FUNCTIONAL CAPACITY OF ECTOPIC PITUITARY TRANSPLANTS IN THE TELEOST *POECILIA FORMOSA*, WITH A COMPARATIVE DISCUSSION ON THE TRANSPLANTED PITUITARY

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[Plates 18 to 21]

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The capacity of the pars distalis to secrete its hormones when removed from connexions with the hypothalamus has been assessed in the teleost *Poecilia formosa*. Mortality, integumentary characters, body growth, fin regeneration, freshwater adaptation, liver reserves, fat stores, haematology, and the histology of the thyroid gland, interrenal tissue and ovary, have been studied in intact fish, in hypophysectomized fish, and in hypophysectomized fish bearing a pituitary homotransplant in the musculature of the caudal peduncle. Growth, fin regeneration, liver reserves, fat stores, interrenal and ovary, have been studied also in sham-hypophysectomized fish.

It is concluded that the ectopic pituitary transplant in this fish secretes thyrotrophin (*TSH*) at a higher rate than normal, adrenocorticotrophin (*ACTH*) at a subnormal rate, and growth hormone (*GH*) only in very small amounts. The transplant secretes the prolactin-like hormone essential for freshwater adaptation, but does not secrete gonadotrophin.

The extensive literature on the ectopic pituitary has been surveyed, and the present results were compared with those obtained for other vertebrate groups. The hypothalamic influence on *TSH* secretion in *Poecilia* appears to be inhibitory, in contrast to the stimulatory relationship in mammals, and the pituitary of this teleost seems to have greater autonomy in the secretion of *ACTH* than the mammalian gland. The hypothalamic control of *GH*, prolactin and gonadotrophin, however, is probably essentially the same in *Poecilia* as in mammals.

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Introduction

Much of the work behind the current concept of the control of the adenohypophysis by hypothalamic neurosecretion has involved analysis of the function of the mammalian pituitary gland when transplanted to sites remote from the hypothalamus. This technique has produced in the hands of many investigators results that are in the main consistent, indicative of considerable but selective loss of secretory function in such *ectopic* mammalian pituitary tissue, though there are some more-or-less minor disagreements that will be considered in the Discussion of this paper. The comparative aspects of this field are of obvious interest, particularly as recent studies on amphibians and birds have suggested that there may exist in the lower vertebrates hypothalamo–hypophysial relations fundamentally different from those which obtain in mammals. The function of ectopic pituitary tissue has not previously been investigated in fishes, so we were particularly interested in the opportunity for studying this topic that arose with the development (Ball 1962) of a technique for hypophysectomy of a teleost fish in which tissue and organ homografts can readily be established (Kallman 1962*a*), the small viviparous *Poecilia* (*Mollienesia*) *formosa* (Girard) (Cyprinodontiformes, Poeciliidae).

Material and methods

Poecilia formosa is an all-female species which reproduces gynogenetically after mating with males of related species, so that the descendants of a given female constitute a clone, all having identical genotypes. Tissue and organ transplants made between members of the same clone are accepted and survive indefinitely (Kallman 1962*b*). The fish used in this investigation belonged to a number of clones originally isolated from a drainage ditch near Brownsville, Texas, and which were maintained in the laboratory for several generations by matings with *P. sphenops* and *P. vittata*.

In the main part of the investigation (Series 1) three groups of fish were established: intact fish; hypophysectomized fish; and hypophysectomized fish bearing an established pituitary transplant in the caudal musculature (*grafted fish*). Activity of the pituitary graft was assessed by comparing the histology of the endocrine organs in the three groups, and by observations of various parameters known or presumed to be dependent ultimately on pituitary hormones. All the fish were judged on size and appearance to be sexually mature at the beginning of the experiment, and they were 3·0 to 4·5 cm in length.

Pituitary grafts were established 3 to 4 weeks prior to hypophysectomy of the host. Each grafted fish received a single whole pituitary gland from a donor fish of the same clone, the gland being extirpated at the stalk and so including the entire adenohypophysis plus neurohypophysis. The pituitary graft was inserted into a horizontal pocket made with a fine glass needle in the musculature of the caudal peduncle. The slight pressure exerted by the sides of the pocket held the transplant in place, and the mouth of the pocket healed within a few days. The fish were kept in 0·6% saline until the wound healed, and were then returned to their home-tanks of freshwater.

Preliminary experiments had shown that hypophysectomy abolished the ability of *P. formosa* to live in freshwater, but that hypophysectomized fish can live indefinitely in dilute seawater. Accordingly, 1 week before hypophysectomy all the fish were transferred

to dilute seawater (12 parts per thousand), and they were kept in this medium until the end of the experiment. Using the opercular approach, and with tricaine methane sulphonate (MS 222 Sandoz; 1:2000) as an anaesthetic, the pituitary was removed from 26 non-grafted fish and from 16 fish with pituitary grafts. Completeness of hypophysectomy was ultimately confirmed in all fish by examination of 10 μm serial sections of the head. At the same time, 14 non-grafted fish were set aside as intact controls. The standard length of each fish was measured, and 3 to 5 mm was removed from the distal margin of the caudal fin, to allow study of regeneration. The fish were then kept for 4 weeks in the dilute seawater, at 25 ± 1 °C, with 9 h illumination per day. They were housed in groups of three or four, in all-glass tanks, allowing 2 l. of water per fish, and were individually identified by pectoral fin-clippings. About three-quarters of the water in each tank was discarded each week and replaced by clean water. The fish were fed *ad libitum* twice daily with Aronson's mixture enriched with a minute trace of iodine, sufficient to prevent the development of goitre (Pickford 1954*a*), and thrice weekly they were fed a supplement of fresh-frozen *Artemia*. Faeces and uneaten food were removed from the tanks each day. Once each week the fish were measured, the state of their eyes and skin was noted, and the length of the regenerated portion of the caudal fin was determined.

After 4 weeks, all the fish were sacrificed. Each fish was lightly anaesthetized and weighed. The pericardial cavity was opened and blotted dry, and blood was taken by cardiac puncture from the auricle. The blood was withdrawn into a fine heparinized glass pipette by oral suction, transferred to a measuring pipette,* and diluted with the fluid of Rees & Ecker (Slicher 1961). Within a few hours, the numbers of erythrocytes, leucocytes and thrombocytes were determined by standard haematological methods using a Levy counting chamber with improved Neubauer double ruling. The liver was removed, weighed and fixed in 10% formalin in 95% ethyl alcohol. The ovary was weighed and fixed in sublimated Bouin–Hollande (Herlant 1956). The mesenteric fat associated with the gut was scraped away and weighed, and any internal abnormalities were noted. The thyroid region was fixed in alcoholic Bouin, while the head, anterior trunk (containing the interrenal tissue), and the region of the pituitary graft, were all fixed in sublimated Bouin–Hollande. All the tissues except the ovaries and the thyroid regions were subsequently decalcified electrolytically before paraffin embedding and sectioning. The tail region of each fish was fixed in formol–alcohol for determination of its melanin content by a turbidometric method (Pickford & Kosto 1957).

Thyroid regions were sectioned at 5 μm and stained by a Masson technique, periodic acid-Schiff (*PAS*), Cleveland–Wolfe (trichrome), and haemalum–erythrosin. Ovaries were sectioned at 5 μm and stained with Masson, Cleveland–Wolfe, and *PAS*. Interrenal tissue was studied in 4 and 5 μm sections stained with *PAS*, haemalum–erythrosin and Cleveland–Wolfe.

The pituitary grafts were recovered in 4 or 5 μm serial sections of the caudal peduncle, and stained with various special techniques. The cytology of the pituitary grafts in relation to the cytophysiology of the normal pituitary (Olivereau & Ball 1963*a*, *b*; 1964) will be described fully in another paper.

* 'Unopettes', supplied through the courtesy of Dr H. W. Gerarde and Becton, Dickinson and Co., Rutherford, New Jersey.

Two subsidiary experiments were performed, using fish not included in Series 1 and from different clones. Results of the main experiment showed that the grafted fish developed some of the deficiency symptoms characteristic of hypophysectomized fish. To be certain that these deficiencies did indeed reflect partial disfunctioning of the pituitary graft, and were not the results of the surgical trauma of hypophysectomy, it was decided to assess the effects of sham-hypophysectomy. In Series 2, nine mature fish (length 3·6 to 4·5 cm) were sham hypophysectomized, the pituitary being exposed but left *in situ*. Nine unoperated fish (length 3·7 to 4·3 cm) served as controls. These two groups were kept for 4 weeks under conditions as nearly as possible the same as in the main experiment, though it was impossible to feed the supplement of fresh-frozen *Artemia*. These fish were used to study growth, fin regeneration, liver state, fat stores, interrenal tissue and ovarian state.

In Series 3, mature hypophysectomized fish and mature grafted fish (lengths 3·4 to 4·0 cm), living in dilute seawater, were tested for ability to withstand transfer to freshwater.

Results

(*a*) *Mortality*

During the 4 weeks of the main experiment (Series 1), 12 of the 26 hypophysectomized fish died. Six of these fish, dying more than 1 week after the operation, had large numbers of renal calculi in the kidneys and renal ducts, which probably caused death (Pickford 1953*a*). The deaths of 4 other fish, during the first week after hypophysectomy, were probably due to a bacterial infection encountered in other experiments with *Poecilia*, the main symptoms of which are superficial haemorrhages (commonly in the vent region), and intense inflammation of the intestine. Blood smears from infected fish show large numbers of cocci. The other 2 fish died from unknown causes and displayed neither kidney stones nor symptoms of the bacterial infection.

No deaths occurred among the intact controls. Of the 16 grafted fish, 3 died within 24 h of hypophysectomy, probably as a result of surgical damage or haemorrhage, and the remaining 13 lived to the end of the experiment.

In Series 2, all the 9 sham-hypophysectomized fish survived to the end of the experiment, while one of the intact fish died, of the bacterial infection mentioned above. This fish contained no visible kidney stones at autopsy.

These mortality records suggested the possibility that the pituitary graft was functional and was able in some way to prevent or retard the development of kidney stones after hypophysectomy, and to maintain resistance against bacterial infection.

At autopsy, all the fish were checked for the presence of macroscopically visible stones in the kidneys and ducts. The records bear out the possibility of the graft acting to retard the development of renal lithiasis: kidney stones were seen in 2 out of the 14 intact fish, in one of the 13 grafted fish, but in 11 out of 14 hypophysectomized fish. Subsequently, sections of the head kidneys of the fish were available, and were examined for small stones, such as could not have been seen at autopsy. All the fish recorded as containing stones at autopsy also displayed small stones in the sections of head kidney, but in addition small stones were found in fish that did not display frank renal lithiasis. Small stones, resembling

those illustrated by Pickford (1953*a*) were seen in sections of 13 out of 14 intact fish, in 9 out of 13 grafted fish, and in 11 out of 14 hypophysectomized fish. In most cases the stones were small and widely scattered in the kidney tissue, so that not every section contained a stone, and where present there were usually only one or two stones in a section. It appears that small stones were formed in both intact and grafted fish without accreting and accumulating to form macroscopically visible bodies, but that the formation of large stones in the kidneys and ducts was accelerated by hypophysectomy and retarded by the pituitary graft.

No stones were recorded in the fish of Series 2 at autopsy, but small stones were found in the sections of the head kidneys of these fish, as in the intact fish of Series 1.

(*b*) *Integumentary characters*

By the fourteenth post-operative day the skin of all the 14 surviving hypophysectomized fish had lost its normal silvery appearance and become dull and grey, presumably as a result of a reduction in its guanine content. In strong contrast, the 13 grafted fish retained to the end of the experiment the same silvery skin as the intact controls. At the same time, the skin of the hypophysectomized fish took on a coarsened appearance, while that of the grafted fish retained its normal texture. Parallel to these changes in the skin, one or both corneas of the hypophysectomized fish became partly or wholly white and opaque by day 14, a condition we have termed 'cloudy eyes'. No grafted fish developed this peculiar feature, nor has it ever been seen in intact *Poecilia*, nor in the sham-operated fish of Series 2. As far as we could judge, this corneal opacity did not seriously impair vision. It seems probable from the work of Smelser (1962) that cloudy eyes develop as a result of hydration changes within the cornea itself, in turn dependent upon some failure of the normal permeability properties of the corneal epithelium and endothelium. Pickford (personal communication) has noticed cloudy eyes in a percentage of hypophysectomized *Fundulus*, and both corneal opacity and roughness of the skin have been seen in hypophysectomized *Xiphophorus* (Schreibman & Kallman 1963).

Later work (Ball 1963) indicates that both cloudy eyes and the skin dullness may be indices of thyroid deficiency following hypophysectomy. A group of 11 *Poecilia formosa*, hypophysectomized and then kept for 14 days in a thyroxine solution, retained normal silvery skins, and 9 of the fish retained transparent corneas. The absence of these post-hypophysectomy changes in the grafted fish, therefore, points to the secretion of thyrotrophin (*TSH*) by the pituitary transplants, an indication which is borne out by thyroid histology (section *f*).*

There were no obvious differences in melanin pigmentation between the three groups; in contrast to the case of *Fundulus* (Pickford & Kosto 1957), possibly because of the shorter duration of our observations, our hypophysectomized fish appeared no paler than the intact controls and the grafted fish. In view of the established part played by the pituitary in regulating the number of melanophores and melanin content in *Fundulus*, we thought

* [*Footnote added in proof* 19 *January* 1965.] Recent work (Ball & Slicher, unpublished) on hypophysectomized *Poecilia latipinna* indicates that the prolactin-like hormone (section *c*) may maintain skin texture. In the same experiments, thyroxine treatment failed to prevent the development of cloudy eyes; thus, interpretation of the effect of the grafted pituitary on this character is uncertain.

it necessary to confirm these visual impressions by determining the melanin content of the tails of the fishes by a turbidometric method, the melanin content being expressed as arbitrary Klett units (for method, see Pickford & Kosto 1957). Melanin contents were, for intact fish, $251 \pm 11{\cdot}4$ Klett units; for hypophysectomized fish, $243 \pm 7{\cdot}8$; and for grafted fish, $243 \pm 16{\cdot}1$. The figure for intact fish is not significantly higher than that for hypophysectomized fish ($t = 0{\cdot}5$, $p = 0{\cdot}5$ to $0{\cdot}6$). In contrast to this finding is the situation in another poeciliid, *Xiphophorus*, in which hypophysectomy results in a loss of melanin after 6 weeks or less (Schreibman & Kallman 1963).

(*c*) *Adaptation to freshwater*

Preliminary observations showed that *Poecilia formosa*, like *P. latipinna* (Ball 1962) cannot live in freshwater without the pituitary gland, hypophysectomized individuals of both species developing symptoms of acute distress within a day or two of entering freshwater from dilute seawater. In *P. latipinna* this distress was found to be accompanied by extreme depression of plasma sodium levels (Ball 1963). At this stage the fish will recover if they are returned to dilute seawater, but they will die within a few hours if left in freshwater. Intact fish transferred to freshwater show a milder depression of plasma sodium followed in 2 or 3 days by a return to the normal range; during this period of adaptation, intact fish never show signs of distress and continue to eat and behave normally. In nature, *P. formosa* occurs only in freshwater, while *P. latipinna* lives in freshwater, in brackish water and in seawater. Thus, there can be little doubt that as in *Fundulus heteroclitus* (Burden 1956) and *Xiphophorus* (Schreibman & Kallman 1963), the pituitary secretes some hormone or hormones that is, or are, indispensable to these fishes for survival in freshwater. The studies on *Fundulus* by Pickford and her collaborators (Burden 1956; Pickford & Phillips 1959; Pickford, unpublished results), together with studies on the histophysiology of the pituitary in *Poecilia* (Ball 1963; Olivereau & Ball 1964) and *Fundulus* (Ball & Pickford 1964), and a recent demonstration that ovine prolactin promotes freshwater survival in hypophysectomized *Poecilia* (Ball & Olivereau 1964), provide evidence that this 'freshwater factor' in both fishes is in fact a prolactin-like hormone (hereafter referred to as prolactin). The presence of prolactin in the fish pituitary is indicated by biological tests (Lehrman 1956; Grant & Pickford 1959). Recent work of Stanley & Fleming (1963) also implicates prolactin in restoration of normal kidney function in hypophysectomized *Fundulus kansae*.

A subsidiary experiment (Series 3) was performed to see if the grafted pituitary could influence survival in freshwater. Eight hypophysectomized *Poecilia formosa*, which had lived with every sign of vigour in dilute seawater for 2 weeks after the operation, were transferred to freshwater. Five were found dead within 48 h of the transfer; the other three, found in distress within 36 h, were returned to dilute seawater and recovered to live for a further 4 weeks or longer. In contrast, 9 grafted-hypophysectomized fish showed no evidence of distress when transferred to freshwater. They remained healthy and vigorous in freshwater for 2 or 3 weeks and were then returned to dilute seawater. Three of these grafted fish were kept for several months and were then tested again in freshwater, with the same result. Thus, the pituitary appears to secrete the 'freshwater factor', presumably prolactin, as readily when transplanted to the tail musculature as when in its normal position attached to the hypothalamus.

(*d*) *Growth*

Weight changes were not followed in this investigation, partly because repeated weighing of the fish would have involved excessive handling which might have diminished any growth response, and partly because it is clear from Pickford's work on *Fundulus* (Pickford 1953*b*, 1954*a*) that changes in length provide better criteria of true growth than changes in weight, which are usually erratic and which express factors other than growth increments, such as gonad development and accumulation of food stores. The length changes during the 28 days, expressed as percentages of the initial lengths, are given in table 1.

TABLE 1. GROWTH, FIN REGENERATION AND METABOLIC PARAMETERS

	number of fish	length changes (% in 28 days)	fin regeneration (mm in 14 days)	*HSI**	liver reserves		*PFI*†
					glycogen	fat	
			Series 1				
intact	14	13·0 ± 1·1‡	4·1 ± 0·1	3·3 ± 0·2	3·2§	0·3§	1·0 ± 0·2
hypophysectomized	14	(−1·7 ± 0·2)	2·8 ± 0·2	3·7 ± 0·2	2·9	2·4	1·6 ± 0·2
grafted	13	2·2 ± 0·3	2·9 ± 0·2	4·1 ± 0·3	3·6	2·2	3·0 ± 0·2
			Series 2				
intact	8	3·6 ± 0·2	3·7 ± 0·1	3·1 ± 0·3	3·1	0	1·2 ± 0·3
sham-hypophysectomized	9	3·5 ± 0·2	3·6 ± 0·1	2·9 ± 0·2	3·3	0	1·2 ± 0·1

* Hepatosomatic index, $HSI = \frac{\text{liver weight} \times 100}{\text{body weight} - \text{ovary weight}}$.

† Perivisceral fat index, $PFI = \frac{\text{weight of perivisceral fat} \times 100}{\text{body weight} - \text{ovary weight}}$.

‡ Mean ± standard error.

§ Mean histological ratings on arbitrary five-point scale, 0–4.

In agreement with earlier work on other species of fish (Pickford 1953*a*; Ball 1962) the hypophysectomized *Poecilia formosa* stopped growing and actually decreased in length, though under the same conditions the intact controls grew vigorously. The grafted fish, however, increased in length, though much more slowly than the intact controls. In Series 2, both intact and sham-hypophysectomized fish grew at the same rate ($t = 0{\cdot}09$, $p > 0{\cdot}9$), though more slowly than the intact fish in Series 1, reflecting perhaps the absence of frozen *Artemia* in the diet of the Series 2 fish, and also the fact that the fish in the two series came from different clones. However, it is certain that sham-hypophysectomy had no significant effect on growth, so that we may conclude from the Series 1 results that the transplanted pituitary secretes a growth-factor, but at a much slower rate than when in its normal position.

Examination of the vertebrate literature showed that we have to consider the possibilities that this growth factor could be thyrotrophin (*TSH*) (e.g. Geschwind & Li 1955; Scow 1959), prolactin (see below), or growth hormone (*GH*) *per se*. In seeking to interpret our findings, it seems that *TSH* can be excluded, since neither *TSH* injections in *Fundulus* (Pickford 1954*b*) nor thyroxine treatment in *Poecilia* (Ball 1963) will induce growth in hypophysectomized fish, and other evidence indicates abundant secretion of *TSH* by the transplanted pituitary (§§*b* and *f*). Prolactin and *GH* appear to be closely related,

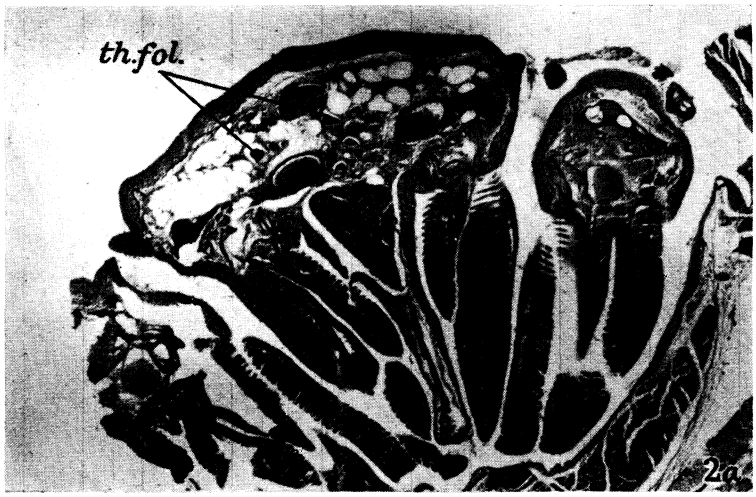
possibly even identical in some species; thus primate *GH* has been claimed to contain inherent prolactin activity (Ferguson & Wallace 1961; Li 1963), but this has recently been questioned, and the two activities may yet prove to be due to two separable factors of the primate pituitary (Brauman & Brauman 1963; Reisfield, Hallows, Williams, Brinkman & Steelman 1963; Pasteels 1963). The hypophysectomized pigeon respond either to prolactin or to *GH* by a weight increase (Bates, Miller & Garrison 1962), and Hublé (1956) has suggested that in birds prolactin assumes the function of mammalian *GH*. According to Etkin (1963) and Berman *et al.* (1964), prolactin may also be the amphibian growth-promoting hormone, although tadpoles are known to respond to injections of mammalian *GH* (Enemar & von Mecklenberg 1962). Even in the rat, there is some evidence that ovine prolactin has some growth-promoting potency (Reisfield, Tong, Riches & Brink 1961; Cargill Thompson & Crean 1963). In fish, however, repeated experiments entailing the chronic treatment of hypophysectomized *Fundulus* with ovine prolactin have never yielded evidence of growth-induction (Pickford & Kosto 1957; Pickford, personal communication). Further, the graft in our experiments gave no evidence of a deficiency in the secretion of prolactin (§*c*, and Discussion). On the other hand, beef *GH* readily stimulates growth in hypophysectomized *Fundulus* (Pickford 1953*b*, 1954*a*) and in hypophysectomized *Poecilia formosa* (Ball 1963). Thus, we conclude that the growth-promoting factor secreted in extremely reduced amounts by the transplanted pituitary was *GH* itself, probably potentiated by the concomitant thyroid stimulation (cf. Scow 1959; Pickford & Atz 1957, p. 99) due to *TSH* secreted by the transplants (§§*b* and *f*).

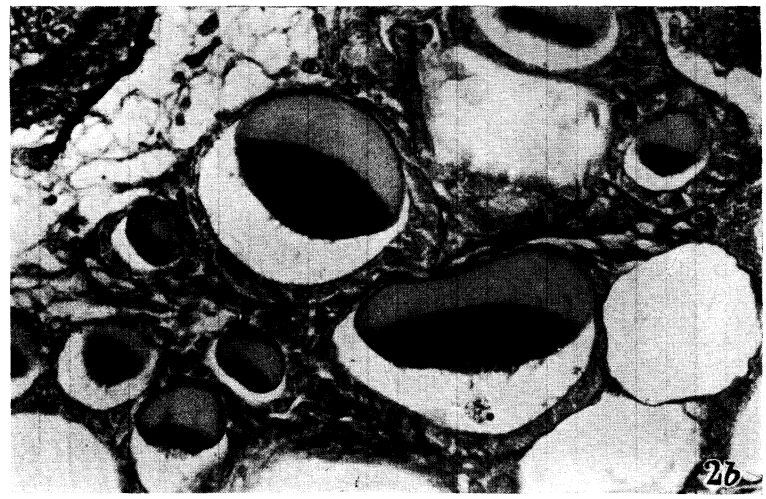
(*e*) *Regeneration of the caudal fin*

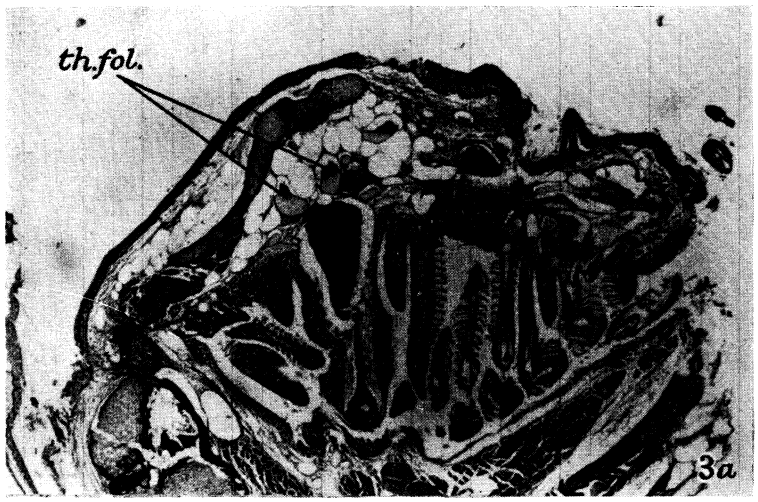
Regeneration of the amputated caudal fin was followed for the first 2 weeks of the experiment by measuring the new tissue formed in mid-line from the line of amputation to the tip of the regenerated fin-rays. In Series 2, sham-hypophysectomy had no detectable effect on fin-regeneration (table 1). In Series 1, the grafted and the hypophysectomized fish regenerated the fin at the same low rate (table 1), much more slowly than the intact animals (compared with the intact group, $p < 0{\cdot}001$ for both grafted and hypophysectomized fish). Thus the grafted pituitary was defective in the secretion of the hormone(s) chiefly necessary for normal fin-regeneration. Earlier work, using intact fishes (Buser–Lahaye 1953) had indicated that the thyroid and *TSH* stimulated fin regeneration in various species, and this is true also for regeneration of the caudal fin in intact *P. latipinna* (Ball 1963). But thyroxine treatment failed to stimulate regeneration in hypophysectomized *P. formosa*, whereas bovine *GH*, given in conjunction with thyroxine, significantly accelerated regeneration in hypophysectomized fish in the same experiment (Ball 1963). We conclude therefore that fin regeneration, like body growth, was defective in the grafted fish (despite activation of the thyroid by the transplants; see §*f*), because of a very low rate of secretion of *GH* by the transplanted pituitary.

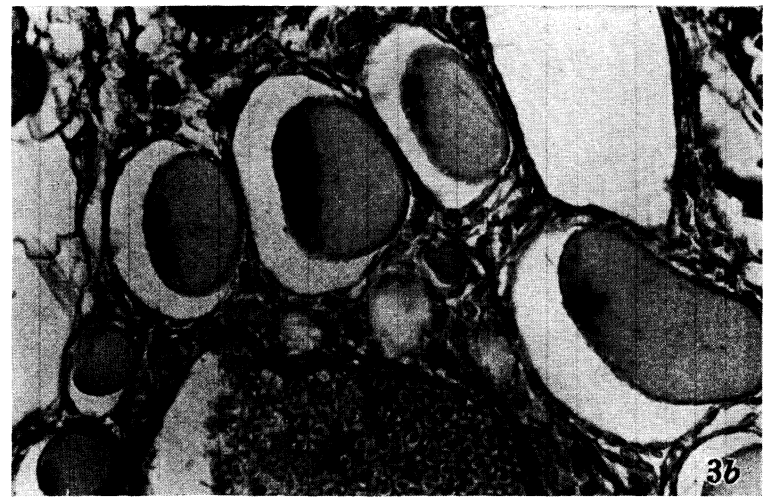
(*f*) *Thyroid gland*

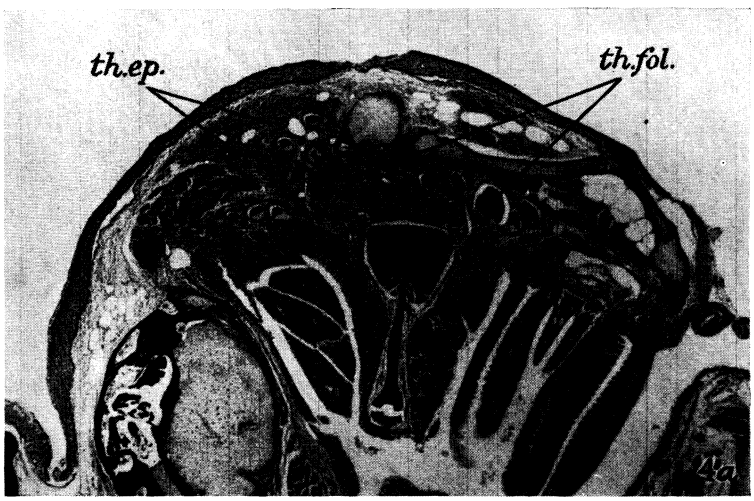
As in most teleosts, the thyroid in *Poecilia* is not encapsulated but consists of follicles scattered in the gular region, associated with the inferior jugular veins and the ventral aorta and contained in a mass of loose connective tissue. In some individuals, both intact











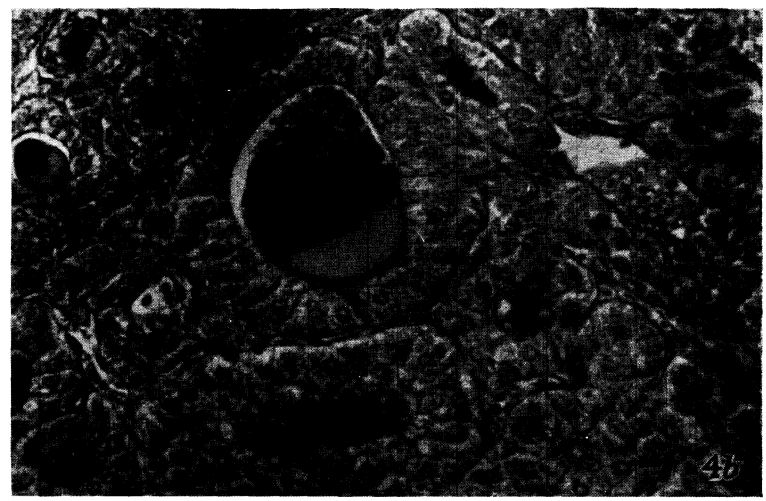


FIGURE 2*a*. Transverse section of the thyroid region of an intact fish. Floor of pharynx above, roots of gill-arches below. Thyroid follicles (*th.foll.*) scattered in loose connective tissue (× 50).

b. Higher magnification of the thyroid shown in figure 2*a*. Cuboidal follicular epithelial cells, partly granulated colloid (× 500).

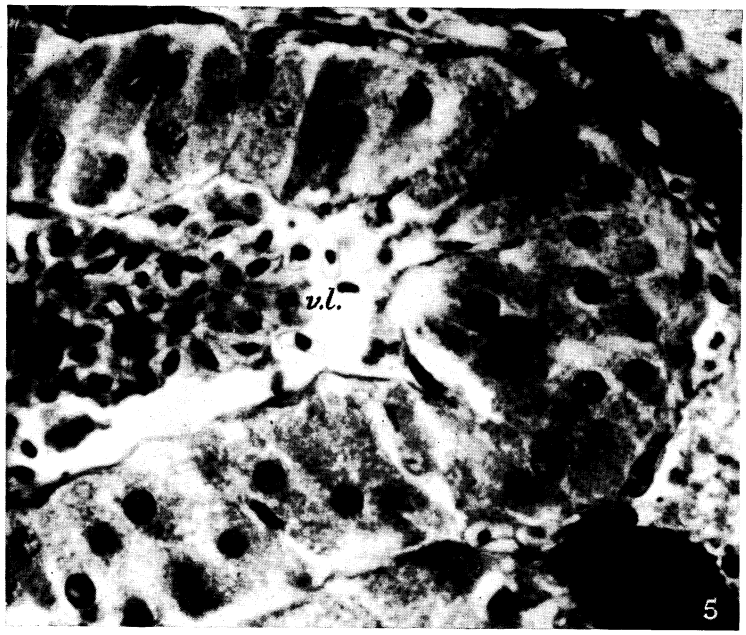
FIGURE 3*a*. Transverse section of the thyroid region of a hypophysectomized fish. Scattered follicles (× 50).

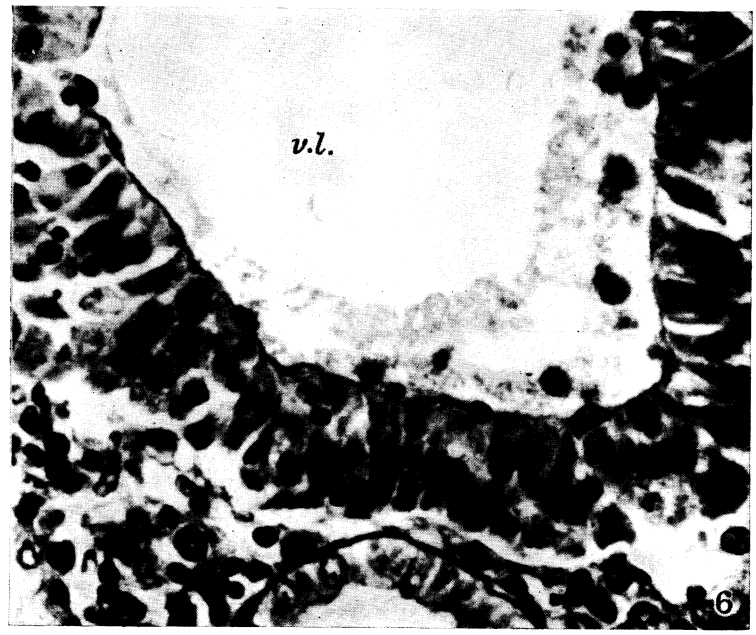
b. Higher magnification of the thyroid shown in figure 3*a*. Squamous follicular epithelial cells, dense non-granulated colloid (× 500).

FIGURE 4*a*. Transverse section of the thyroid region of a grafted-hypophysectomized fish. Marked hyperplasia of thyroid, with sheets of thyroid epithelial cells (*th.ep.*) and follicles almost filling the gular region (× 50).

b. Higher magnification of thyroid shown in figure 4*a*. Columnar thyroid epithelial cells, with rounded nuclei, prominent nucleolus, mainly granulated colloid, numerous resorption vacuoles (× 500).

(*Facing p.* 76)





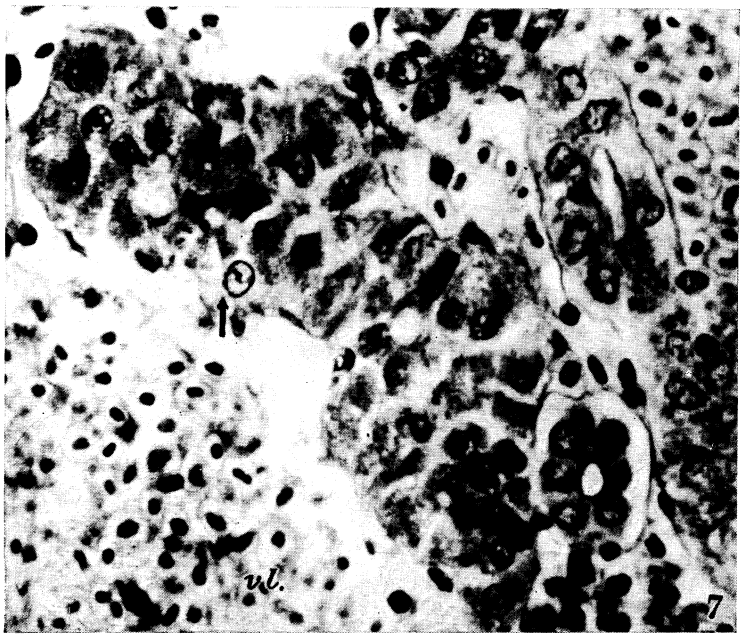
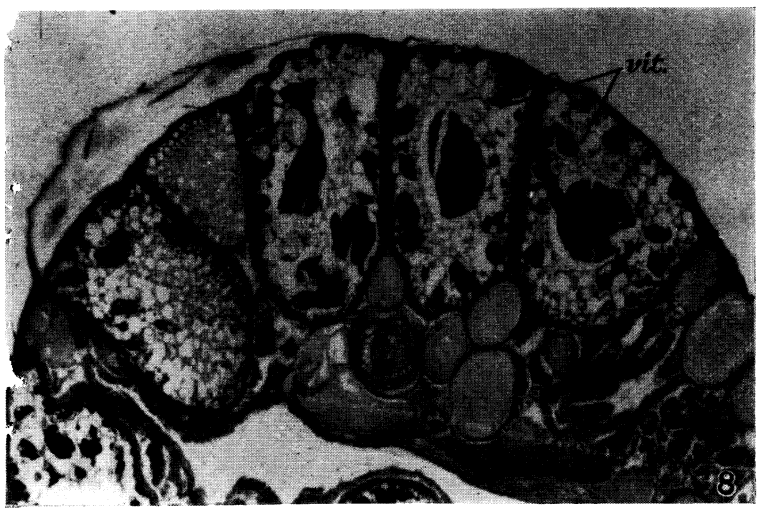
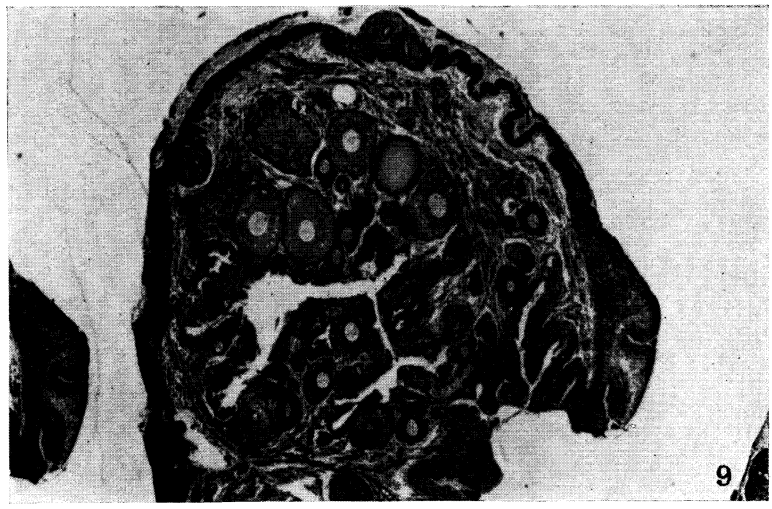


FIGURE 5. Interrenal cells of an intact fish around a vein lumen (*v.l.*). Pale cytoplasm, large round nuclei, prominent nucleoli (× 1250).

FIGURE 6. Interrenal cells of a hypophysectomized fish. Small cells, dark cytoplasm, dense small nuclei (× 1250).

FIGURE 7. Interrenal cells of a grafted-hypophysectomized fish. Morphology intermediate between that in intact fish (figure 5) and in hypophysectomized fish (figure 6). Arrow points to a chromaffin cell (× 1250).





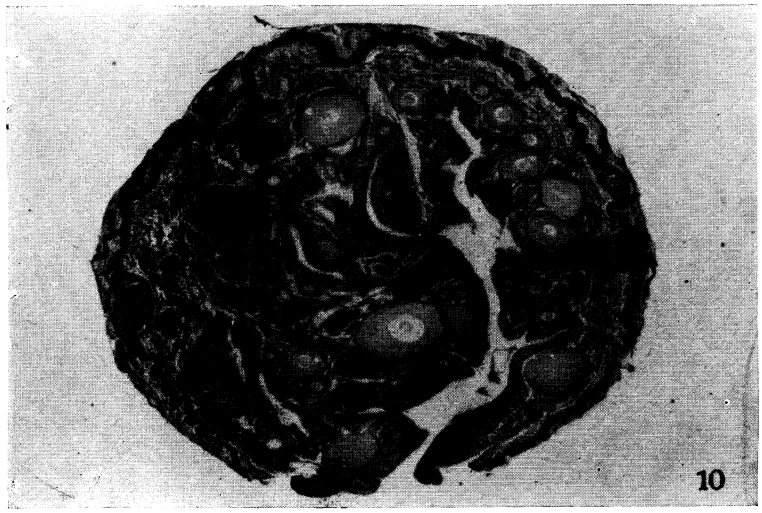


FIGURE 8. Section of the ovary of an intact fish. Large oocytes (*vit.*) in early stages of vitellogenesis (pituitary-dependant), smaller pre-vitellogenic oocytes (× 50).

FIGURE 9. Section of the ovary of a hypophysectomized fish. Absence of vitellogenesis, persistence of healthy pre-vitellogenic oocytes in voluminous connective tissue stroma (× 50).

FIGURE 10. Section of the ovary of a grafted-hypophysectomized fish. Note similarity to the ovary of a hypophysectomized fish (figure 9) (× 50).

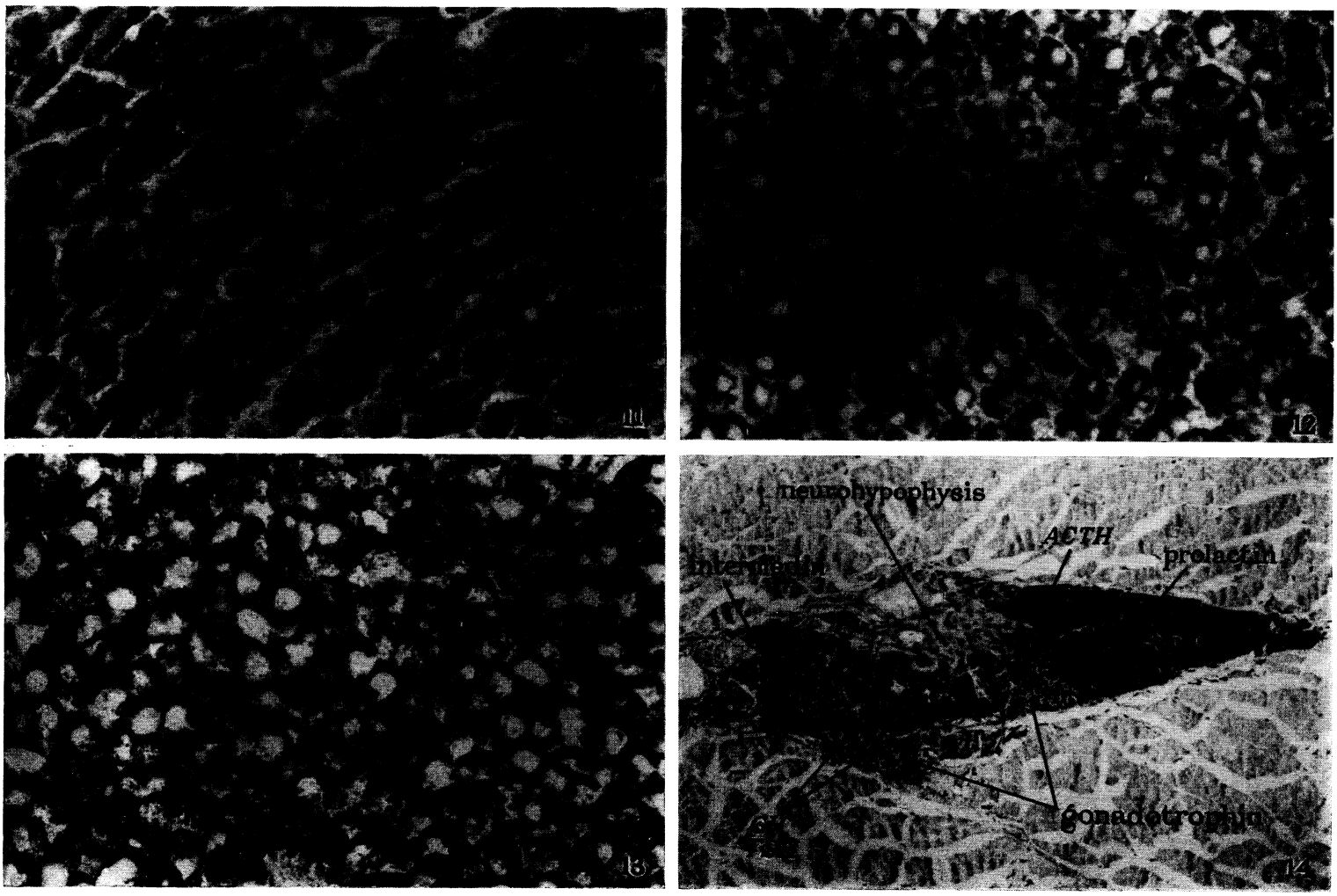


FIGURE 11. Section of the liver of an intact fish. Abundant glycogen (dark), little fat (× 500).

FIGURE 12. Section of the liver of a hypophysectomized fish. Numerous large vacuoles, representing dissolved fat (× 500).

FIGURE 13. Section of the liver of a grafted-hypophysectomized fish. Numerous fat vacuoles(× 500).

FIGURE 14. Longitudinal section of a pituitary transplant *in situ* in caudal musculature, anterior end of gland to the right, to show the location of the various cell-types in the pituitary (see text, pp. 84–85).

and hypophysectomized, a few thyroid follicles have also been found in the region between the heart and the oesophagus.

Histological features of the thyroid in the three groups of fish in Series 1 are summarized in table 2, and typical glands are illustrated in figures 2, 3 and 4, plate 18. It is clear that the thyroid was more stimulated in the grafted fish than in the hypophysectomized fish.

TABLE 2. SUMMARY OF THE HISTOLOGICAL CHARACTERISTICS OF THE THYROID GLANDS IN SERIES 1

The figures, other than those for cell height, show the number of fish in which the listed characters were predominant.

	intact	hypophysectomized	grafted
number of fish	14	14	13
hyperplasia of thyroid	4	0	8
thyroid epithelial cells			
squamous	1	12	2
cuboidal	5	2	6
columnar	8	0	5
nuclei rounded	12	0	9
nucleoli prominent	10	0	9
mean lowest cell height	$2{\cdot}7 \pm 0{\cdot}3$ μm	$1{\cdot}6 \pm 0{\cdot}2$ μm	$2{\cdot}9 \pm 0{\cdot}7$ μm
mean greatest cell height	$6{\cdot}3 \pm 0{\cdot}6$ μm	$2{\cdot}8 \pm 0{\cdot}2$ μm	$7{\cdot}3 \pm 1{\cdot}0$ μm
number of fish having cells 7·8 μm or more high	2	0	6
colloid			
strongly *PAS* positive	14	14	2
granulated	10	0	9
resorption vacuoles			
many	9	0	8
few	5	9	4
none	0	5	1

Furthermore, it appears that the thyroid was more strongly stimulated in the grafted fish than in the intact controls, in which the gland displayed the same grade of activity as in wild fish (Olivereau, unpublished); among the grafted fish, twice as many displayed frank hyperplasia of the thyroid, with follicles and masses of thyroid epithelial cells invading the bases of the gill arches; three times as many contained follicles with epithelial cells 7·8 μm or more in height; and whereas the thyroid colloid was strongly *PAS* positive in all the intact fish (indicating a relatively low rate of release of thyroid hormone; see Krompecher, László & Oláh 1962), it was strongly *PAS* positive in only two of the grafted fish.

The findings from the histological state of the thyroid gland, then, are in agreement with the evidence of thyroid activity furnished by the retention by the grafted fish of normal skin and corneas. It appears that the grafted pituitary can secrete *TSH* at a higher rate than normal. The implications of this finding have been considered in a previous paper (Ball, Olivereau & Kallman 1963).

(*g*) *Interrenal tissue*

The interrenal tissue of teleosts is the homologue of the adrenal cortex of mammals, and considerable evidence shows that the structure and activity of the interrenal cells are under the control of pituitary adrenocorticotrophin (*ACTH*) (see Chester Jones 1957;

Pickford & Atz 1957). In *Poecilia*, the interrenal tissue consists of one or several layers of polyhedral cells forming a collar round the posterior cardinal veins on both sides, embedded in the anterior kidney. Intermingled with the interrenal cells are chromaffin cells, sometimes scattered, sometimes in groups, which have a clear and vacuolated cytoplasm and a vesicular nucleus with an obvious nucleolus (figure 7, plate 19). The chromaffin cells were always easily distinguishable from the much more numerous interrenal cells, and they presented the same appearance in all three groups of fish.

TABLE 3. SUMMARY OF THE HISTOLOGICAL CHARACTERISTICS OF THE INTERRENAL CELLS IN SERIES 1

	intact	hypophysectomized	grafted
nuclear diameter	3·5–4·5 μm	2·5–3·5 μm	3·0–4·0 μm
nucleolus	prominent	rarely prominent, indistinct in most cells	generally prominent
chromophilia of nucleoplasm	faint, clear with distinct chromatin masses	densely staining, chromatin masses indistinct	faint, clear with distinct chromatin masses
chromophilia* of cytoplasm	faintly staining	deeply staining	intermediate

* Erythrosinophilia, assessed using the cytoplasm of adjacent kidney cells as standard.

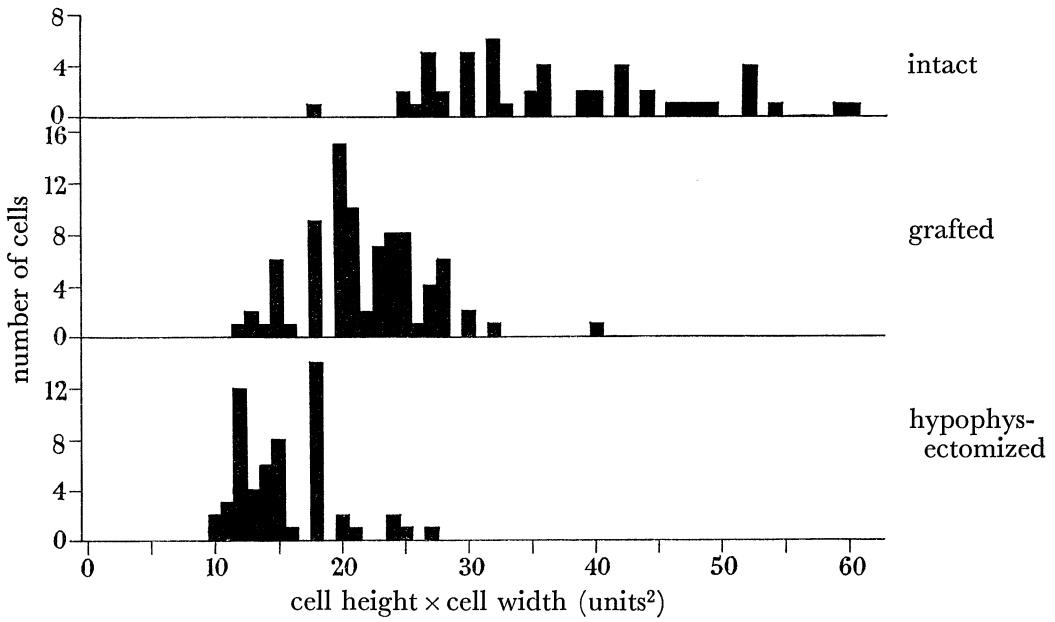


FIGURE 1. Interrenal tissue. Frequency histograms of the product cell height × cell width, measured in ocular scale units (1 unit = 1·66 μm). Based on measurements of 50 cells in three intact fish, 85 cells in 6 grafted fish, and 57 cells in 3 hypophysectomized fish. Interrenal cells tend to be larger in grafted fish than in hypophysectomized fish, though smaller than in the intact group.

Table 3 summarizes the histological state of the interrenal tissue, and typical areas of interrenal cells are shown in figures 5, 6 and 7, plate 19. The hypophysectomized fish appeared to have less interrenal tissue than the intact controls, though this was difficult

to evaluate precisely because of great variation in the number of cell layers from place to place along the vein, and from fish to fish in all the groups. In none of the fish did the interrenal cells display pronounced cytoplasmic vacuolation, though this is a characteristic feature of mammalian adrenal cortical cells. The features listed in table 3 are the ones that were finally selected, after careful study of sections of intact and hypophysectomized fish, as being the most reliable indicators of the functional state of the interrenal cells. The results of measurements of the greatest cell height and cell width in fish of the three groups (figure 1) confirm the differences in the cell dimensions between the three groups that were noticed in examination of the sections.

In Series 2, no morphological differences could be detected between the interrenals of sham-hypophysectomized and intact fish.

By the various criteria, it can be seen that the pituitary grafts maintained the interrenal cells in a functional state below that in the intact fish, but higher than in the hypophysectomized group, indicating considerable, though subnormal, output of *ACTH* by the grafts.

(*h*) *Ovary*

The ovary of *Poecilia* is a median hollow sac, with a lumen that continues via the lumen of the oviduct to the exterior. The ovarian follicles act as brood-chambers for the developing young: the mature egg is not ovulated but is retained in the follicle, and the embryo then develops to full-term on top of the yolky egg. Many embryos usually develop at the same time within the ovary, and when fully developed they are released by rupture of the follicles and are all expelled, by way of the oviduct, as a brood. Following the birth of a brood, some of the partly grown oocytes remaining in the ovary embark on rapid growth and accumulation of yolk (vitellogenesis) and are fully mature within a few days. Under the conditions of our experiments, intact females produced broods successively at intervals of about 28 days. Hypophysectomy did not interfere with gestation, nor prevent the birth of the current brood; but it did prevent the vitellogenic growth of any more oocytes after the current brood had been born. The ovarian cycle, and the lack of dependence of gestation and birth on adenohypophysial hormones, resembles the case of *P. latipinna*, which was described previously (Ball 1962).

In the light of what is known about pituitary gonadotrophic function in female fishes (Ball 1960; Ramaswami 1962), our concern was to determine the effects of the transplanted pituitary on vitellogenic (second phase) oocyte-growth. Table 4 gives the relevant data. A hypophysectomized fish of Series 1 and a sham-hypophysectomized fish of Series 2 were pregnant at autopsy and are excluded from table 4. No other fish were pregnant.

The ovaries were significantly smaller in both hypophysectomized and grafted fish than in the intact group ($p = 0{\cdot}01$ to $0{\cdot}025$ in both cases). Sections showed that vitellogenesis was completely absent in the grafted and the hypophysectomized groups, though proceeding normally in 9 of the intact fish. In the hypophysectomized and the grafted fish, the ovary in most cases consisted of fibrous connective tissue, containing many first-phase oocytes, oogonia, corpora atretica (Ball 1960) and, in a few instances, evacuated follicles from which young fish had recently been expelled (figures 9 and 10, plate 20). Prominent fibrous tissue was not to be found in the ovaries of the intact fish, which consisted mostly of developing oocytes, many in various stages of vitellogenesis (figure 8,

plate 20). Thus in all essential features, the ovaries of the grafted fish were indistinguishable from those of the hypophysectomized group.

The ovaries of most of the hypophysectomized and the grafted fish were heavily infected with a parasitic fungus, though this was rarely found in the ovaries of the intact fish. This fungus is usually found to some extent in all *Poecilia* in captivity, but its enormous spread in these ovaries was exceptional. However, infection of the kidneys, brain, liver and viscera was not increased in the hypophysectomized and the grafted fish, so that the heavy ovarian infection cannot be attributed directly to pituitary deficiency. It seems likely that the degenerating fibrous ovary, deprived of gonadotrophic support, offers ideal conditions for the spread of the fungus. The identitity of the fungus is uncertain, but it closely resembles *Ichthyophonus* as described by Schäperclaus (1954).

TABLE 4. CONDITION OF THE OVARY

	number of fish	*GSI**	number of fish with vitellogenesis	number of fish with infective fungus in the ovary†
		Series 1		
intact	13‡	$2{\cdot}9 \pm 0{\cdot}9$	9	2
hypophysectomized	10‡	$0{\cdot}4 \pm 0{\cdot}1$	0	10
grafted	12‡	$0{\cdot}4 \pm 0{\cdot}1$	0	10
		Series 2		
intact	8	$9{\cdot}0 \pm 2{\cdot}0$	8	1
sham-hypophysectomized	8	$3{\cdot}8 \pm 2{\cdot}0$	8	1

* Gonosomatic index, $GSI = \frac{\text{weight of ovary} \times 100}{\text{weight of body}}$.

† See text, p. 80, above.

‡ Ovaries of one intact, three hypophysectomized and one grafted fish not processed.

In Series 2, all the fish, both intact and sham-hypophysectomized, displayed stages in vitellogenesis. The lower *GSI* of the sham-hypophysectomized group (table 4) was not significantly below that of the intact fish ($t = 1{\cdot}7, p = 0{\cdot}1$), and obviously the pituitary in both groups was secreting sufficient gonadotrophin to maintain vitellogenesis. It is possible, nevertheless, that the stress of the operation might have caused a temporary reduction in the output of gonadotrophin, resulting in slightly retarded vitellogenesis 1 month later. Stress in *Poecilia*, as in mammals, probably induces hypersecretion of *ACTH* (Ball & Slicher 1962), and repeated stress in the rat induces also a retention of gonadotrophin (Klastersky 1963). However, the total absence of vitellogenesis and the ovarian degeneration found equally in the hypophysectomized and the grafted fish of Series 1 cannot be ascribed to surgical trauma, and point clearly to the conclusion that the transplanted pituitary secretes little gonadotrophin, if any.

In 3 grafted-hypophysectomized fish, extra to the present experiments, that were kept and autopsied 7 months after the graft was established, the ovaries were also regressed and without vitellogenesis, though the state of the skin and eyes attested to the functional integrity of the grafts; a finding of some interest in view of reports of a resumption of gonadotrophin secretion, after long periods of inactivity, in pituitary transplants in the rat (Courrier & Colonge 1957; Martinowitch, Pavic & Zivkovic 1963).

(i) Liver state and fat reserves

The weight of the liver and its content of fat and glycogen, together with the amount of stored perivisceral fat, were determined for each fish, with the intention of obtaining information about the ability of the transplanted pituitary to influence these metabolic parameters. Pickford (1953*a*) found accumulation of fat and glycogen in the enlarged livers of hypophysectomized male *Fundulus*, corresponding to the condition in intact fish during sexual regression, and showed that depletion of these stores, corresponding to the condition in the males during the breeding season, was produced by treatment with methyl testosterone (Pickford 1952), while earlier observations on *P. latipinna* suggested an accumulation of both hepatic and perivisceral fat after hypophysectomy (Ball 1963). In the present work, liver size was expressed as a *hepatosomatic index* (*HSI*: see table 1), with allowance for the pronounced variations in the size of the ovary both within the intact group and between the groups. Similarly, the amount of stored fat around the viscera was expressed as a *perivisceral fat index* (*PFI*).

Hepatic glycogen and fat were estimated visually in 5 μm paraffin sections stained with *PAS*-orange *G*-haemalum. Fat was assessed from the number of distinctive rounded vacuoles in these sections. By this method, small amounts of fat would probably not be detected, but large differences in fat content were readily seen. Frozen sections of the livers of some of the fish confirmed that the prominent vacuoles in the original paraffin sections did indeed represent dissolved fat. Control sections for glycogen were incubated with saliva at 37 °C for 30 min. Both fat and glycogen were assessed on a 5-point scale, from 0 (none present) to 4 (very abundant), and the scores were averaged to give an index for each group of fish (table 1). A second reading of the sections, made at a later date by an independent observer, agreed closely with the original assessments.

Hypophysectomy possibly increased liver size, (table 1) though the difference in mean *HSI* for intact and hypophysectomized fish is not statistically significant ($t = 1{\cdot}4, p = 0{\cdot}2$). The livers of the grafted fish were significantly larger than those of intact fish ($t = 2{\cdot}1$, $p = 0{\cdot}05$), though not significantly larger than the livers of the hypophysectomized fish ($t = 1{\cdot}0$, $p = 0{\cdot}2$ to $0{\cdot}4$). All the fish in both Series 1 and 2 had abundant glycogen stores, but fat storage was obvious only in the hypophysectomized and the grafted fish (figures 11, 12, 13). Thus the increased liver size in the grafted fish can be attributed to accumulation of fat together with ample stores of glycogen.

Perivisceral fat was slightly increased after hypophysectomy (table 1), though the *PFI* difference was not quite significant ($t = 1{\cdot}8$, $p = 0{\cdot}05$ to $0{\cdot}1$). However, all the grafted fish had significantly more perivisceral fat than either of the other groups ($p = 0{\cdot}001$ in both comparisons).

The tendency towards storage of fat in the liver and viscera of the hypophysectomized fish, and the frank storage displayed by the grafted fish, most probably reflect in both cases the deficiency of some pituitary hormone(s) normally active in the mobilization of this fat. On the evidence of the present work, the grafted fish were markedly deficient in growth hormone and gonadotrophin, but probably not grossly deficient in other pituitary hormones. Growth hormone is known to decrease fat stores in mammals (Buckle 1962; Engel 1962) and possibly in amphibians (Larsen 1963), but seemingly has little or no

effect on hepatic fat reserves in hypophysectomized *Fundulus* (Pickford 1953*b*). Several facts argue against an important role for *GH* in depleting the fat reserves in *Poecilia*. Thus, hepatic and perivisceral fat is extremely abundant in immature rapidly growing fish, and treatment of hypophysectomized *P. formosa* with bovine *GH* failed to reduce the fat stores, though it induced vigorous growth (Ball 1963). In immature fish, with their large fat stores, the pituitary gonadotrophs are either undifferentiated or inactive; while in adult females, the fat stores are depleted as vitellogenesis proceeds, the gonadotrophs being intensely active during vitellogenesis (Ball 1963). Other workers have demonstrated that hepatic fat is depleted during vitellogenesis in other poeciliids, *Xiphophorus* and *Poecilia* (*Lebistes*) *reticulata*, and that this depletion can be induced in male *Xiphophorus* by

TABLE 5. BLOOD CELL COUNTS FOR SERIES 1 FISH

	number of fish	erythrocytes ($\times 10^6$)	leucocytes ($\times 10^3$)	thrombocytes ($\times 10^3$)
intact	14	$6{\cdot}44 \pm 0{\cdot}21$*	$90{\cdot}53 \pm 7{\cdot}64$†	$333{\cdot}57 \pm 14{\cdot}78$
hypophysectomized	12‡	$4{\cdot}68 \pm 0{\cdot}32$	$31{\cdot}72 \pm 4{\cdot}91$	$242{\cdot}67 \pm 32{\cdot}15$
grafted	13	$6{\cdot}09 \pm 0{\cdot}17$	$75{\cdot}88 \pm 4{\cdot}82$	$437{\cdot}69 \pm 33{\cdot}64$

* Mean ± standard error.

† Mean leucocyte count for four fully mature fish, *GSI* > 6·0, was $119 \pm 13{\cdot}48$. Mean count for the remaining 10 partly-mature fish was $79{\cdot}04 \pm 6{\cdot}52$. The difference is statistically significant ($t = 2{\cdot}5$, $p = 0{\cdot}02$ to $0{\cdot}05$).

‡ Blood not taken from two of the hypophysectomized fish.

treatment with oestrogen but not with androgen (Clavert & Zahnd 1956, 1957). In elasmobranchs, the variations in liver weight during the sexual cycle are more marked in the female than in the male (Olivereau & Leloup 1950). Furthermore, in the intact group of our Series 1, the two fish that displayed some hepatic fat had pre-vitellogenic ovaries. The weight of the evidence, then, inclines us to believe that the accumulation of hepatic and perivisceral fat in the grafted fish was a consequence of the failure of the grafted pituitary to secrete gonadotrophin.

(*j*) *Haematology*

Hypophysectomy resulted in a decrease of all the blood cells (pancytopenia) as in *Fundulus* (Slicher 1961). The depression of both erythrocytes and leucocytes was highly significant ($p < 0{\cdot}001$ in both cases), while the reduction in thrombocytes was significant at $p = 0{\cdot}05$ (table 5). An interesting incidental finding from the figures for the intact group was that the total number of leucocytes increases as vitellogenesis proceeds. In the four intact fish with fully mature ovaries (*GSI* = 6 or higher) the mean leucocyte count was significantly raised above that in the other, partly mature, fish, in which the ovary was either previtellogenic or in the earlier stages of vitellogenesis, suggesting that ovarian hormones might elevate the leucocyte count.

Although the pituitary transplant secreted little or no gonadotrophin, so that any ovarian effects on the leucocytes were excluded in the grafted fish, the graft, nevertheless, maintained the leucocyte count at normal levels (table 5), not significantly below that for all the intact fish ($p = 0{\cdot}1$ to $0{\cdot}2$), and essentially the same as the mean for the partly mature intact fish ($p = 0{\cdot}5$). Similarly (table 5), the erythrocyte count of the grafted

fish was within the range for intact fish ($p = 0{\cdot}3$) and the thrombocyte count in the grafted group was actually higher than normal ($p = 0{\cdot}01$ to $0{\cdot}02$).

The pituitary graft, then, completely prevented the pancytopenia of hypophysectomy. The possible mechanism of this effect will be considered in the Discussion.

Discussion

The results have revealed a selective retention of some of the functions of the pituitary gland of *Poecilia* in ectopic transplantation. The evidence is that the transplanted gland is able to secrete *TSH* and prolactin in considerable amounts, moderate *ACTH*, growth hormone in very reduced amounts, and no gonadotrophin. One or more of the hormones maintained a normal haematological picture; the mechanism of this effect is undoubtedly complex, and requires consideration at this point.

In mammals, the pituitary erythropoietic factor, once considered separable from all the other known adenohypophysial hormones, is now considered to be *ACTH* (van Dyke 1959); however, the anaemia of hypophysectomized rats cannot be alleviated completely by corticosteroids (Vollmer, Gordon & Charipper 1942; Crafts & Meineke 1959), and other pituitary hormones are involved. Complete restoration of the peripheral red cell counts to normal is achieved by a combination of thyroxine and cortisone, but in addition growth hormone (*GH*) is needed for repairs of the atrophic bone marrow (Crafts & Meineke 1959). It is probable, then, that the mammalian pituitary normally influences erythropoiesis by three of its hormones, *ACTH*, *TSH* and *GH*, with *ACTH* as the most potent single factor as far as the peripheral red cell count is concerned (Fisher & Crook 1962; van Dyke 1959). The pituitary hormones apparently enhance erythropoiesis mainly indirectly, by their stimulatory effects on general metabolism (Crafts & Meineke 1959; van Dyke 1959; Evans, Rosenberg & Simpson 1961; Fisher & Crook 1962). In toads, but to a lesser degree than in rats, treatment with *ACTH* or cortisone partly alleviated the anaemia of hypophysectomy (Nakao & Shirakura 1961).

Our knowledge of the endocrine control of erythropoiesis in fish comes largely from work on *Fundulus heteroclitus*, in which hypophysectomy results in anaemia (Slicher 1961). Chronic treatment with *GH* had no beneficial effect (Pickford 1953*b*, 1954*a*; Slicher & Pickford, unpublished), but *ACTH* completely alleviated this condition (Slicher 1961). *TSH* also elevated the red cell count in hypophysectomized fish, though this could be attributed to the known contamination of the *TSH* preparation with luteinizing hormone (*LH*), since the preparation contained enough *LH* to stimulate the testes of the recipient fish and methyl testosterone strongly enhances erythropoiesis in *Fundulus* (Slicher 1961; see also Pickford & Atz 1957). The thyroid and *TSH* are probably not of paramount importance in erythropoiesis in *F. heteroclitus*, since hypothyroidism in this species had no effect on the red cell count (Harris 1959; Slicher 1961). Thus the maintenance of the red cell counts in our grafted fish can be ascribed with some certainty to the secretion of *ACTH* by the graft, possibly acting in combination with the *TSH* also secreted.

Hypophysectomy in the rat generally leads to an increased total white cell count (Crafts & Meineke 1959; Piliero 1959), but with a tendency towards lymphopenia (Piliero 1959), though in certain strains of rats removal of the pituitary may cause a reduction in all the white cells (Gordon 1954). The overwhelmingly predominant white cell in

Poecilia is a lymphocyte, as in *Fundulus* (Slicher 1961), so that the leucopenia (lymphopenia) of hyphysectomy in these fishes is in essential agreement with the findings on the rat. In hypophysectomized *Fundulus*, chronic treatment with purified *ACTH* maintained the white cell count at normal levels; *TSH* was ineffective, but intermedin had a beneficial effect, possibly a reflexion of its structural overlap with *ACTH* (see Li 1962). It is probable that prolactin has a mildly beneficial effect on the leucopenia of hypophysectomized *Fundulus* (Slicher 1961), though the matter needs further investigation. Since the white cell counts were consistently higher in *Fundulus* adapted to freshwater than in specimens adapted to seawater (Slicher 1961), and since prolactin is the principal pituitary hormone involved in freshwater adaptation in this species (Pickford & Phillips 1959; Pickford, unpublished; Ball & Pickford 1964), then an influence of prolactin on the white cell count seems to be a possibility. It seems probable, however, that the pituitary–adrenal axis is the main endocrine influence on the number of circulating white cells. In this connexion, it is of interest that the white cell count of intact *Poecilia latipinna* was lowered by immersion for 24 h in a solution of the adrenocortical inhibitor SU-4885 to a mean value of $21\,000 \pm 1454$, the figure for control fish being $27\,746 \pm 2241$ (data of Ball & Slicher 1962; $t = 2{\cdot}5$, $p = 0{\cdot}02$ to $0{\cdot}05$), suggesting that corticosteroids maintain the number of white cells at normal levels. At present, then, the normal levels of circulating white blood cells in the grafted fish can be ascribed tentatively to corticosteroid secretion, stimulated by *ACTH* from the transplanted pituitary, with the reservation that the role of prolactin is not yet settled.

The mechanism in fish by which the numbers of circulating thrombocytes are regulated is at present unknown (Slicher 1961).

It is clear from this discussion that the grafted pituitary most probably maintained the normal haematological picture by its secretion of *ACTH*, acting via the interrenal, but that other hormones secreted by the transplanted gland (*TSH*, prolactin) may also be involved.

Studies now in progress on the cytology of the grafts show that the partial retention of function by the transplanted pituitary is precisely paralleled by the selective persistence, in an active state, of only some of the cell-types of the normal pituitary. An account of the pituitary of *Poecilia*, with experimental evidence for the allocation of functions to the various cells, is given elsewhere (Olivereau & Ball 1964). The grafts vary greatly in the extent to which the normal pituitary morphology is maintained. Some grafts resemble the normal pituitary most strikingly (figure 14, plate 21), while in others the surviving cells are disposed in a much less orderly fashion. Despite such variations, we have found that the grafts display a common cytology. Active prolactin cells and *TSH* cells are abundant, and active *ACTH* cells are present, though not always easy to identify. Few or no active *GH* cells can be found, and the gonadotrophic region of the graft is occupied by inactive chromophobic cells (figure 14). Thus, the cytology of the grafts, in relation to their functional capacity, provides independent confirmation of the functional identification of the pituitary cell-types of *Poecilia* that we have previously made on other evidence (Olivereau & Ball 1963*a*, *b*, 1964; Ball 1963).

By analogy with the established situation in the higher vertebrates, the failure of the grafts to display some of the normal pituitary functions is most plausibly ascribed to the

severance of the hypothalamo–hypophysial connexion, thereby preventing stimulatory hypothalamic neurosecretory material from reaching the pituitary cells. There are, however, some objections to this interpretation, which should be considered at this point. It might be claimed that the deficient output of *GH* and gonadotrophin by the grafts resulted from damage inflicted on the secretory cells during the transplantation. This becomes unlikely when one considers the anatomical location of these cells in the pituitary (figure 14, plate 21; see Olivereau & Ball 1964), since though the gonadotrophs are indeed peripheral, so that the outermost layers of these cells could readily suffer mechanical damage, the *GH* cells are the most central secretory elements, well protected from damage, and mixed intimately with those *TSH* cells that undergo functional hypertrophy. Further the prolactin cells are peripheral at the widest part of the gland (figure 14), so even more susceptible to mechanical damage than the gonadotrophs, yet we have seen that prolactin appears to be secreted abundantly by the pituitary grafts. Again, these deficiencies could be supposed to arise from poor or delayed vascularization of the grafts; but sections show the grafts to be well-vascularized at autopsy, and a deficient blood supply would not be likely to produce a regular pattern of deficiencies, the same for all the grafted fish, but a variable pattern, depending on the extent and location of ischemic damage. Then too, the gonadotrophs, thyrotrophs and growth hormone cells lie in close proximity, the last two largely intermingled (figure 14). Why should the thyrotrophs in every case remain active while the other two cell-types degenerate, if the changes were brought about by interruption of the blood supply to this part of the gland? In the case of the rat, the objection that the extensive loss of function in the ectopic pituitary could be the result of damage inflicted during the transplantation, or to ischemia, has been entirely refuted by the beautiful work of Nikitowitch-Winer & Everett (1957, 1958*b*, *c*, 1959), who demonstrated renewal of function and cytological differentiation in pituitary grafts when they were removed from the kidney (where function and cytological differentiation were confined to the secretion of prolactin), and re-transplanted beneath the median eminence, the connexions with the hypophysial portal system being thereby restored.

The possibility that the functional deficiencies in our pituitary grafts could be caused by the surgical stress of hypophysectomy is countered by the results from the Series 2 fish. Since the surgical damage to the roof of the mouth in sham hypophysectomy did not result in failure of secretion of growth hormone and gonadotrophin by the immediately adjacent *in situ* pituitary gland, it is hardly likely that this damage could interfere with the function of the distantly situated pituitary graft.

The neurohypophysis in the grafts was completely devoid of stainable neurosecretory material at autopsy, a fact of some interest since it makes it very unlikely that any of the signs of pituitary function that we observed could have been due to the peripheral action of neurohypophysial hormones from the graft.

If we then accept that the results demonstrate the different abilities of the various adenohypophysial cell types to function when anatomically separated from the hypothalamus, the question arises whether the cells in the graft are truly *functionally* separated from the hypothalamic neurosecretory cells that in the normal situation are believed to modulate the activity of the pituitary cells (Pickford & Atz 1957, p. 228; Arvy, Fontaine & Gabe 1959; Stahl & Leray 1962; Öztan 1963; Dodd & Kerr 1963), or whether the

neurosecretory products of these hypothalamic cells may reach the pituitary graft by way of the general circulation. This point, of course, arises with equal pertinence in considering the findings on pituitary graft functions in mammals, and indeed a hypothalamic *ACTH*-releasing factor has been reported in the peripheral blood of hypophysectomized rats, though undetectable in blood from intact animals (Brodish & Long 1962). Thus the assumption that pituitary grafts in the rat are necessarily functionally isolated from the hypothalamus is questionable, particularly as regards the reports of *ACTH*-secretion by such grafts (cf. Critchlow, Lipscomb & Guillemin 1963). Little is known about this field in fishes; we may speculate that there is little likelihood of much leakage of hypothalamic neurosecretory material into the blood in teleosts, where according to a recent review, the vascular (portal) route of hypothalamo–adenohypophysial transmission, so well developed in tetrapods and just possibly present in primitive actinopterygians, is replaced by an entirely neural link (Dodd & Kerr 1963). Follenius (1961) has confirmed the absence of a portal system in cyprinodonts. It is reasonable to suppose at the present time that even should the hypothalamic neurohumours reach the pituitary cells in the graft by way of the general circulation, they will do so in concentrations much less than in the normal case. On this assumption, and supposing the various neurohumours to have the same biological half-life, the different fates of the different cell-types in the graft would still indicate the relative extents to which the various pituitary functions are dependent on the hypothalamus, just as well as if the cells in the graft were completely isolated functionally from the brain.

With these considerations in mind, we may consider the performance of the ectopic pituitary in *Poecilia* in relation to data from the other vertebrate groups. Considerations of space prevent a survey of the allied literature on lesions and stimulation of the hypothalamus in mammals.

Gonadotrophic function

The disappearance of gonadotrophic function from the ectopic pituitary in our experiments is in accordance with the greater part of the evidence for other vertebrates. The secretion of gonadotrophin is generally held to be the function of the mammalian pituitary that is most sensitively dependent upon the hypothalamus (see Harris 1955; Nikitowitch-Winer & Everett 1958*b*), and factors that stimulate the release of gonadotrophins by the pituitary have been demonstrated in the mammalian hypothalamus (Courrier, Guillemin, Jutisz, Sakiz & Aschheim 1961; Igarashi & McCann 1964). Most investigators of the function of pituitary grafts in female mammals have reported that the ovaries and accessory organs of the hosts gave no evidence of the secretion of gonadotrophin by the grafts (Westman & Jacobsohn 1940; Harris & Jacobsohn 1952; Greer, Scow & Grobstein 1953; Everett 1956; Ifft 1957; Nikitowitch-Winer & Everett 1958*b*; Sanders & Rennels 1959; Meunier, Bousquet & Mayer 1961; Dao & Gawlak 1963; Desclin 1963). The few cases in which the ovary gave evidence of gonadotrophin secretion by the grafted pituitary concerned local effects produced by intraovarian grafts, in which the amount of secreted gonadotrophin was probably very small (Petrovic & Lavillaureix 1958*a*; Moszkowska 1959; Moszkowska & Kordon 1960), or else depended on the maintenance of ovarian weight, which could be due to the abundant secretion of prolactin (see below) acting on the corpora lutea rather than on the follicular apparatus (Hertz 1960; Blanquet, Meunier,

Croizet, Mayer & Meyniel 1961). Hertz (1960) also showed that *four* grafted pituitaries could secrete enough gonadotrophin to augment the ovarian weight response to chronically administered chorionic gonadotrophin; since the augmented ovarian weights were very high, much greater than the ovarian weights of untreated intact controls, it can be accepted that what the grafts secreted was really gonadotrophin, but probably only a small amount from each graft. Failure of gonadotrophin output by pituitary grafts in male mammals has also been reported (Fortier 1951; Martini, De Poli, Pecile, Saito & Tani 1959; Knigge 1961), but several workers have claimed the secretion of gonadotrophin by ectopic pituitary tissue in the male in small amounts (Goldberg & Knobil 1957; Ahrén, Arvill & Hjalmarson 1962), or in considerable quantities (Courrier & Colonge 1957; Petrovic & Aron 1958); while local gonadotrophic effects have resulted from the establishment of pituitary grafts in the testis (May 1955; Marescaux & Deminatti 1955; Aron, Aron, Petrovic & Marescaux 1956; Petrovic & Lavillaureix 1958*b*). Martinowitch, Pavic & Zivkovic (1963) were able to demonstrate considerable gonadotrophic activity in hypophysectomized male rats bearing *multiple* grafts of infant rat pituitaries. The curious phenomenon of an initial loss of gonadotrophic potency followed by a resumption of gonadotrophin secretion by ectopic pituitary grafts in male rats was described by Courrier & Colonge (1957) and confirmed by Martinowitch *et al.* (1963). The consensus is that the *single* mammalian pituitary does not continue to secrete much gonadotrophin when grafted ectopically; in the experiments of Courrier & Colonge (1957) and Petrovic & Aron (1958), it is probable that the effects of small amounts of gonadotrophin secreted by the graft were augmented by large quantities of prolactin, which can reinforce the action of gonadotrophin on the testis (e.g. Woods & Simpson 1961), and which the grafts would be secreting in quantity (see below). The histology of the grafts in the experiments of Courrier & Colonge lends support to this interpretation (Courrier, Colonge, Herlant & Pasteels 1961).

Cockerels bearing pituitary autotransplants beneath the kidney capsule displayed atrophy of the comb to the same extent as hypophysectomized controls, indicating failure of gonadotrophic function in the grafted pituitary (Ma 1963); correspondingly, section of the hypothalamo–hypophysial portal vessels in the duck resulted in atrophic testes that could not be stimulated by continuous illumination (Benoit & Assenmacher 1955; Assenmacher & Tixier-Vidal 1959), while transection of the pituitary stalks in laying hens led to ovarian atrophy (Shirley & Nalbandov 1956). Hypothalamic lesions have also been found to suppress gonadotrophin release in birds, both male (McFarland 1959) and female (Ralph 1959; Egge & Chiasson 1963).

There are indications in the literature that the amphibian ectopic pituitary retains more gonadotrophic potency than the mammalian and avian glands. Pasteels (1960) reported the total cessation of gonadotrophic activity in ectopic pituitary autotransplants in both male and female *Pleurodeles*, but in another urodele, *Triturus*, such autotransplants maintained a very low rate of gonadotrophin output in both sexes (Vivien 1959; Mazzi & Peyrot 1962–3). In frogs too, Vivien found similar indications of a slight gonadotrophic function in pituitary autografts. Schott (1960) confirmed the results on the frog presented by Vivien, and also demonstrated a feeble local gonadotrophic action of intratesticular grafts in the frog, recalling the situation in mammals. Pituitary grafts placed beneath the median eminence in both *Triturus* and the frog retained normal gonadotrophic activity and mediated the

usual seasonal changes in gonadal function (Vivien 1959; Schott 1960). In the toad *Bufo bufo*, there is evidence, from the study of testis and thumb-pad, of the persistent secretion of an *FSH*-like factor by the ectopic pars distalis over a considerable period (about 2 months), though the output of an *ICSH*-like factor eventually ceased. Transplants on the median eminence continued the normal secretion of both factors (Lofts 1963). Extirpation of the hypothalamic preoptic nucleus scarcely interfered with gonad function and secondary sexual characters in male and female *Rana temporaria*, save for the abolition of ovulation in the female (Dierickx 1963*a*, *b*), while hypothalamic and median eminence lesions had little effect on gonadotrophin secretion in *Triturus* (Mazzi 1958; Mazzi & Peyrot 1960).

One generalization that seems implicit in the literature is that the testis is a more sensitive indicator for small amounts of gonadotrophin than the ovary, so that could we have assessed the effects of our grafts on testis functions we might have been able to detect a low residuum of activity.

Prolactin

There is near-universal agreement that the rat or mouse pituitary is able to secrete prolactin in abundance when removed from its hypothalamic connexions. This finding, due originally to Desclin (see Desclin 1963), was confirmed first by Everett (1954, 1956), and subsequently by many workers; as evidence of prolactin secretion these investigators have relied on signs of luteotrophic activity (Everett 1954, 1956; Desclin 1956; Alloiteau 1958; Nikitowitch-Winer & Everett 1958*a*; Mühlbock & Boot 1959; Sanders & Rennels 1959; Quilligan & Rothchild 1960; Meunier, Bousquet & Mayer 1961; Browning & White 1963; Dao & Gawlak 1963; Montemurro & Gardner 1963; Wolthuis & de Jongh 1963; Zeilmaker 1963), or on the demonstration of the mammotrophic properties (Desclin 1956; Mühlbock & Boot 1959; Ahrén 1961; Dao & Gawlak 1963; Montemurro & Gardner 1963) or lactogenic properties (Meunier, Bousquet & Mayer 1961) of the grafted pituitary. In addition, several workers have demonstrated the secretion *in vitro* of prolactin, detected by its effects on the pigeon crop, by the pituitary of rat (Meites, Kahn & Nicoll 1961; Nicoll & Meites 1963; Pasteels 1963), guinea-pig, rabbit, mouse and monkey (Nicoll & Meites 1962) and man (Pasteels 1963). Thus the ability to secrete prolactin, defined in the four possible ways, is a highly characteristic feature of the mammalian pituitary when separated from the hypothalamus. There are opposing reports for the guinea pig pituitary *in vivo* (Aron & Marescaux 1962; Russell 1962), though this gland *in vitro* behaves like the rat pituitary (Nicoll & Meites 1962). The possibility that the grafted pituitary actually secretes prolactin at a higher rate than normal has frequently been raised, and was recently amply confirmed by Wolthuis & de Jongh (1963) and Zeilmaker (1963). Thus the proposition that the hypothalamus in mammals actually *inhibits* the secretion of prolactin is supported by the behaviour of the transplanted pituitary, as well as by evidence from other sources, notably from experiments involving hypothalamic lesions, and from *in vitro* studies which have shown that hypothalamic tissue or extract will inhibit the abundant secretion of prolactin by the mammalian pituitary in tissue culture (Pasteels 1963; Talwalker, Ratner & Meites 1963).

The only information about the hypothalamic control of prolactin secretion in birds is that the pigeon pituitary *in vitro* secretes little or no prolactin, which raises the possibility

of a fundamental difference between birds and mammals in this respect (Nicoll & Meites 1962).*

For amphibians, Masur (1962) finds that pituitary grafts in the red eft (*Diemyctylus viridescens*) will restore the water-drive after hypophysectomy. There is good evidence that the water-drive in this newt is induced by prolactin (Grant & Grant 1958), so that Masur's work constitutes evidence for the secretion of prolactin by pituitary grafts in an amphibian.

The reasons for considering that the 'freshwater factor' in *Poecilia* is prolactin have been given earlier. Accepting this identity, it is apparent that the secretion of abundant prolactin by the grafted pituitary in our experiments is in keeping with the behaviour of the mammalian and amphibian pituitary. In the dilute seawater used in our work, the putative prolactin cells in the normal pituitary (Olivereau & Ball 1964) have mainly a rather inactive appearance, suggestive of a low persistent rate of prolactin secretion. In the intact *P. latipinna*, there is a marked increase in the activity of these cells within 24 h of entering freshwater. This increased activity coincides with the return of the initially depressed plasma sodium concentrations to normal values (Ball 1963). Obviously, the grafted pituitary is able to secrete prolactin in amounts sufficient to guarantee survival in freshwater, and it remains to be determined whether the prolactin cells in the graft are able to respond directly with increased activity to entry into freshwater (dilution of plasma sodium?), or whether, as in mammals, these cells in the graft maintain a constantly higher-than-normal output of prolactin.

Growth hormone (GH)

Hypothalamic control of the secretion of *GH* has been relatively neglected in comparison with the attention given to control of the other pituitary hormones. Maintenance of growth was one of the effects of *multiple* pituitary grafts in hypophysectomized rats; in reporting this finding, Martinowitch *et al.* (1963) have drawn attention to the highly variable behaviour of pituitary grafts in different sites in the rat, and to other sources of the variable results that are found in the literature. One of the most important variables is clearly the absolute amount of pituitary tissue that is grafted. Martinowitch *et al.* (1963), and earlier Martinowitch (1954), have used multiple grafts and have thereby obtained evidence of considerable function in the ectopic pituitary, including the secretion of *GH*. Again, Hertz (1959, 1960) reported the secretion of moderate levels of *GH* by four pituitary grafts per recipient in the rat. The experiments directly comparable to our own, however, are those in which the capacities of the *single* grafted gland have been assessed, and the evidence from these experiments indicates little or no autonomous capacity for secretion of *GH* in the mammalian pituitary (Harris 1955, for the earlier work). In experiments in which single pituitaries of foetal or neonatal rats were transplanted into hypophysectomized recipients, the transplants apparently released small amounts of *GH* (Goldberg & Knobil 1957) though Knigge (1961) in similar experiments could detect no

* [*Footnote added in proof* 19 *January* 1965.] Section of the hypophysial portal vessels in the duck caused severe atropy of the prolactin cells in the pars distalis, an observation that 'plaide contre l'existence d'un centre hypothalamique inhibiteur de la prolactinogenèse hypophysaire'. (Assenmacher & Tixier-Vidal 1964).

such activity. The adult mammalian pituitary transplanted in this way has been reported to retain very little growth-promoting potency (Fortier 1951; Martini *et al.* 1959; Sanders & Rennels 1959; Knigge 1961; Khazin & Reichlin 1961; Ahrén *et al.* 1962; Foster & Rothchild 1962; Dao & Gawlak 1963). Everett (1956) found that pituitary grafts could maintain body weight in hypophysectomized rats, but apart from the fact that body weight alone is not an ideal index of new growth, there is some possibility that this could have been an effect of the abundant secretion of prolactin by these grafts, since sheep prolactin has growth-maintaining properties (Reisfield *et al.* 1961; Cargill *et al.* 1963).

In work that hinged on the synergic role of *GH* in the response of the rat mammary gland to gonadal hormones, Ahrén (1961, 1962) and Ahrén *et al.* (1962) concluded that the ectopic pituitary could secrete very little *GH*. When the pituitary is removed and grafted in the 'hypophysiotrophic' area of the rat hypothalamus, *GH* output is greater than from grafts in other ectopic positions, though still less than from the *in situ* pituitary (Halász, Pupp, Uhlarik & Tima 1963). There is also evidence that hypothalamic lesions can interfere with the output of *GH* by the intact pituitary (Spirtos, Ingram, Bogdanove & Halmi 1954; Reichlin 1960; Szentágothai, Flerkó, Mess & Halász 1962), and a *GH*-releasing factor has been demonstrated in extracts of the hypothalamus of pig (Franz, Haselbach & Libert 1962) and rat (Deuben & Meites 1964), so that a considerable degree of dependence of *GH* secretion in mammals on the hypothalamus is indicated. The rat pituitary *in vitro* has, however, been reported to secrete *GH*, detected by the tibia test (Meites, Hopkins & Deuben 1962), but since prolactin can give a positive result in the tibia test (Cargill Thompson & Crean 1963), it is possible that the rat pituitary *in vitro* was actually secreting little *GH* but abundant prolactin.*

Retention of growth-promoting potency by the ectopic pituitary has been found in amphibians (Pasteels 1960; Pehlemann 1962). Etkin & Lehrer (1960) reported that hypophysectomized tadpoles with pituitary transplants grew even faster than intact controls, an effect, however, that Etkin (1963) has since ascribed to prolactin rather than to *GH* itself.† Guardabassi (1961) showed that growth promoting potency was largely retained by the larval pituitary in *Xenopus* after removal of the hypothalamus, and hypothalamectomized *Alytes* tadpoles grew to giant size (Bounhiol & Remy 1962), while lesions of the median eminence had no effect on the growth of newts (Mazzi 1958).

Thus, most of the evidence indicates a drastic reduction of complete loss of somatotrophic potency by the mammalian pituitary when removed from its hypothalamic connexions, but the retention of higher levels of potency in the transplanted or isolated amphibian gland. Since our results indicate plentiful secretion of *TSH* by the grafts, which would presumably permit maximal expression of growth, it appears that the pituitary of *Poecilia* resembles the mammalian gland in secreting *GH* only in very reduced amounts after transplantation.

* [*Footnotes added in proof* 19 *January* 1964.] More recently, evidence has been published that indicates higher levels of somatotrophic potency in rat pituitary transplants than would appear from this survey (Kastin & Gittes 1964; Meites & Kragt 1964).

† Ovine prolactin strongly promoted growth in frog tadpoles (Berman *et al.* 1964); thus in the papers cited, the amphibian pituitary transplants could perhaps have maintained growth by secreting prolactin rather than growth hormone *per se*.

Thyrotrophic hormone (TSH)

The evidence from the present work, it will be seen, suggests that the hypothalamo–hypophysial relationship in *Poecilia* may be essentially the same as in mammals with respect to the control of secretion of gonadotrophin, prolactin and *GH*. In contrast, the indications are that with further work the hypothalamic control of *TSH* and *ACTH* in this teleost may prove to be rather different from the mammalian pattern.

The greater part of the mammalian literature demonstrates a severe reduction in the secretion of *TSH* when the pituitary is severed from the hypothalamus (Harris & Jacobsohn 1952; Harris 1955; Courrier & Colonge 1957; Sanders & Rennels 1959; Hertz 1959; Harris 1962), though many workers have emphasized the persistence of a considerable residuum of activity (Schweizer & Long 1950; Hertz 1959; Goldberg & Knobil 1957; Rebel & Marescaux 1958; Martini *et al.* 1959; Petrovic & Lavillaureix 1959; Florsheim & Knigge 1960; Blanquet *et al.* 1961; Meunier, Blanquet & Boussagol 1961; Khazin & Reichlin 1961). In mice (Greer *et al.* 1953) and rabbits (Brown-Grant, Harris & Reichlin 1957), the pituitary when isolated from the hypothalamus was estimated to maintain thyroidal radio-iodine uptake at about two-thirds the normal level, and an even higher rate was later demonstrated in mice (Scow & Greer 1955) and rats (Martini *et al.* 1959). Despite this, the grafts failed to maintain thyroid weight in mice (Greer *et al.* 1953; Scow & Greer 1955), though were partially effective in this respect in rats (Martini *et al.* 1959). Reduced but considerable secretion of *TSH* by the ectopic pituitary was also demonstrated for rabbits by Euler & Holmgren (1956*a*) and for rats by Knigge (1961).

Ma (1963) found that ectopic pituitary autotransplants in the cockerel maintained nearly normal levels of *TSH* secretion, judged by thyroid weight and radio-iodine uptake. Similarly, stalk-section in hens did not modify thyroid histology and weight (Shirley & Nalbandov 1956), though hypothalamic lesions may reduce thyroid activity (Egge & Chiasson 1963). However, the complexity of the hypothalamus–pituitary–thyroid relationship in birds is illustrated by the results of section of the pituitary portal vessels in the duck. Though this operation reduced the rate of secretion of thyroid hormone into the blood stream, thyroid histology was unaffected, and radio-iodine uptake and rate of hormone synthesis remained within the normal range, despite atrophy of the *TSH* cells in the pituitary. Hypophysectomy, in contrast, greatly reduced the uptake of ^{131}I. Obviously, the duck pituitary, like that of the cockerel (Ma 1963) retains considerable *TSH*-potency when separated from the hypothalamus (Assenmacher & Tixier-Vidal 1959, 1963).

In amphibians, normal or near-normal rates of secretion of *TSH* may continue from the ectopic pituitary (Jørgensen & Larsen 1963; Etkin 1963; Schotté & Tallon 1960), though there are reports of a moderate (Rosenkilde 1963; Pehlemann 1962), severe (Vivien 1959) or total (Pasteels 1957, 1960) loss of thyrotrophic activity in such pituitary grafts. Hypothalamectomy did not reduce the output of *TSH* in larvae of *Xenopus* (Guardabassi 1961) and *Rana* (Chang 1957), and median eminence lesions had only a slight depressant effect in *Triturus* (Mazzi & Peyrot 1960). However, in the hypothalamectomized *Rana* tadpoles, as in stalk-sectioned *Ambystoma* larvae (Etkin & Sussman 1961), the isolated pituitary was unable to secrete the extra amounts of *TSH* needed for the climax of metamorphosis.

This last finding has been confirmed by other workers (Bounhiol & Rémy 1962; Rémy 1962; Voitkevitch 1962; Disclos 1963).

The relation between the mammalian hypothalamus and the *TSH* cells of the pituitary appears to be primarily one of stimulation (though Euler & Holmgren (1956*b*) postulated two co-existent hypothalmic mechanisms controlling *TSH* secretion, one stimulatory, the other inhibitory), and in some confirmation a *TSH*-releasing factor has recently been demonstrated and isolated from the mammalian hypothalamus (Guillemin, Yamazaki, Gard, Jutisz & Sakiz 1963). The feedback response of the mammalian thyrotrophs to circulating thyroid hormone is mediated primarily at the pituitary level (Scow & Greer 1955; Euler & Holmgren 1956*a*; Brown-Grant *et al.* 1957; D'Angelo 1958; Knigge 1961; Meunier, Croizet, Blanquet & Meyniel 1963), hypothalamic stimulation being superimposed on this balance. A similar situation may exist in birds (Ma 1963), though against this, division of the pituitary portal vessels in the duck completely abolished the goitrous response to propyl-thiouracil (Assenmacher & Tixier-Vidal 1959). The relationship between the hypothalamus and *TSH* secretion in amphibians is not at present clear. Some of the work mentioned above indicates a stimulatory relationship, as in mammals, and such a mechanism certainly seems to operate during the metamorphic climax (Etkin 1963). The thyroid–*TSH* feedback in amphibians probably operates to a great extent at the level of the pituitary cells, as in mammals, though some involvement of the hypothalamus in this balance has been indicated (Mazzi & Peyrot 1960). However, on the basis of the failure of the ectopic pituitary to respond to injections of thyroxine in *Ambystoma* (unlike the rat ectopic gland: Meunier *et al.* 1963), Jørgensen & Larsen (1963) were driven to postulate 'a hypothalamic factor inhibiting the release of thyrotrophin from the pars distalis', though the situation in *Ambystoma* seems to be complicated by the fact that the ectopic pituitary in the absence of thyroxine appeared to secrete *TSH* at about the same rate as the normal gland, and not at a clearly enhanced rate, which suggests that the action of the injected thyroxine was on the hypothalamus rather than directly on the pituitary.

It is apparent that the case of *Poecilia* is exceptional, and we have postulated an inhibitory hypothalamic influence on *TSH* secretion, to account for the output of *TSH* by the grafted pituitary at higher levels than normal in a system in which normally *TSH* and thyroid hormone are in negative feedback balance (Ball *et al.* 1963).

Adrenocorticotrophin (*ACTH*)

The *ACTH*-potency of the ectopic pituitary in mammals has been investigated recently by several groups of workers. Earlier workers had claimed that ectopic transplantation of the pituitary results in atrophy of the adrenal cortex, but that the ability of the pituitary tissue to respond to stress with *ACTH*-secretion is nevertheless retained (see Ganong 1959; Foster & Rothchild 1962). Many investigators, however, have reported better adrenal weight or structure in animals bearing transplanted pituitaries than in hypophysectomized controls without transplants (Schweizer & Long 1950; Fortier 1951; Harris & Jacobsohn 1952; Golberg & Knobil 1957; Hertz 1959; Martini *et al.* 1959; Khazin & Reichlin 1961; Yoshida & Sayers 1961; Martinowitch, Bacq, Pavitch & Simitch-Sladitch 1961; Blanquet *et al.* 1961; Foster & Rothchild 1962). This may indicate a low persistent rate of autonomous

ACTH-secretion, although there is some possibility that adrenal weight could be maintained by low levels of *GH*, or perhaps by prolactin (Lanman & Dinerstein 1960; see too, Foster & Rothchild 1962), and that adrenal weight alone is not an adequate criterion of *ACTH*-secretion (Kovács, David & László 1962; Critchlow *et al.* 1963). There is also the possibility that this apparently residual output of *ACTH* could be not truly autonomous, but a response to hypothalamic *ACTH*-releasing factor in the systemic circulation (Brodish & Long 1962). This idea was put forward by Critchlow *et al.* (1963), who found in their own experiments that pituitary grafts in the rat did not maintain a persistent liminal secretion of *ACTH* (using corticosteroid secretion as a criterion), but that, nevertheless, such grafts could respond with *ACTH*-synthesis and secretion when chronically or acutely stimulated by purified hypothalamic *ACTH*-releasing factor (*CRF*). The absence of a persistent output of *ACTH* by rat pituitary grafts was also reported by Courrier & Colonge (1957), Aron & Petrovic (1958), Timmer, Sanders & Rennels (1959), Knigge (1961), Dill & Rennels (1962), Dao & Gawlak (1963) and by Greer, Kendall & Duyck (1963), these last workers also reporting that stress did not induce *ACTH*-secretion by the grafts, in agreement with Foster & Rothchild (1962) and Dill & Rennels (1962), but in disagreement with Fortier (1951), Timmer *et al.* (1959) and Martinowitch *et al.* (1961). Obviously there is no complete agreement concerning the state of *ACTH*-function in the ectopic mammalian pituitary, some of the discrepancies no doubt being due to differences of technique, including site of the pituitary grafts and methods of assessing *ACTH*-secretion. The most reasonable interpretation of this confusing field is that ectopic pituitary grafts in mammals retain a store of *ACTH* but do not normally release the hormone in any quantity (Foster & Rothchild 1962), but that *ACTH*-secretion by these grafts can be induced by lowering the levels of circulating corticosteroids (Hertz 1960), by injections of vasopressin or *CRF* (Martini *et al.* 1959; Yoshida & Sayers 1961; Critchlow *et al.* 1963), or under certain conditions by stress-stimulation (Timmer *et al.* 1959; Fortier 1951; Martinowitch *et al.* 1961); and that the *ACTH* content of the grafted pituitary can be raised by Pitressin (Yoshida & Sayers 1961) and *CRF* (Critchlow *et al.* 1963) and reduced by corticosteroids (Yoshida & Sayers 1961). The generalization seems permissible that the mammalian ectopic pituitary secretes little *ACTH*, if any, as a spontaneous persistent function, a conclusion corroborated by the disappearance of *ACTH* output by the rat pituitary gland cultivated *in vitro* (Guillemin & Rosenberg 1955), though the viability of the pituitary *in vitro* is attested by its ample secretion of prolactin.

For birds, we have the observations that adrenal histology and weight in hens remained normal after pituitary stalk-section (Shirley & Nalbandov 1956), and that the adrenals appeared grossly normal after various diencephalic lesions (MacFarland 1959; Egge & Chiasson 1963). More recently, however, Resko, Norton & Nalbandov (1964) have found that the ectopic pituitary in the chicken, as in mammals, is unable to secrete *ACTH*.

Results reminiscent of those of Critchlow *et al.* were obtained for an amphibian by Jørgensen & Larsen (1963), who showed that the ectopic pars distalis of the toad *Bufo bufo* did not release detectable amounts of *ACTH* spontaneously, but could be induced to release large amounts of *ACTH* by systemic injections of various neurohypophysial octopeptides which presumably were effective mimics of the native toad *CRF*. This finding is in opposition to those of Pasteels (1960), in that he showed that *ACTH*-output of ectopic

pituitary tissue in the urodele *Pleurodeles* remained in the normal range even after many months; similar findings were reported for frogs and newts by Vivien (1959). We should, however, note that Jørgensen & Larsen used criteria of corticosteroid secretion (induction of moulting) to detect *ACTH* output by their pituitary grafts, whereas both Pasteels and Vivien judged *ACTH* secretion by the morphological state of the interrenal. It does not seem likely, though, that the divergent results can be attributed to dissociation between two functions of *ACTH*, particularly since Schotté & Tallon (1960) and Mazzi & Peyrot (1962–3), using criteria of corticosteroid secretion in a newt, obtained evidence of *ACTH* secretion by the autotransplanted ectopic pituitary. It seems more probable that different species of amphibians vary in the extent to which their ectopic pituitary can maintain *ACTH* secretion.

It may be permissible to read into these results with amphibians the possibility that *ACTH*-secretion in the lower vertebrates will prove to be in general less completely dependent on hypothalamic stimulation than appears to be the case in mammals, and our results with *Poecilia* would certainly support this interpretation. In addition to the morphological evidence of *ACTH* secretion afforded by the partial preservation of interrenal structure in our grafted fish, our haematological findings point to the maintenance of corticosteroid secretion above hypophysectomy levels by the pituitary grafts; thus two lines of evidence support the proposition that the pituitary of *Poecilia* has greater autonomy in secreting *ACTH* than the mammalian pituitary.

Pigmentation

Finally, we must emphasize that our very limited observations on pigmentation will allow us to conclude little about the functions of the grafts in relation to melanogenesis. The pituitary control of melanogenesis has not yet been determined in *Poecilia*; the present results show that there is no hypersecretion of the hormone(s) concerned with melanogenesis, such as occurs with the ectopic pituitary in amphibians (Etkin 1962; Pehlemann 1962; Jørgensen & Larsen 1963), but they will not permit further discussion.

Conclusions

Considering our findings for *Poecilia* in relation to the evidence from other vertebrates, the conclusion seems permissible that the hypothalamic control of the secretion of prolactin and gonadotrophin is essentially the same in the groups investigated, with perhaps some local variations, such as the paucity of prolactin secretion by the pigeon pituitary *in vitro*, in contrast to the abundant prolactin secretion by ectopic grafts in mammals, a newt, and probably *Poecilia*; and the possibility that the gland in some amphibians has greater autonomy in the secretion of gonadotrophin than the mammalian and avian pituitaries. In both *Poecilia* and the rat, the ectopic pituitary produces very little *GH*, but the amphibian gland appears to secrete large amounts of this factor when separated from the hypothalamus.* In birds and in some amphibians, the pituitary seems to retain relatively more *TSH*-potency than the mammalian gland when removed from the hypothalamus, though normally subject to a stimulatory hypothalamic influence, while in *Ambystoma* and in

* [*Footnote added in proof* 19 *January* 1965.] This could, however, be a result of prolactin secretion; see footnote, p. 90.

Poecilia there is evidence of an inhibitory hypothalamic influence on *TSH* secretion. The secretion of *ACTH* in *Poecilia* and in some amphibians appears to be less completely dependent on the hypothalamus than in the rat.

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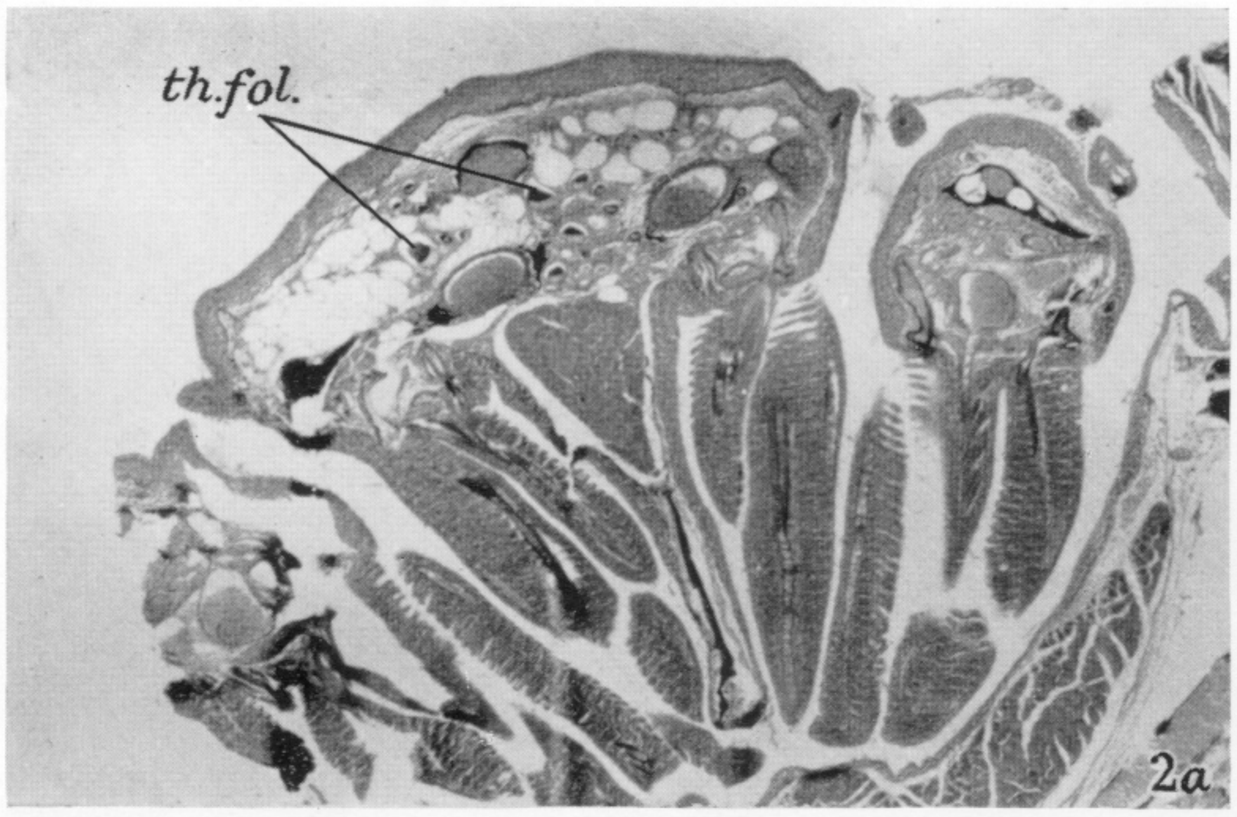
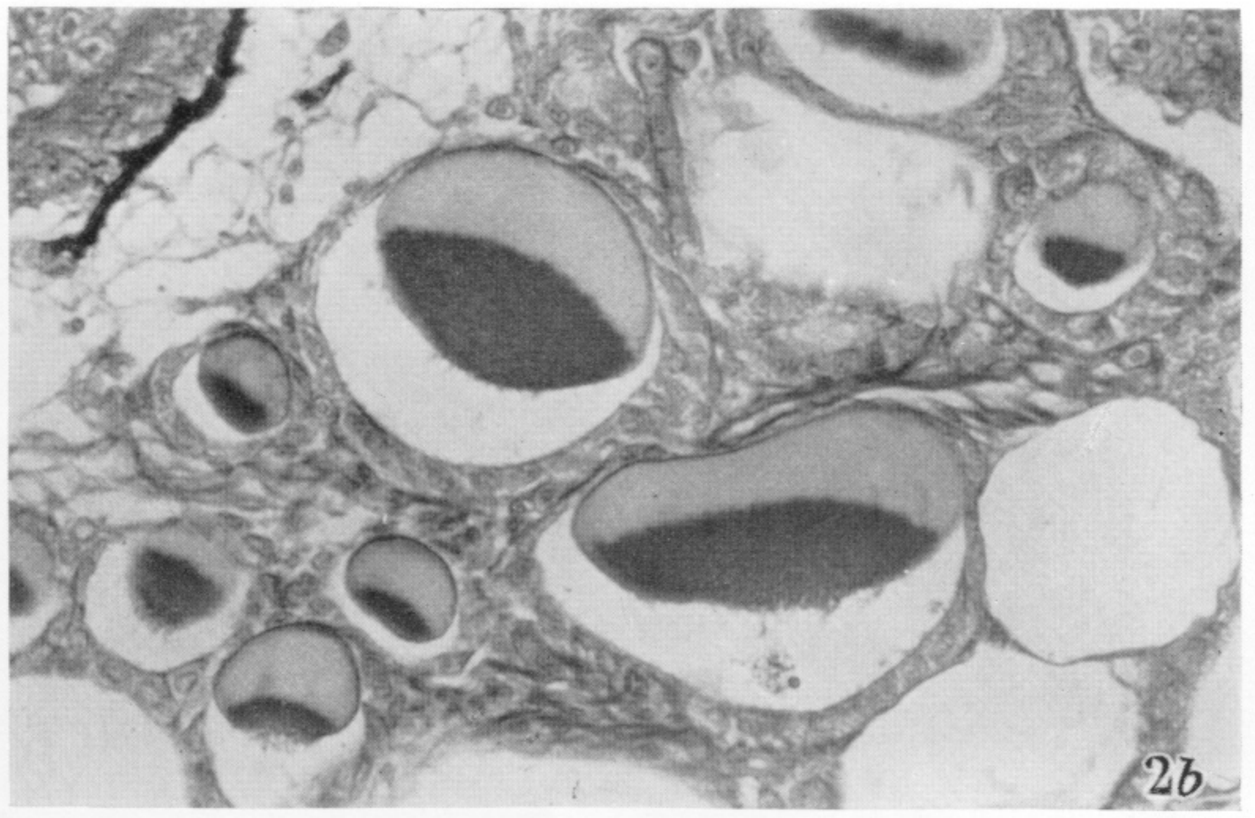
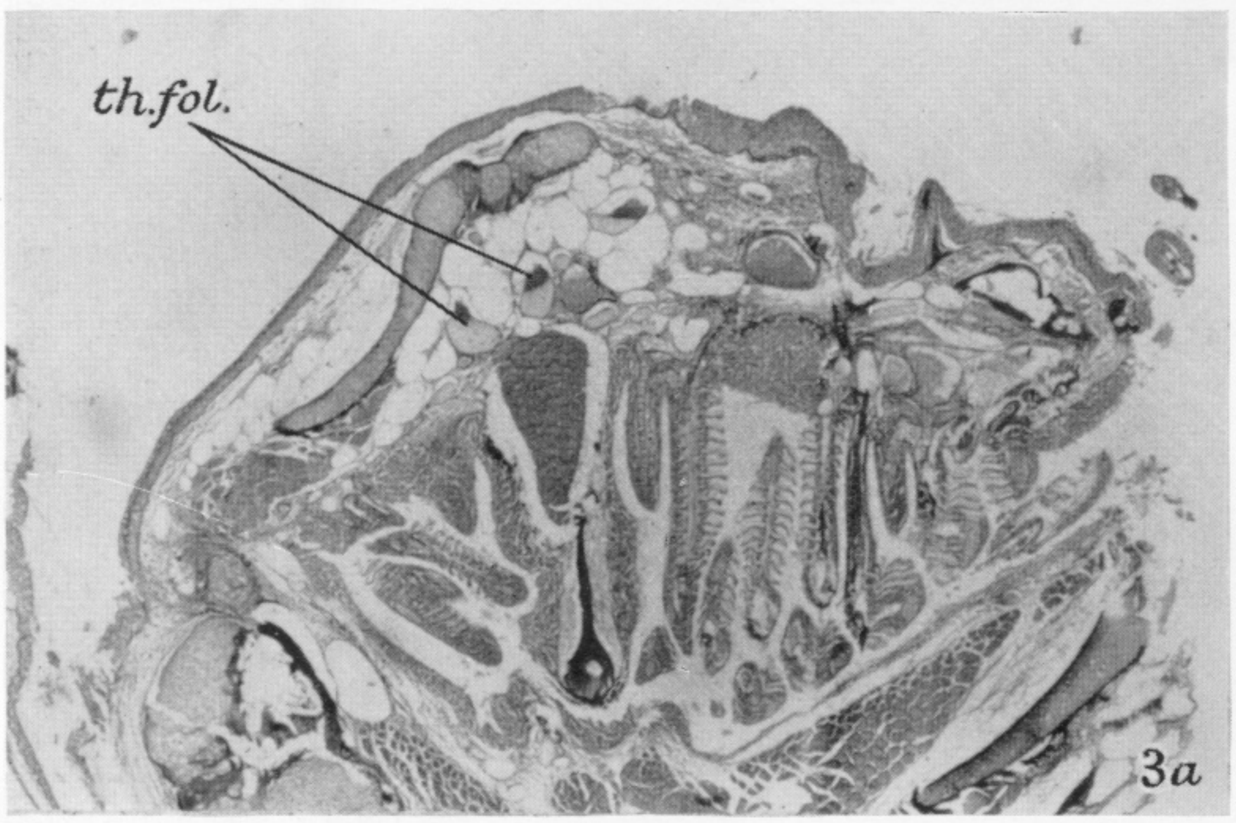
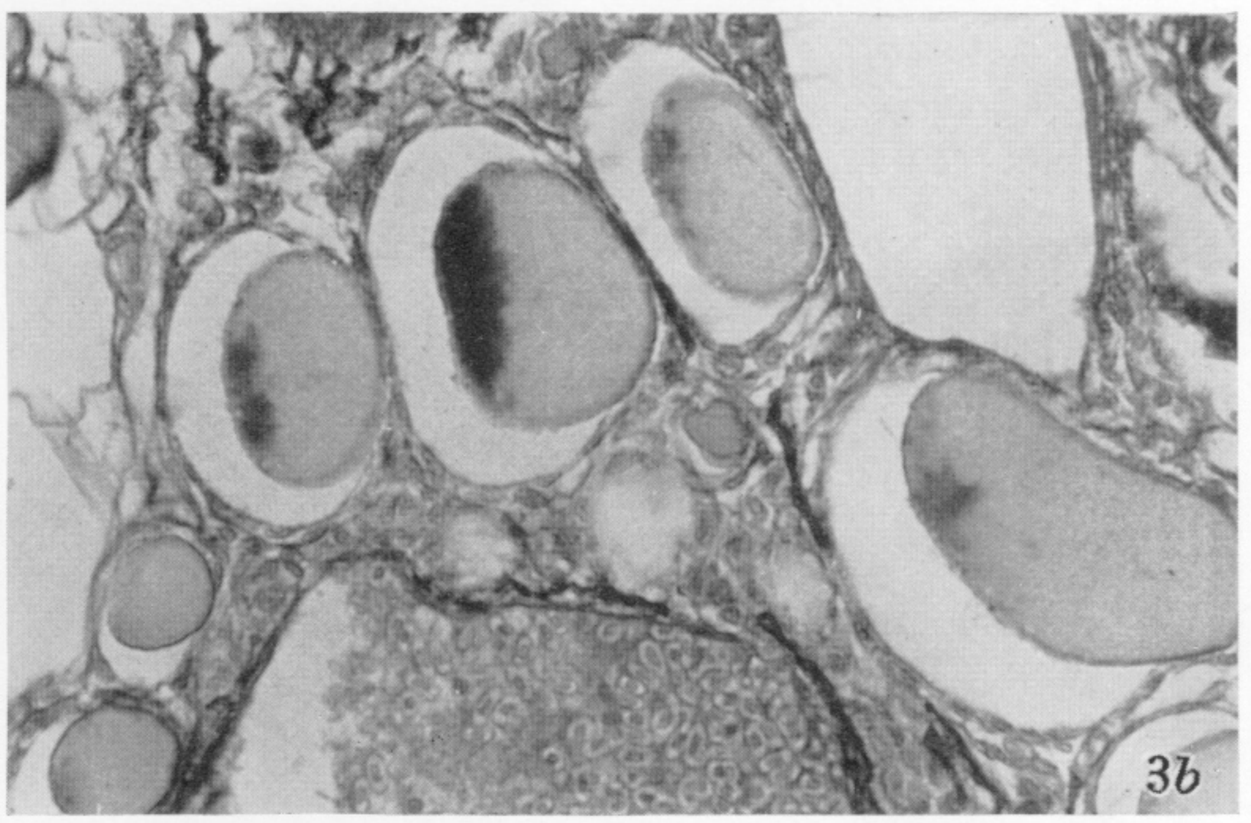
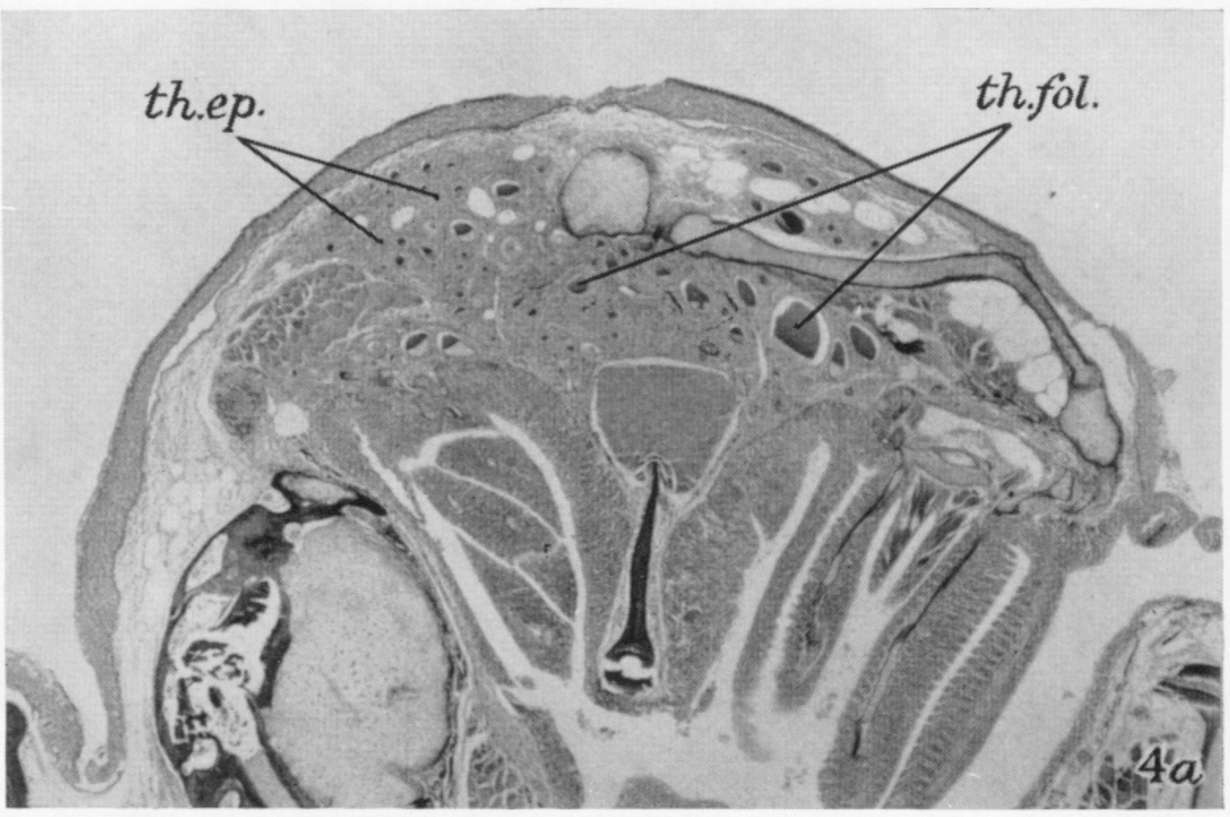










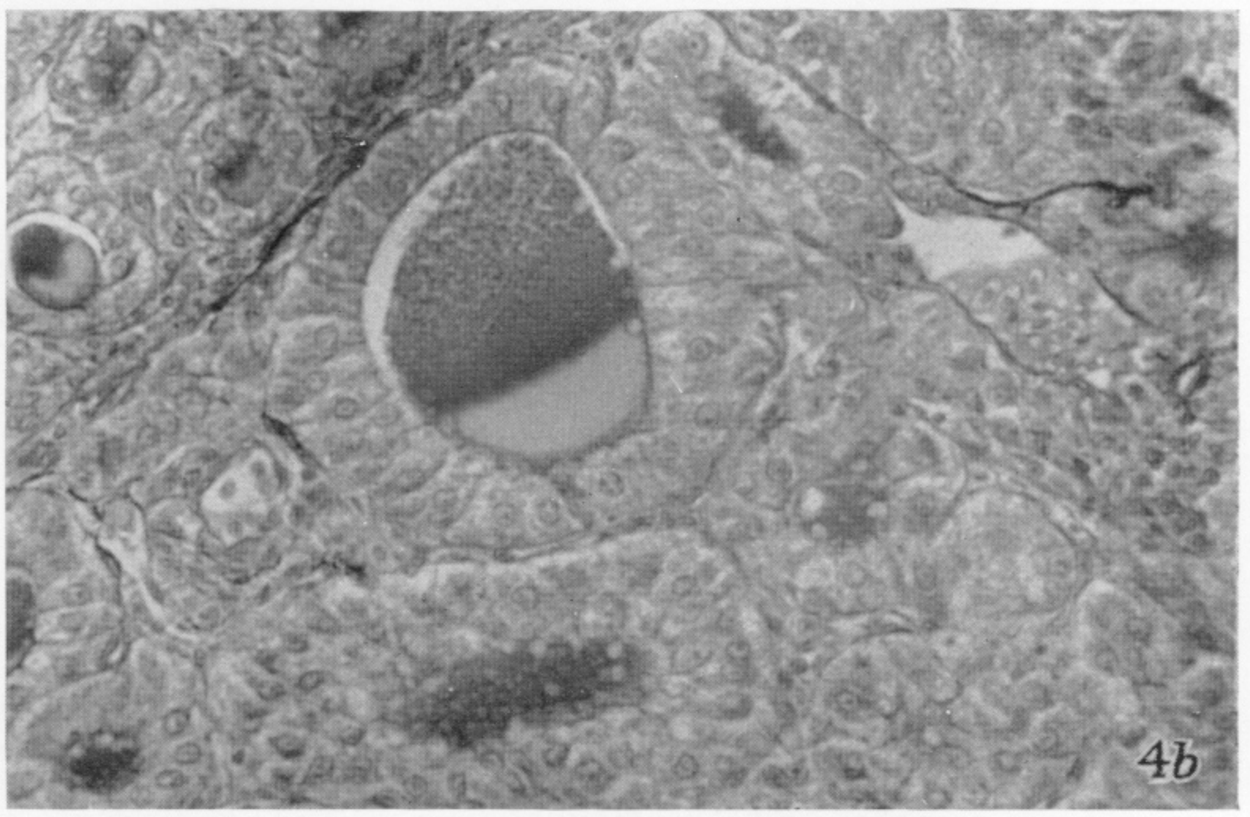


FIGURE 2*a*. Transverse section of the thyroid region of an intact fish. Floor of pharynx above, roots of gill-arches below. Thyroid follicles (*th.foll.*) scattered in loose connective tissue (× 50).

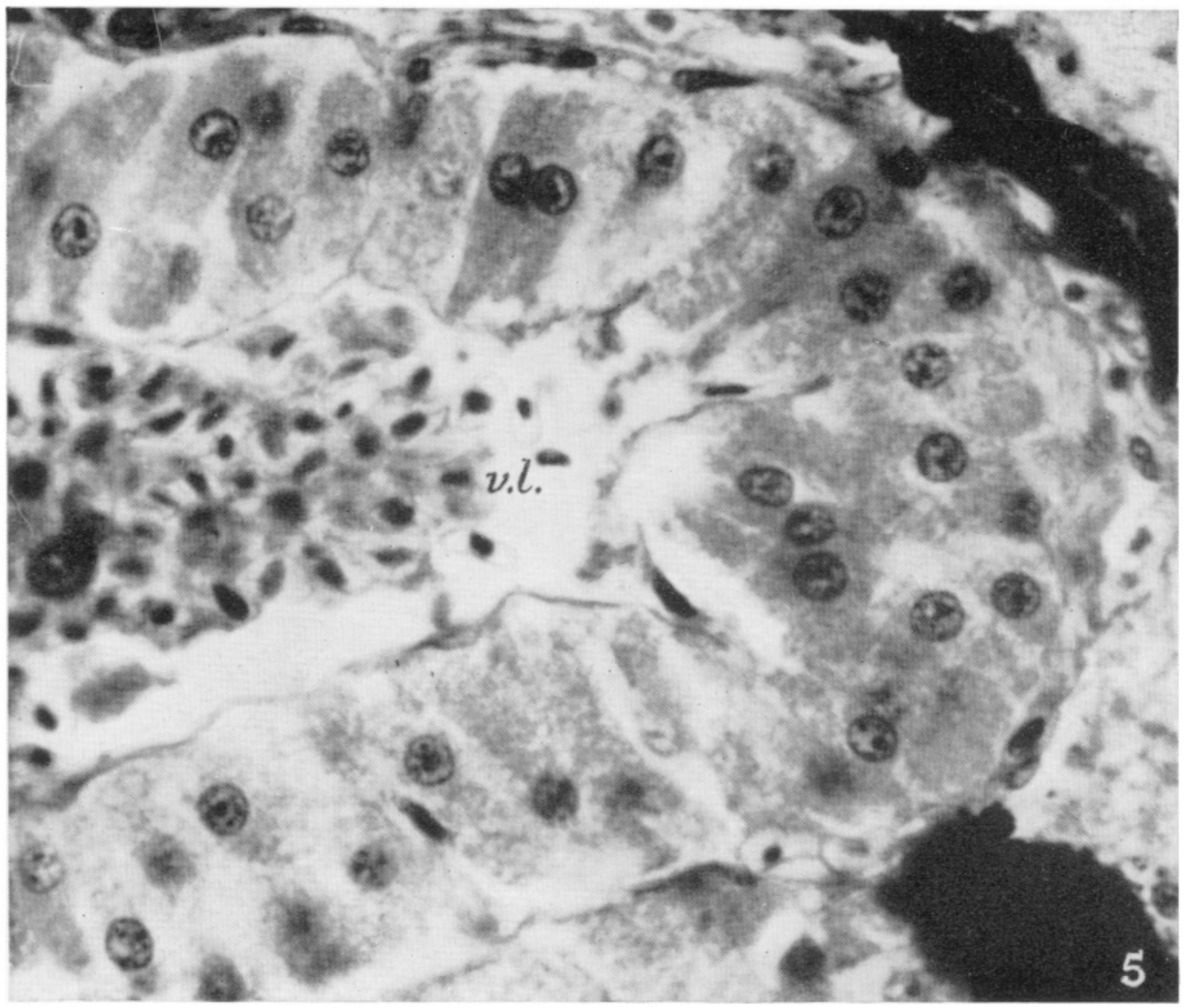
b. Higher magnification of the thyroid shown in figure 2*a*. Cuboidal follicular epithelial cells, partly granulated colloid (× 500).

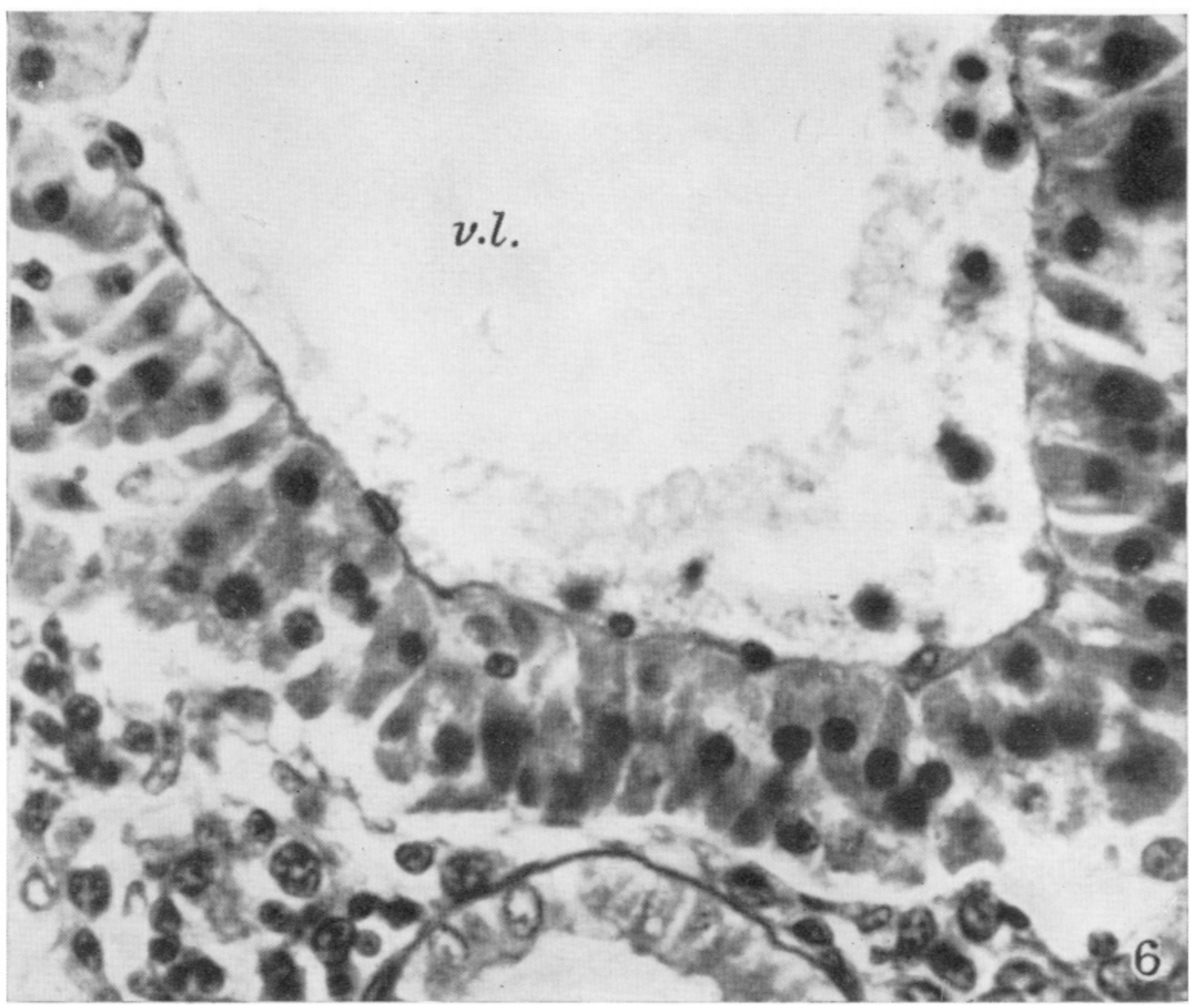
FIGURE 3*a*. Transverse section of the thyroid region of a hypophysectomized fish. Scattered follicles (× 50).

b. Higher magnification of the thyroid shown in figure 3*a*. Squamous follicular epithelial cells, dense non-granulated colloid (× 500).

FIGURE 4*a*. Transverse section of the thyroid region of a grafted-hypophysectomized fish. Marked hyperplasia of thyroid, with sheets of thyroid epithelial cells (*th.ep.*) and follicles almost filling the gular region (× 50).

b. Higher magnification of thyroid shown in figure 4*a*. Columnar thyroid epithelial cells, with rounded nuclei, prominent nucleolus, mainly granulated colloid, numerous resorption vacuoles (× 500).





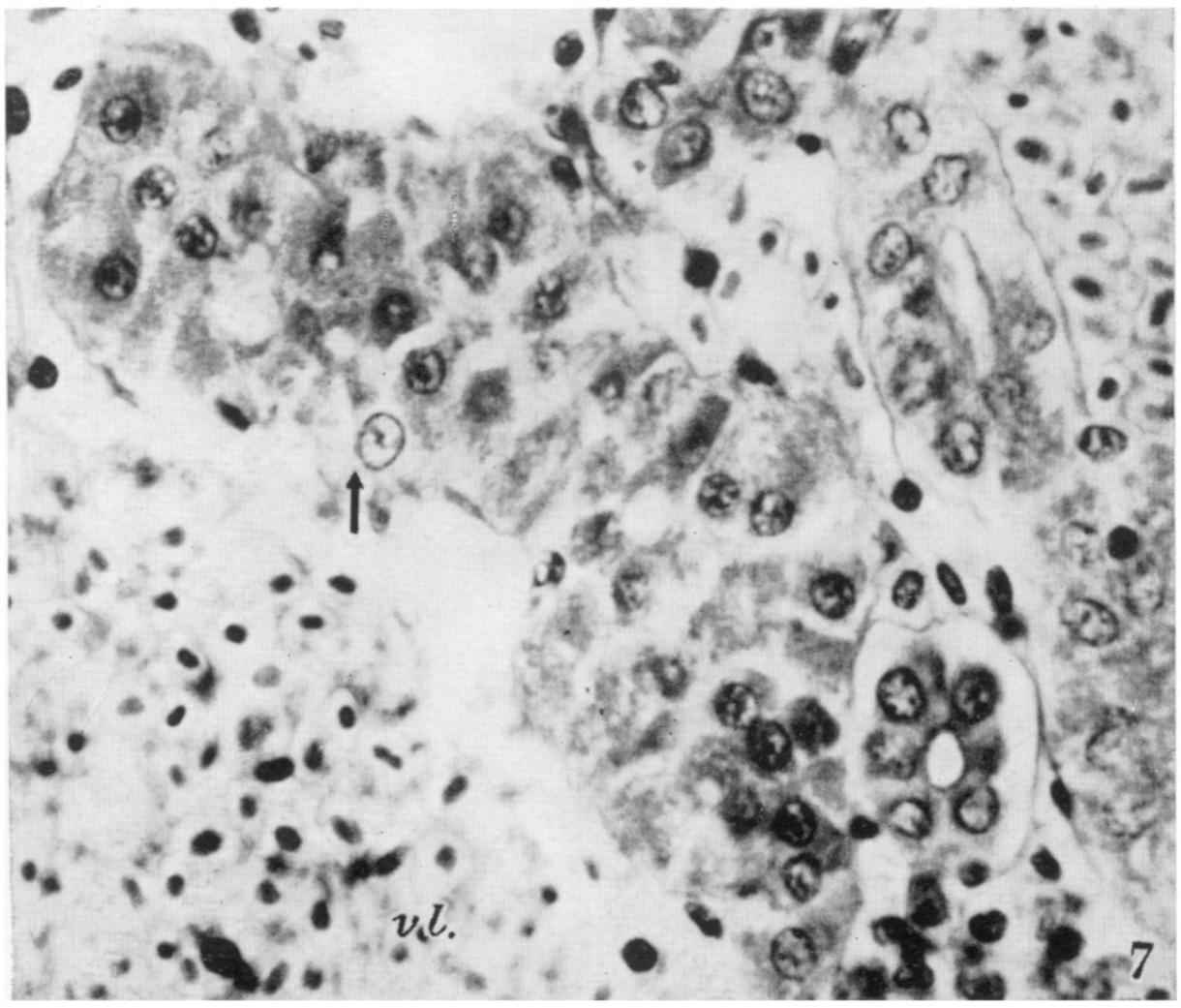
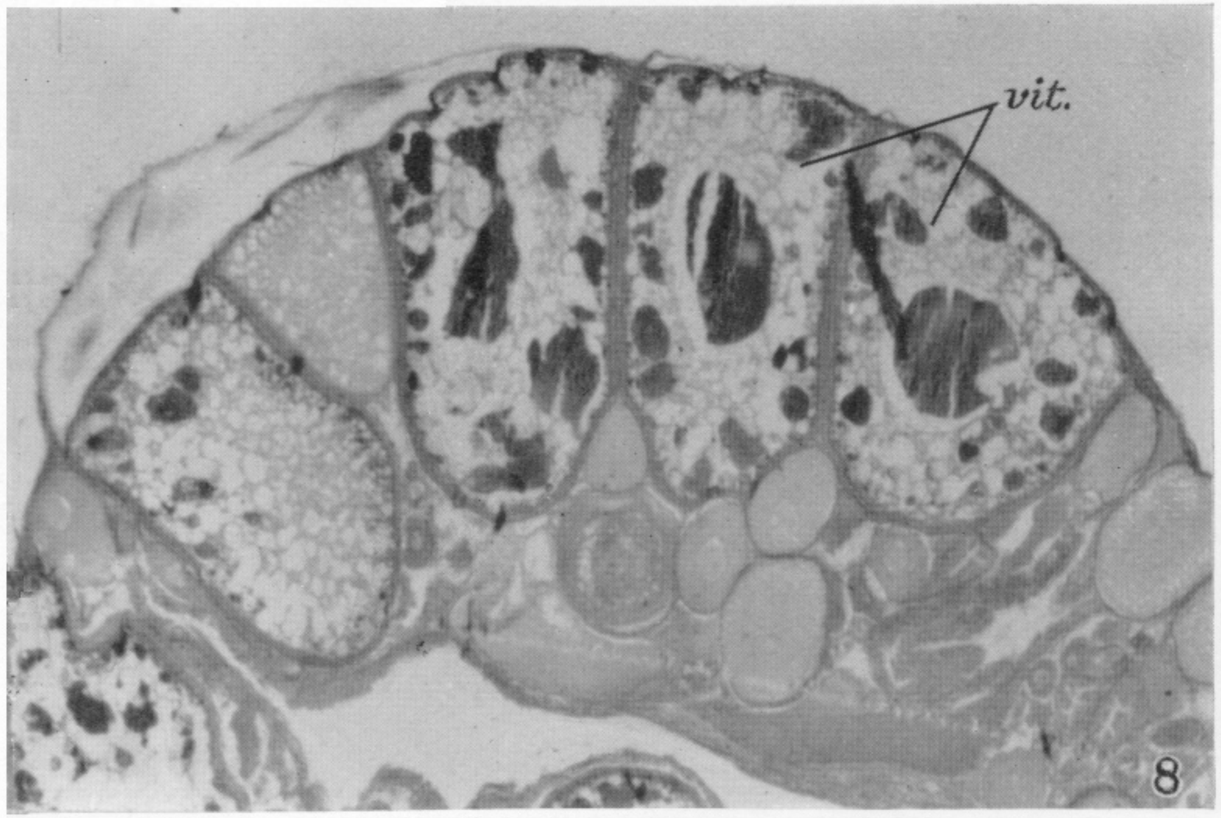
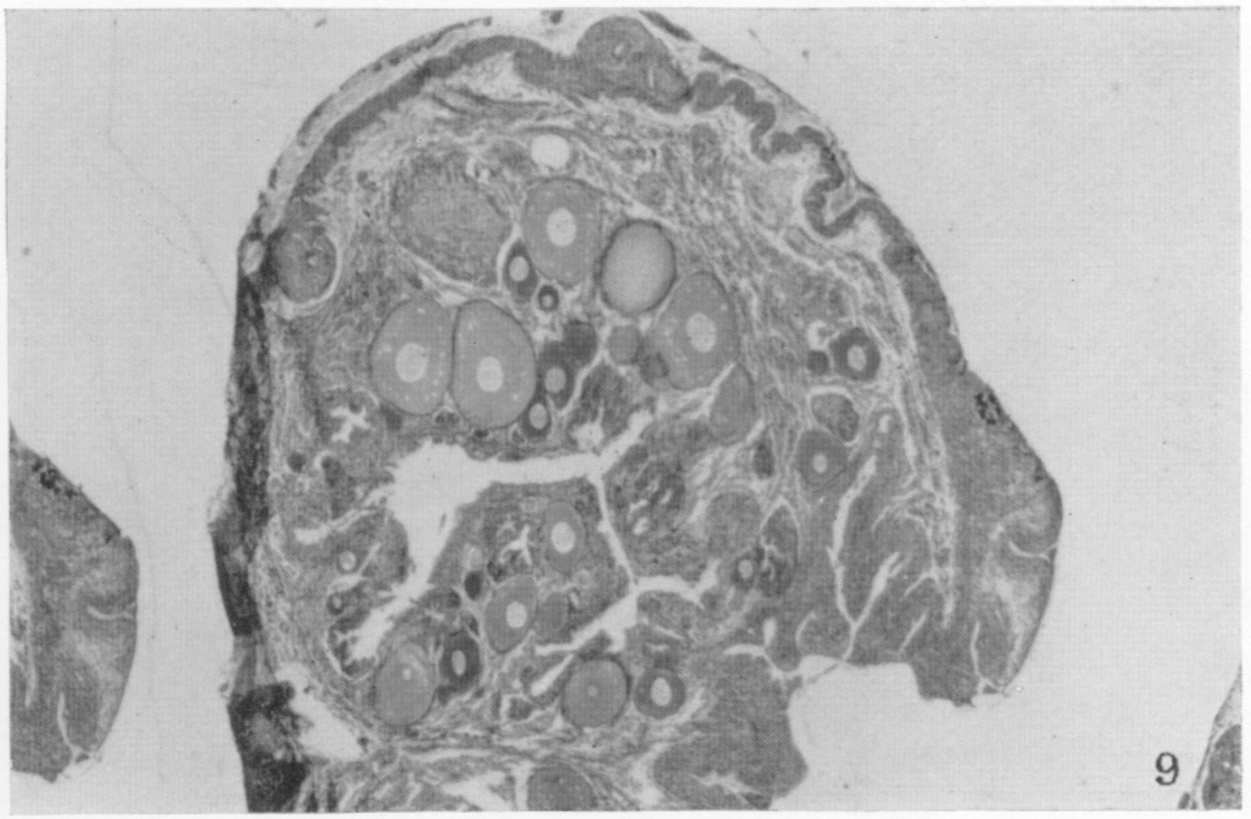


FIGURE 5. Interrenal cells of an intact fish around a vein lumen (*v.l.*). Pale cytoplasm, large round nuclei, prominent nucleoli (× 1250).

FIGURE 6. Interrenal cells of a hypophysectomized fish. Small cells, dark cytoplasm, dense small nuclei (× 1250).

FIGURE 7. Interrenal cells of a grafted-hypophysectomized fish. Morphology intermediate between that in intact fish (figure 5) and in hypophysectomized fish (figure 6). Arrow points to a chromaffin cell (× 1250).





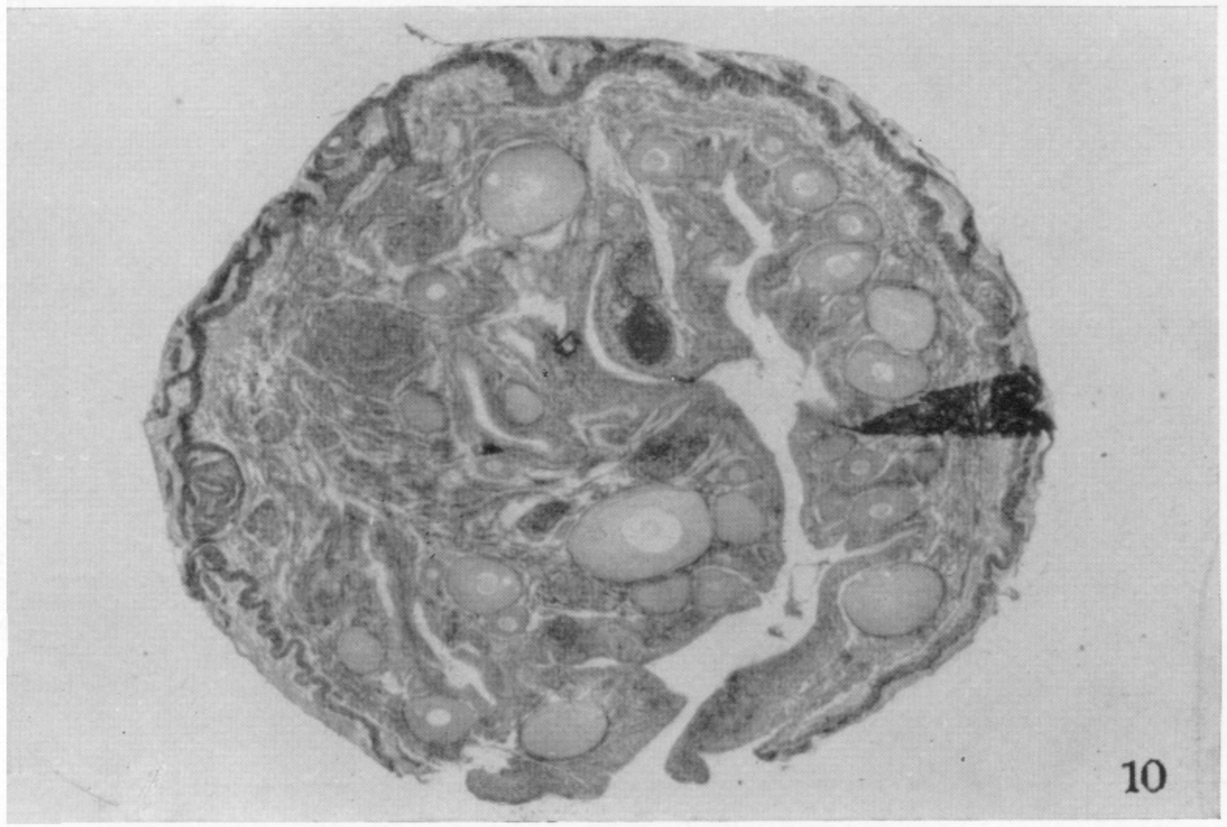
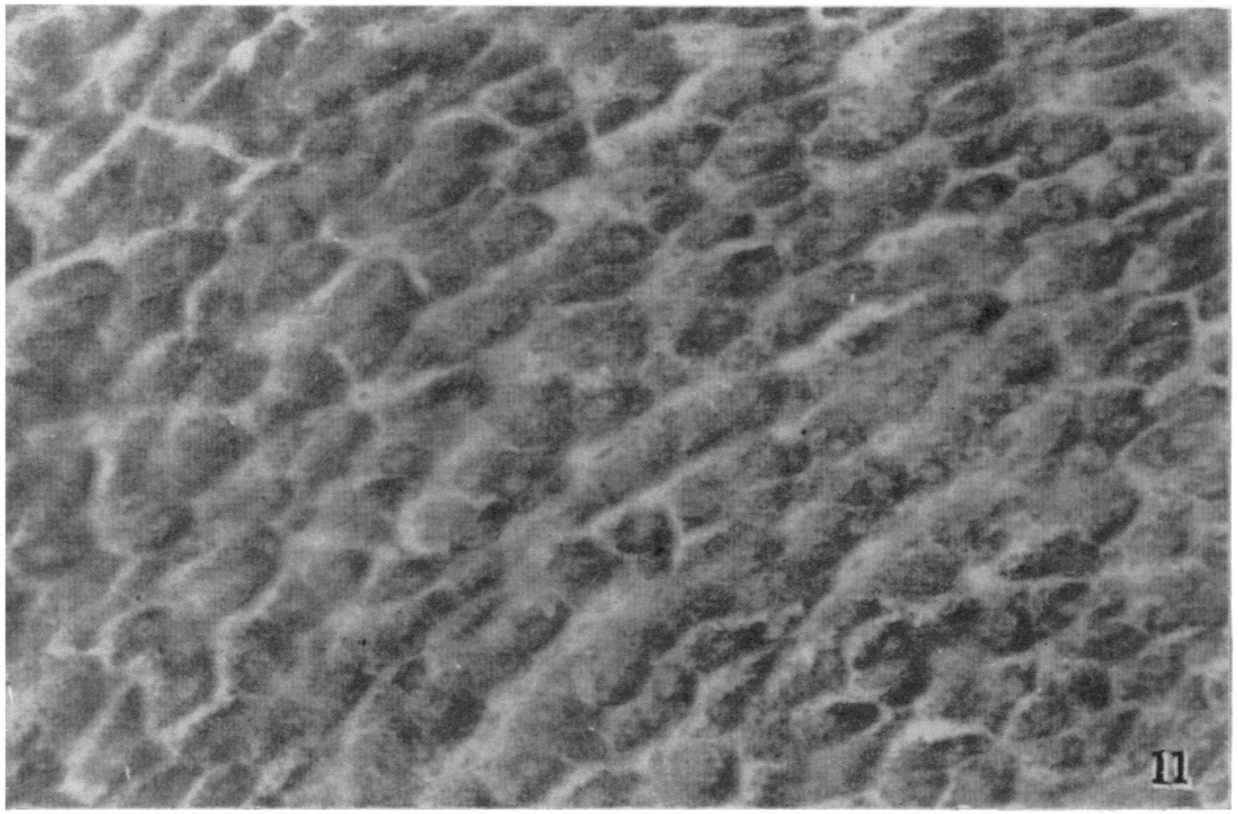
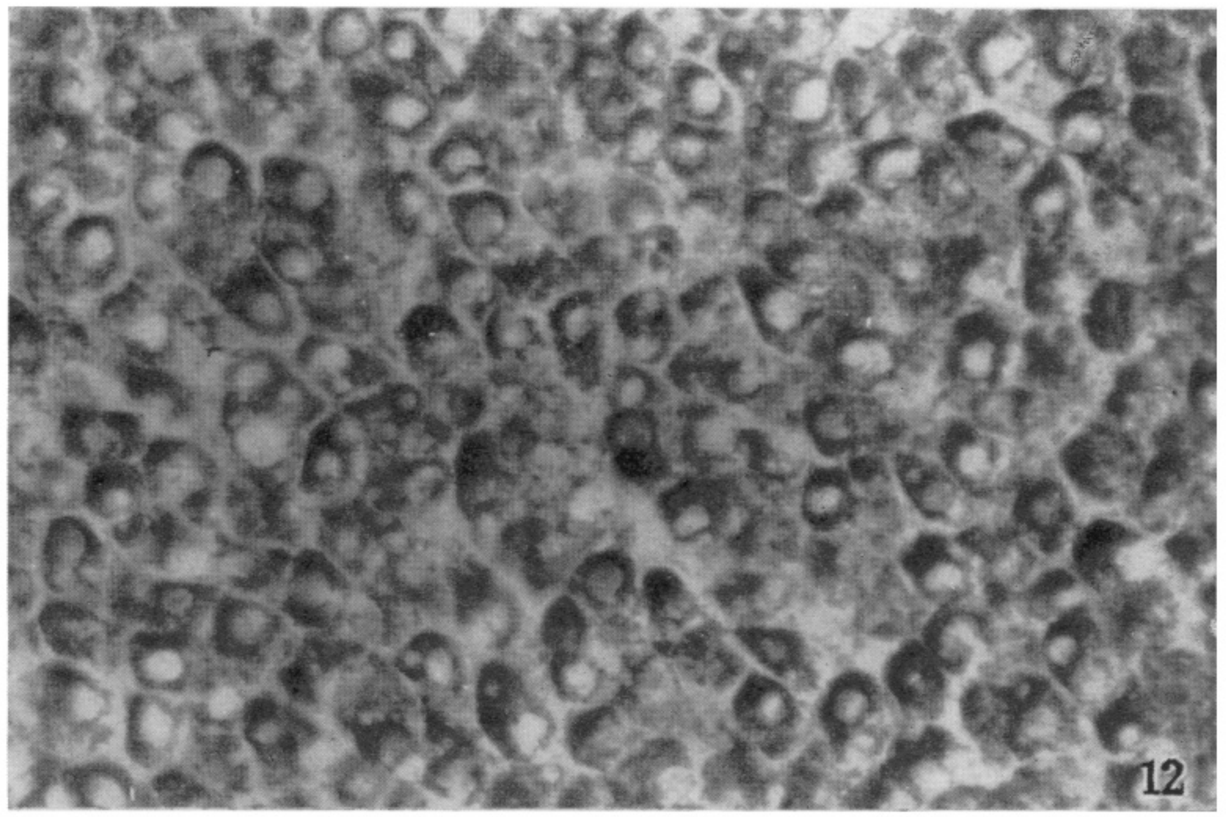


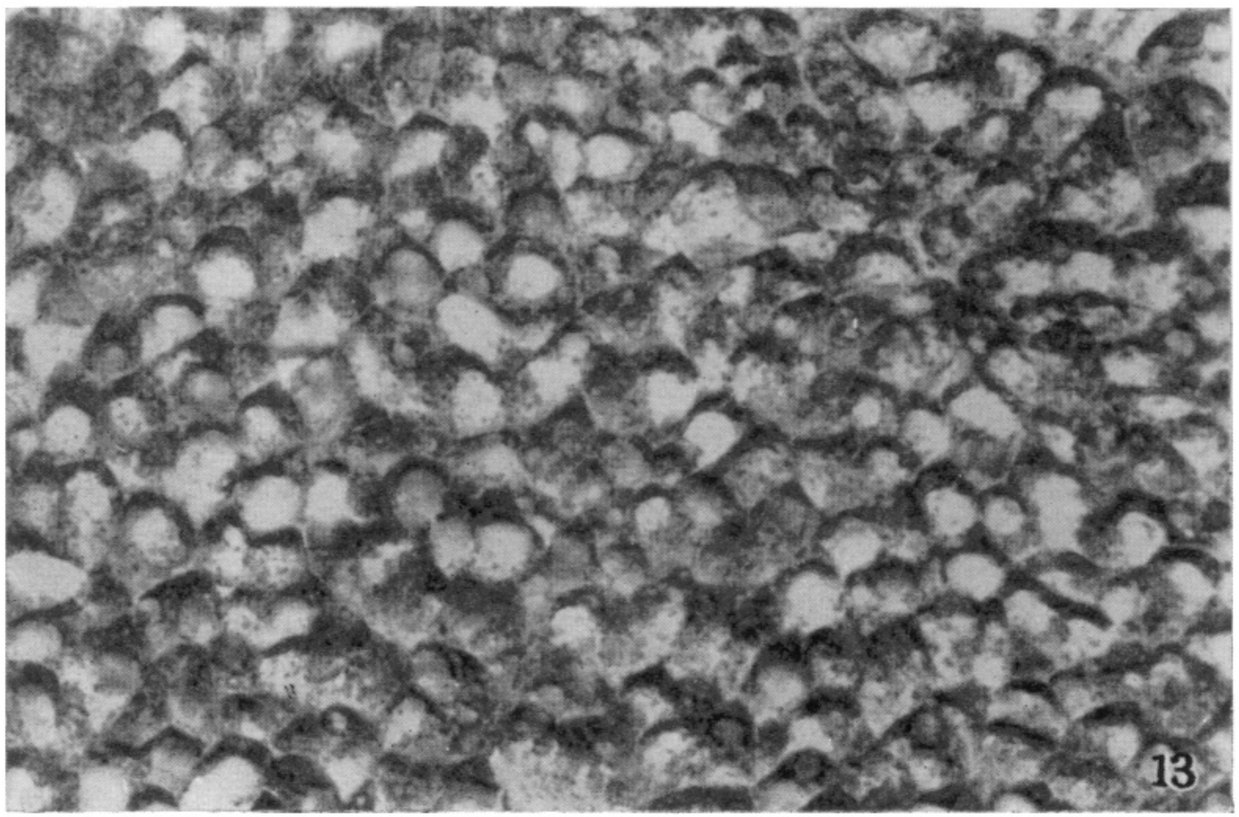
FIGURE 8. Section of the ovary of an intact fish. Large oocytes (*vit.*) in early stages of vitellogenesis (pituitary-dependant), smaller pre-vitellogenic oocytes ($\times 50$).

FIGURE 9. Section of the ovary of a hypophysectomized fish. Absence of vitellogenesis, persistence of healthy pre-vitellogenic oocytes in voluminous connective tissue stroma ($\times 50$).

FIGURE 10. Section of the ovary of a grafted-hypophysectomized fish. Note similarity to the ovary of a hypophysectomized fish (figure 9) ($\times 50$).







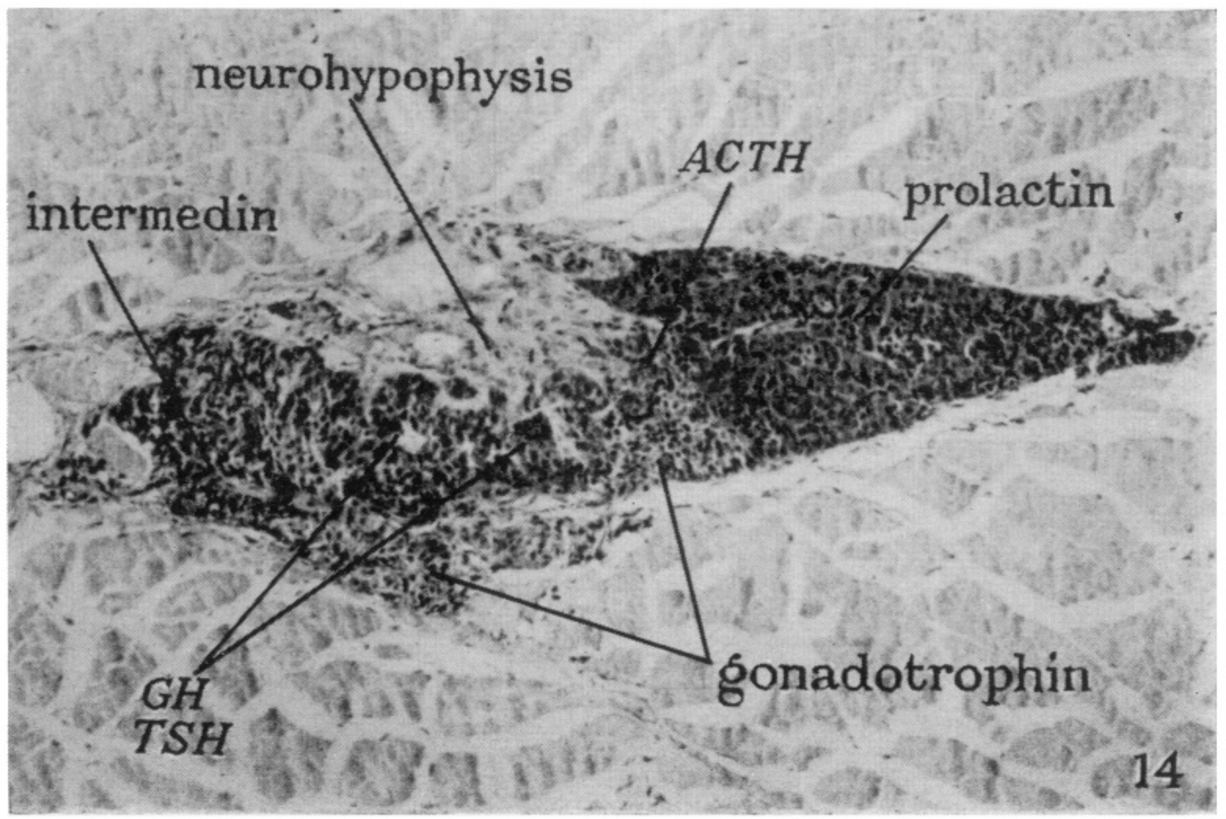


FIGURE 11. Section of the liver of an intact fish. Abundant glycogen (dark), little fat (× 500).

FIGURE 12. Section of the liver of a hypophysectomized fish. Numerous large vacuoles, representing dissolved fat (× 500).

FIGURE 13. Section of the liver of a grafted-hypophysectomized fish. Numerous fat vacuoles (× 500).

FIGURE 14. Longitudinal section of a pituitary transplant *in situ* in caudal musculature, anterior end of gland to the right, to show the location of the various cell-types in the pituitary (see text, pp. 84–85).