THE EFFECT OF TEMPERATURE CHANGES ON THE THYROID–PITUITARY RELATIONSHIP IN TELEOSTS

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(With Plate 14)

INTRODUCTION

An increase in the thyroid activity of teleosts correlated with an increase in environmental temperature has been recorded by Blanc & Buser (1949), Fortune (1953, 1955), and Barrington & Matty (1954); this has not been confirmed by Delsol & Flatin (1956) who found no effect, or by Olivereau (1950, 1954, 1955*a*, *b*, *c*) who demonstrated that increased temperature tended rather to decrease thyroid activity.

The action of the thyrotrophic hormone (TSH) is intensified with increasing temperature (Olivereau, 1955*c*; Delsol & Flatin, 1956). This is confirmed below. Such an action would be expected to lead to a consequent increase in thyroid hormone (TH) production with increased temperature, unless a compensatory decrease in TSH production takes place. Apart from seasonal changes (e.g. Fontaine, 1953; Olivereau, 1954) no information is available on the effect of temperature on the cells of the teleostean anterior pituitary which secrete thyrotrophic hormone (thyrotrophs).

The experiments described here were designed to investigate this aspect and to extend previous observations on the effect of temperature on thyroid activity.

MATERIAL AND METHODS

Phoxinus laevis was used as the main experimental animal; for certain comparative aspects *Carassius auratus* was included.

The fish were kept in groups of six in large glass jars of aerated water. They were fed daily on dried daphnia and the water was changed twice weekly. For chemical thyroidectomy they were immersed in 0·05% thiourea, which was also changed twice weekly. TSH was injected in the form of 'Ambinon' B (Organon Laboratories) which also contains gonadotrophins. 0·05 ml. of Ambinon diluted with Ringer's solution and containing approximately 1–3 Heyl-Laqueur units of thyrotrophin were injected into the body cavity immediately under the pectoral fin. In each experimental series the identical dilution was used. Controls were injected with similar quantities of Ringer solution. The fish were subjected to three environmental temperatures: (*a*) less than 10° C., (*b*) 20° C. (± 1° C.), (*c*) 25° C. (± 1° C.).

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The experiments were carried out during the period December–early February, and in each specimen both the thyroid and the pituitary were examined.

Thyroid activity was estimated on a histological basis according to the ratio of the area covered by the follicular epithelium to the total follicular area (*e/f* ratio) (Fortune, 1955).

Thyrotrophic activity was also estimated on a histological basis. The specimens were fixed for prolonged periods in Bouin's fluid, which also decalcified them, and the brain and attached pituitary were embedded and sectioned at 5 μ in the usual way. Attention was centred on the basophils of the middle glandular region. As this region is small in sagittal section but extends laterally upwards and outwards (Kerr, 1942) only central sections which included the stalk, or those immediately lateral to this region were considered, in order to give comparable pictures of the middle glandular region. The sections were stained with periodic acid Schiff (PAS) method (Pearse, 1953), Gomori's aldehyde–fuchsin (AF) (Dawson, 1953) or Mallory's trichrome. It is possible to distinguish two types of basophil on the basis of their inclusions and staining intensity. One type stains heavily with flocculated inclusions, and cells of this type are regarded as the gonadotrophs; the other type stains less intensely, has finely granular inclusions and a prominent rounded nucleus, and cells of this type are regarded as the thyrotrophs (Kerr, 1942; Atz, 1953; Barrington & Matty, 1955).

Barrington & Matty (1955) found that PAS stained all the basophils whilst AF stained only some of the PAS-positive cells; the latter were experimentally identified as the thyrotrophs. Following the fixation used here this distinction was not valid, and all the basophils stained with both stains (cf. Atz, 1953).

Under certain experimental conditions the staining intensity of the thyrotrophs decreased, accompanied by degranulation and increased vacuolation. This general emptying of the cell was interpreted as indicating increased TSH production. Three grades were recognized: an inactive gland in which the majority of the thyrotrophs showed no marked change in staining intensity or loss of cellular inclusions, an active gland in which the majority of the cells showed pronounced vacuolation and loss of cell contents and an intermediate stage in which at least 50% of the thyrotrophs showed some change indicating emptying of the cells, although this had rarely proceeded as far as the active stage which showed pronounced vacuolation. These are arbitrary grades which merely served to give some measure of comparison between different glands.

EXPERIMENTAL

Thyroid. Effect on thyroid activity of:

(*a*) *'Ambinon' injections. Phoxinus* was acclimatized over a period of 3 days to either 10° C. or 25° C. Twelve fish were used in each experimental group, with twelve controls injected with Ringer and twelve uninjected controls. 'Ambinon' was injected twice weekly (on opposite sides of the body) and two specimens were killed 24 hr. after each injection. Controls were injected and killed at the same

Table 1. *Effect of 'Ambinon' injections at different temperatures*

e/f ratio after injection	Controls (not injected)		Controls (injected Ringer)		Ambinon injected	
	Temp. 10° C.	Temp. 25° C.	Temp. 10° C.	Temp. 25° C.	Temp. 10° C.	Temp. 25° C.
1	36·5	43·4	41·6	32·6	38·8	50·0
2	33·8	44·0	37·6	35·2	37·0	75·0
3	30·7	36·3	42·8	42·5	44·5	97·9
4	32·5	38·2	32·7	31·2	40·3	89·0
Mean *e/f*	33·4	39·7	39·2	35·4	40·1	75·5
σ	2·47	3·92	4·51	5·03	3·20	23·7
S.E.	1·24	1·96	2·26	2·52	1·60	11·9

σ is the standard deviation, estimated with one degree of freedom, and S.E. is the standard error of the mean.

intervals. Results of a typical experiment are shown in Table 1. At 25° C. there was an immediate change in the *e/f* ratio after the first injection and this was intensified with further injections. At 10° C. a change in the *e/f* ratio was found after four injections. These results show that the same dose of 'Ambinon' has more effect on the thyroid activity at 25° C. than at 10° C. It was further found that the controls injected with Ringer's fluid tended to show an increase in *e/f* ratio over the uninjected controls. It is also confirmed that the *e/f* ratio of the controls tends to rise with increasing environmental temperature.

(*b*) *Transference from a high or a low temperature to an intermediate temperature.* In each experiment twelve specimens of *Phoxinus* were placed at room temperature (approximately 17° C.) and over a period of 3 days the temperature was gradually raised to 25° C. A further twelve fish were kept under comparable conditions at 10° C. The fish were maintained at these temperatures for a period of 14 days. Half of each group was then immersed in thiourea and all animals were transferred to 20° C. Specimens were killed and fixed at intervals of 4, 7, 12, and 14 days after the transfer to 20° C. These experiments were repeated through the period December–February, and results of a typical experiment are shown in Table 2.

Table 2. *Effect of previous acclimatization temperature on thyroid activity*

Days	Thiourea-treated		Controls	
	Temp. 25° C.	Temp. 10° C.	Temp. 25° C.	Temp. 10° C.
4	35·2	36·1	38·7	28·9
7	44·0	40·5	32·3	35·0
12	43·4	45·5	36·6	30·9
14	50·0	44·7	30·2	38·7
Mean	43·0	41·7	34·4	33·4
σ	3·16		2·93	
P	0·7		0·8—0·7	

The temperature refers to the pretreatment temperature. σ is the standard deviation of the combined means. *P* is the probability determined using the *t* test.

These results show that there was no significant difference ($P = 0{\cdot}7$), in the thyroid reaction of fish to the common temperature and that the thyroid activity was effectively the same in both groups. In each group a noticeable, though not pronounced, increase in epithelial height and decrease in the amount of colloid following thiourea treatment had taken place by the end of the period of the experiment.

(*c*) *Prolonged maintenance under experimental conditions.* Two groups of *Phoxinus* were set up under experimental conditions similar to those in (*a*). One group was immersed in thiourea. They were kept in the aquarium where the temperature approximated to the outside temperature, for 12 weeks during May–August. The controls for this experiment were the stock fish which were kept in large stone sinks in the aquarium in aerated water and were fed twice weekly on dried daphnia or minced meat. Under this treatment the animals survived in apparently good condition for several months. Fish were killed for examination at weekly intervals after 6 weeks had elapsed. The following values for the *e*/*f* ratio were found: control, mean value 45·5 (extremes 31·6–52·2); experimental, mean value 42·8 (26·3–50·1). Those immersed in thiourea showed maximum histological changes after 6 weeks (*e*/*f* ratio 90+), and the majority of follicles were completely occluded with greatly heightened epithelium. This was maintained until the 10th week when small amounts of intra-follicular colloid again became visible and the *e*/*f* ratio fell to below 90.

Pituitary. Phoxinus. Effect on thyrotroph activity of:

(*a*) *'Ambinon' injections.* These injections caused a general reduction in the staining intensity of all basophils. There was no appreciable difference in the speed of this reaction at the temperatures employed, but it appeared to be more intense at higher temperatures.

(*b*) *Variations in environmental temperature.* At temperatures below 10° C. the thyrotrophs remained full and the cell contents stained readily. Only in a few occasional cells were signs of vacuolation and reduction in cell contents noted (Pl. 14A). At 25° C. the thyrotrophs showed some signs of change after a week's exposure to this temperature, and after a fortnight there was a noticeable reduction in the staining intensity and a reduction in the cell inclusions (Pl. 14B). With thiourea at low temperatures some emptying and vacuolation were seen after 14 days' treatment (Pl. 14C). At 25° C, this vacuolation appeared sooner and was more intense, so that after 14 days' treatment the majority of the thyrotrophs were showing all the changes which were interpreted as signs of increased secretion (Pl. 14D).

Table 3. *Effect of temperature in the thyrotrophs*

Treatment	Duration in days	Condition of thyrotrophs
10° C.	14	++
25° C.	14	+−
10° C. + thiourea	14	+−
25° C. + thiourea	14	−−

++, inactive grade; +−, intermediate grade; −−, active gland.

(*c*) *Transference from a high or a low temperature to an intermediate temperature.* In both cases the thyrotrophs showed some degree of activation, which was intensified by thiourea treatment. No difference could be discerned between the two groups.

(*d*) *Prolonged maintenance under experimental conditions.* The control and experimental fish showed no apparent difference. Those treated with thiourea showed progressively increased vacuolation and reduction of cell content until at the end of the experiment, i.e. after 12 weeks, the thyrotrophs could be discerned only as an empty sphere delimited by a faintly staining ring at the periphery.

Carassius. At low temperatures the thyrotrophs stained intensely and appeared full of inclusions. Neither increased temperature or immersion in thiourea caused any appreciable change in 28 days (Pl. 14E).

Thyroid–pituitary relationship

Phoxinus. In specimens which showed intermediate thyrotroph activity there was no change in thyroid activity. In specimens showing active thyrotrophs, due either to exposure to a higher temperature or to immersion in thiourea, there was a slight increase in thyroid activity, the *e/f* rising by 10. In the case of prolonged immersion in thiourea the thyrotrophs reached the completely evacuated stage, whilst the thyroid activity underwent a rise and fall.

Carassius. The environmental changes to which the animals were subjected caused no change in either the thyrotrophic activity or the thyroid (Fortune, 1956) and both presented the normal resting picture.

DISCUSSION

Experimental evidence suggests that the presence of cellular contents in the thyrotrophs is indicative of stored TSH; consequently the emptying or breakdown of the contents can be interpreted as an increased secretion of TSH (Atz, 1953; Barrington & Matty, 1955). This interpretation is largely based on the fact that treatment with thiourea or thiouracil promotes these changes and the vacuolization of these cells becomes prominent, showing a similar reaction to mammalian thyrotrophs.

These changes are shown in *Phoxinus* with increased temperature and with thiourea treatment. The thiourea treatment is more rapidly effective at high temperatures. It can be stated that increased temperature causes increased secretion of TSH.

Following from this it would be expected that thyroid activity would also increase with increased temperature. Contradictory results have been reported. One possible explanation of these is that genuine specific differences exist. This seems to be the case in *Carassius auratus*, which has a thyroid resistant to external change, and explains some of the results where this species has been used as the only teleost representative. It may then be asked why such specific differences exist in related animals under similar environmental conditions. In the case of *C. auratus* the long period of artifical selection is probably adequate to explain many

abnormalities. Until reasons for a different endocrine response are forthcoming for other teleosts further explanation must be sought. One point that is not always sufficiently emphasized is that the range of temperature over which a response is sought must be related to the normal temperature range of the species, and no marked activation of the thyroid with increasing temperature can be expected until the temperature is approaching the upper lethal. Baggerman (1957) has put forward a similar suggestion in that there may be a critical level for each species below which no response to temperature takes place.

A further possibility is that the temperature to which the animal has been subjected prior to the experiment affects its response. This possibility is suggested by experiments on the oxygen consumption where fish kept at 20° C., with an induced high consumption, and fish kept below 12° C., with an induced low consumption, showed a reversal of this when brought to an intermediate temperature (Wells, 1935). Additional experiments, based on respiratory rate and susceptibility to lethal agents, showed that 'differences in physiological activity which had originally been induced by acclimatization to different temperatures were completely reversed when the fishes were brought to a common temperature' (Sumner & Dourodoff, 1938). Pretreatment for a fortnight, at extremes unlikely to be encountered under natural conditions, did not affect the thyroid activity as measured by histological changes. Variations in activity due to this cause may be all over in the first few days, which form a latent period for histological change; further, the method may not be sufficiently sensitive to detect these variations. It is, however, apparent that there is no long-term effect on thyroid activity due to pretreatment temperatures.

When the majority of thyrotrophs are activated there is a slight increase in the *e/f* ratio. It is known from previous experiments that this is the commencement of a gradual rise to a considerably higher *e/f* value. It seems that the thyrotrophs are more sensitive than the thyroid to external conditions and that changes in thyrotroph activity precede changes in thyroid activity. This may partly explain the fact that 'Ambinon' injections at a low temperature affected the thyrotrophs before any change became apparent in the thyroid. An additional factor here is that the TSH is accompanied by gonadotrophins which will also affect the endocrine balance.

One of the most interesting points to emerge was the change in the thyroid on prolonged treatment with thiourea. The epithelial height rose to a maximum and then declined with the reappearance of some colloid. This might be interpreted as adaptation of the gland to thiourea, or exhaustion of TSH causing decline in epithelial height. Neither possibility can be ruled out by the histology of the thyrotrophs. It does, however, indicate that the TH:TSH ratio can be set at different levels, for example high or low production of both hormones. It is known that under normal conditions the negative feed-back maintains the ratio constant. Under disturbed metabolic conditions, due either to temperature extremes or metamorphic changes, the reciprocal control between TSH and TH secretion rates becomes disturbed and, if successful adaptation ensues, becomes set at a new level which is expressed histologically. A changing histological picture would then

indicate active attempted adaptation, whereas a static picture would indicate successful adaptation to the prevailing conditions although actual hormone production might vary according to the setting of the ratio.

SUMMARY

1. It is confirmed that TSH has a more marked effect on the teleost thyroid at high temperatures.

2. The secretion of TSH is increased at high temperatures.

3. The temperature to which the animal is subjected prior to the experiment has no long-term effect on thyroid activity.

4. The thyroid–pituitary relationship is affected by temperature changes, and it is suggested that the TH:TSH ratio may be set at different levels by altering the environmental temperature.

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REFERENCES

ATZ, E. H. (1953). Experimental differentiation of basophil cell types in the transitional lobe of the pituitary of a teleost fish *Astyanax mexicanus*. *Bull. Bingham Oceangr. Coll.* **14** (2), 94–116.

BAGGERMAN, B. (1957). An experimental study of the timing of breeding and migration in the three-spined stickleback (*Gasterosteus aculeatus* L.). *Arch. néerl. Zool.* **7**, 105–318.

BARRINGTON, E. J. W. & MATTY, A. J. (1954). Seasonal variation in the thyroid gland of the minnow *Phoxinus phoxinus* L., with some observations on the effect of temperature. *Proc. Zool. Soc. Lond.* **129**, 89–95.

BARRINGTON, E. J. W. & MATTY, A. J. (1955). The identification of thyrotrophin-secreting cells in the pituitary gland of the minnow (*Phoxinus phoxinus*). *Quart. J. Micr. Sci.* **96**, 193–201.

BLANC, M. & BUSER, J. (1949). Action de la température sur l'osteogénèse répatrice chez les poissons téleostéens. *Bull. Soc. zool. Fr.* **74**, 167–70.

DAWSON, A. B. (1953). Evidence for the termination of neurosecretory fibers within the pars intermedia of the hypophysis of the frog, *Rana pipiens*. *Anat. Rec.* **115**, 63–67.

DELSOL, M. & FLATIN, J. (1956). Action de la température sur l'activité de l'hormone thyreotrope chez deux vertébrés inférieurs; *Rana esculenta* L. and *Carassius auratus* L. *C.R. Soc. Biol., Paris*, **150**, 938–40.

FONTAINE, M. (1953). La fonction hypophyse—thyroïdienne des poissons dans ses rapports avec leur morphologie et leur comportement. *J. Cons. int. Explor. Mer*, **19**, 23–38.

FORTUNE, P. Y. (1953). Thyroid activity in teleosts. *Nature, Lond.*, **171**, 483–484.

FORTUNE, P. Y. (1955). Comparative studies of the thyroid function in teleosts of tropical and temperate habitats. *J. Exp. Biol.* **32**, 504–13.

FORTUNE, P. Y. (1956). An inactive thyroid gland in *Carassius auratus*. *Nature, Lond.*, **178**, 98.

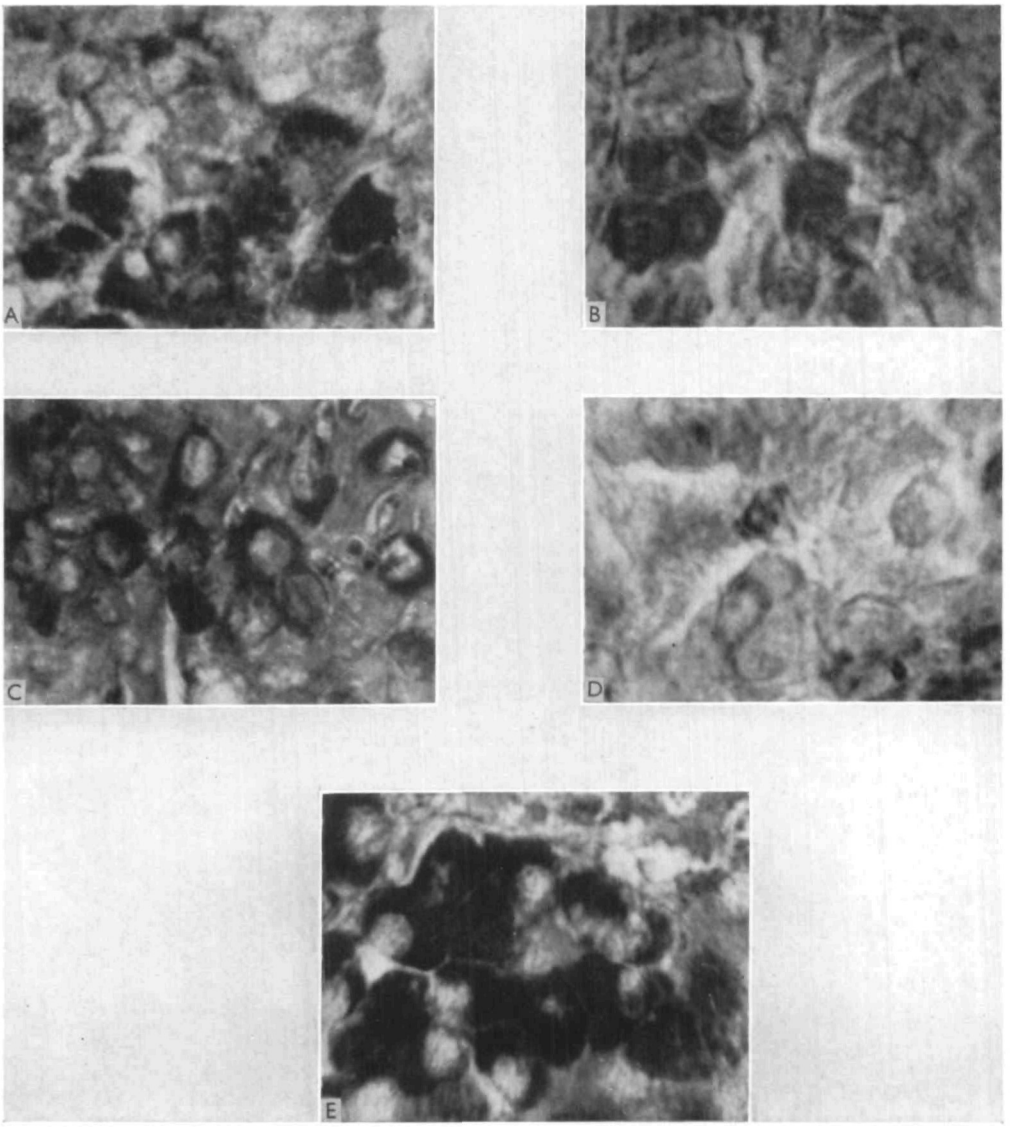
KERR, T. (1942). A comparative study of some teleost pituitaries. *Proc. Zool. Soc. Lond.* A, **112**, 37–56.

OLIVEREAU, M. (1950). Influence d'une augmentation de salinité sur l'activité thyroïdienne de divers téleostéens d'eau douce. *C.R. Soc. Biol., Paris*, **144**, 775–6.

OLIVEREAU, M. (1954). Hypophyse et glande thyroïde chez les poissons. Etude histologique de quelques corrélations endocriniennes, en particulier chez *Salmo salar*. *Ann. Inst. océanogr. Monaco*, **29**, 95–296.

OLIVEREAU, M. (1955*a*). Influence de la témperature sur le fonctionnement thyroïdien de la truite étudié a l'aide de l'iode radioactif. *C.R. Soc. Biol., Paris*, **149**, 536–9.

OLIVEREAU, M. (1955*b*). Influence de la témperature sur l'histologie thyroïdienne de divers téléostéens. *Bull. Soc. zool. Fr.* **80**, 43–52.



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OLIVEREAU, M. (1955*c*). Hormone thyréotrope et température chez la truite arc-en-ciel (*Salmo gairdnerii* L.) et l'anguille (*Anguilla anguilla* L.). *Arch. Anat. micr. Morph. exp.* **44**, 236–64.

PEARSE, A. G. E. (1953). *Histochemistry, Theoretical and Applied.* London.

SUMNER, F. B. & DOURDOROFF, P. (1938). Some experiments upon temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull., Woods Hole*, **74**, 403–29.

WELLS, N. A. (1935). Variations in the respiratory metabolism of the pacific killifish, *Fundulus parvipinnis*, due to size, season and continued constant temperature. *Physiol. Zoöl.* **8**, 318–36.

EXPLANATION OF PLATE

Phoxinus

A. Inactive gland showing thyrotrophs with granular inclusions. Note a group of three at the bottom. Some gonadotrophs are present at the right. AF.

B. Moderately active gland with thyrotrophs showing degranulation. Note reduction in cellular contents in the cells on the right. Taken after exposure to 25° C. for 14 days. AF.

C. Moderately active gland. Same category as (*b*) but thyrotrophs less vacuolated, although showing some changes. Thiourea at 10° C. for 14 days. AF.

D. Active gland showing empty and vacuolated thyrotrophs. Note a group of three at the bottom centre showing extreme emptying and appearing as faintly staining ring. Exposed to thiourea at 25° C. for 14 days. AF.

Carassius

E. Inactive gland with heavily staining thyrotrophs. Exposed to thiourea at 25° C. for 28 days. AF.