotls under experimental conditions such as described above, and are not to be considered as representative of either a whole population of axolotls or of any individual. They, together with the data given in the table, show that the effect of the hormones which accelerate the growth rate is both to increase the food consumed and, at the same time, to raise the proportion anabolized. The actual amount catabolized is roughly the same except in the case of Schockaert's extract and it is rather surprising that this is so, since there were very large discrepancies between the sizes of the animals in tanks 2, 3 and 5, and those in tank 1 towards the end of the experiment. The conclusion drawn is that growth-promoting hormones of the anterior pituitary effect an economy in the utilization of food.

V. DISCUSSION

As has been pointed out above, the ratio used in describing the results can only be taken as an approximation, since errors tending to raise the value arise owing to the unknown proportion of undigestible matter in the food given, and to lower it owing to there being a higher percentage of water in the tissues of amphibia than in ox muscle or liver. König (1903) gives the following values for water content: frog tissue, 81·6 %; ox muscle, 76 %; liver, 73 %. Nevertheless, the figures are of the same order as those obtained for the percentage of feed protein retained in growing farm animals. Calves 16–19 days old may retain 75–81 %, pigs 120 days old 21–34 % of protein fed (Armsby, 1917). The results with axolotls, retention of between 30 and 69 % of the food eaten, are of the same order.

The values are influenced by the nature and quantity of the food supplied and also by the ages of the animals: e.g. in farm animals, the percentage retention diminishes with age, falling nearly to zero in adults. By keeping the nature of the food constant for all experiments, by feeding to repletion daily and by using animals of the same age, the influence of these factors should be reduced to a minimum in the above experiments. It is of interest that a growing axolotl kept under the conditions described by Clements & Howes (1938) and about 23–35 weeks old, takes of the order of 4 g. of food per week.

With regard to the big decrease in the ratio $\frac{\text{food intake}}{\text{weight gained}}$ due to injection of growth-promoting hormones, no direct evidence bearing on its cause has yet been obtained in axolotls, but there is considerable evidence from work on Mammalia that it is due to increased nitrogen anabolism. There may be some water storage.

Bierring & Nielsen (1932) and Lee & Schaffer (1934), all working on rats, found some increase in water content of their animals, but, on the other hand, Teel & Cushing (1930) with dogs and Wadehn (1932) with mice, found no water storage. It is probable that little of the decrease in the ratio is due to increased hydration of the axolotl's tissues. Teel & Cushing (1930) found that injection of growth-promoting hormones caused a lowering of the basal metabolism of rats but, with the exception of the animals injected with Schockaert's extract, there is little evidence of any diminution in the quantity of food catabolized by the axolotls. There appears to be general agreement that injection of growth-promoting hormones decreases the excretion of nitrogen and causes an increase in the total nitrogen in the body (Teel & Watkins, 1929; Bierring & Nielsen, 1932; Wadehn, 1932; Gaebler, 1933; Lee & Schaffer, 1934; Gaebler & Price, 1937) and since the greater part of the growth of an axolotl must be due to increase in cell tissue which is mainly protein in nature, it is most probable that the large decrease in the ratio observed in this investigation may be attributable to this cause. If so, growth-promoting hormones may cause accelerated growth, not necessarily by direct stimulation of cell division, but secondarily, by increasing the amount of nitrogenous material available for cell growth and multiplication.

VI. SUMMARY

1. A method is described by which the food intake of axolotls during a given period can be compared with their growth during the same period.

2. The method has been applied to animals injected with growth-promoting hormone.

3. The ratio $\frac{\text{food intake}}{\text{weight gained}}$ has been calculated for batches of ten axolotls injected with muscle extract, Schockaert's extract of anterior pituitary, phyone, prolactin (Bates & Riddle, 1935), prolactin plus phyone.

4. This ratio was 2.66 ± 0.20 for animals injected with muscle extract, 1.47 ± 0.12 for Schockaert's extract, 1.60 ± 0.11 for phyone, 2.21 ± 0.16 for prolactin and 1.68 ± 0.12 for prolactin and phyone together.

5. The effect is attributed to increased nitrogen anabolism.

REFERENCES

ARMSBY, H. P. (1917). *The Nutrition of Farm Animals.* New York.
BATES, R. W. & RIDDLE, O. (1935). *J. Pharmacol.* **55**, 365–71.
BIERRING, E. & NIELSEN, E. (1932). *Biochem. J.* **26**, 1015–21.
CLEMENTS, D. I. & HOWES, N. H. (1938). *J. exp. Biol.* **15**, 541.
GAEBLER, O. H. (1933). *J. exp. Med.* **57**, 349–63.
GAEBLER, O. H. & PRICE, W. H. (1937). *J. biol. Chem.* **121**, 497–506.
KÖNIG, J. (1903). *Chemie der menschlichen Nahrungs- und Genussmittel*, **1**. Berlin.
LEE, M. O. & SCHAFFER, N. K. (1934). *J. Nutrit.* **7**, 337–63.
SCHOCKAERT, J. E. (1931). *Anat. Rec.* **50**, 383–97.
TEEL, H. M. & CUSHING, H. (1930). *Endocrinology*, **14**, 157–63.
TEEL, H. M. & WATKINS, O. (1929). *Amer. J. Physiol.* **89**, 662–85.
VAN DYKE, H. B. & WALLEN-LAWRENCE, Z. (1930). *J. Pharmacol.* **40**, 413–22.
WADEHN, F. (1932). *Biochem. Z.* **255**, 189–99.