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STUDIES ON INTERNAL SECRETION II.—ENDOCRINE ACTIVITY IN FŒTAL AND EMBRYONIC LIFE.

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CONTENTS

1. Introduction.

The present series of experiments was suggested in the first place by an investigation (Crew, 1922-23) into the pathology of the abortive "bulldog" fœtus which occurs in the Dexter breed of cattle. The possibility that endocrine factors are involved in the characteristic deformities of the Dexter monster encouraged the attempt to explore more fully the time at which fœtal ductless glands assume their functional activity, and, if possible, to obtain data correlating the time of physiological activation with the histological appearance presented by the endocrine organs of the fœtus. Recent progress in our recognition of the rôle of internal secretion in the developmental processes of amphibia endorse the problem with a more catholic interest, since it is along such lines that we may hope to achieve a more coherent grasp of the significance of the ductless glands throughout the vertebrate series.

Previous work in this field has been conducted by two American investigators, Fenger (1912) and M'Cord (1915). The former approached the subject from the purely biochemical standpoint. He was able to detect significant quantities of iodine in the thyroid of domestic ruminants from the sixth week onwards, and by Hale and Seidell's iodic acid method was able to demonstrate adrenaline in the suprarenals at an equally early stage. M'Cord, on the other hand, employed physiological tests for post-pituitary and suprarenal extracts of fœtal (ox) glands. He records a positive action on the guinea-pig uterus from the beginning of the third month in the former and the end of the sixth week in the latter case, agreeing with Fenger as regards adrenaline. Neither of these authors give histological data in connection with their observations.

In the present communication attention has been confined to the same three glands, since in the existing state of knowledge the thyroid, post-pituitary, and adrenals are the only organs to which the term "endocrine" can be justifiably prefixed and in which, moreover, there exist established methods for recognising active secretory products. The methods adopted have been physiological, and are different from those employed by M'Cord. In testing for the presence of active secretion in the fœtal thyroid and pituitary, the glands of oxen and sheep were employed. In addition tests have been carried out on the suprarenals of the chick embryo. The procedure adopted in each instance will be detailed in its appropriate place.

The present research was carried out in the Animal Breeding Research Department and the expenses involved defrayed by a grant from the Medical Research Council.

2. The Fœtal Thyroid in Sheep and Oxen.

Since the work of Gudernatsch (1912-14), the specific action of thyroid feeding upon amphibian metamorphosis, at least as an indicator of the iodine content of the gland (Lenhart, Swingle, 1917-19), has been abundantly confirmed and is now universally accepted. In this country the indigenous amphibia are somewhat unsuitable as critical tests for thyroid-

iodine on account of their extremely variable and very short larval life. In the imported European race of axolotls which originated from the neighbourhood of Mexico city we have, on the other hand, an ideal indicator for thyroid secretion. This form, unlike its related variety from Texas and Colorado, never undergoes metamorphosis if kept in deep water in aquaria: it breeds from generation to generation in the aquatic stage. It has been shown repeatedly by Laufberger (1913), Jensen (1917), Kaufmann (1918), Huxley and Hogben (1920) that a single meal of mammalian thyroid suffices to induce the complete transformation of the axolotl larva into a fully developed terrestrial Amblystoma: hence the action of thyroid feeding on the axolotl is an "all or none" response. The presence of a high iodine content in the amphibian as well as the mammalian thyroid, the clear relation of the presence of iodine to metamorphosis shown by Swingle and Jensen's work (1918-21), and the failure of Cameron (1914) to detect significant quantities of iodine in other vertebrate tissues, amply justify the validity of employing the power of a single meal of fœtal thyroid to initiate metamorphosis in the axolotl as a means of demonstrating the fact that the gland is actually building up—we do not say liberating—its active product. This is the method which we have employed in our researches.

The glands were dissected and weighed within six hours from slaughtering. A preliminary experiment showed that a single meal of fresh thyroid from a full time ox fœtus and a young calf in each case sufficed to transform a pair of medium-sized (year old) axolotls weighing 20 to 25 gms. A corresponding quantity (about 0.6 gm.) of tissue from a fœtus aged two months from the time of fecundation produced absolutely no response in visible bodily conformation when administered to a pair of axolotls of the same size; nor was there any diminution in weight—a highly characteristic indicator of the onset of metamorphosis—at the expected period. Experiments were then carried out with glands from fœtal sheep and oxen as they were obtained. Conditions of feeding and temperature (22° C.) were standardised as described in previous papers by one of the authors. The following particulars, giving the weight and age from fecundation of

the fœtus, total weight of thyroid and weight actually eaten by the experimental animal, and effects on the axolotl itself, sum up the experimental data obtained.

(*a*) **Sheep.*** No. 1 Female. — Approximate age from fecundation five months. Body weight 112 oz., weight of thyroid 2.4 gms. The whole gland was administered at one meal to a black axolotl weighing 67 gms. The dorsal fin was absorbed, gills shrunk, and larval skin shed ten to twelve days later. Metamorphosis complete.

No. 2 Female.—Approximate age from fecundation four months. Body weight 44 oz., weight of thyroid 0.69 gm. The whole gland was administered at one meal to a black axolotl weighing 33 gms. Metamorphosis (shedding of larval skin, etc.) began twelve to fourteen days later, and was complete.

No. 3 Male.—Approximate age from fecundation three months. Body weight 30 oz., weight of thyroid 0.9 gm The whole gland was administered to a black axolotl weighing 20 gms. After eleven days, no change being manifest, the same axolotl was fed with 1.2 gms. fresh thyroid from two male three months' fœtuses each weighing about 30 oz. After a fortnight (from the second meal) the fin was completely resorbed along the back, and subsequently the larval skin was shed in the usual manner.

No. 4 Female.—Approximate age from fecundation two months. Body weight 7 oz., weight of thyroid 0.13 gm. This was given as a single meal to a small black axolotl weighing 11 gms. No change whatever resulted.

No. 5 Male.—Approximate age from fecundation one month. Body weight 4 oz., weight of thyroid 0.02 gm. (approximate). This was given as a single meal to a small black axolotl weighing 12 gms. No change whatever resulted.

(*b*) **Oxen.** No. 1 Male.—Seven months from fecundation.† Weight 1288 oz., weight of thyroid 14.2 gms. Two grams were given to a large black axolotl weighing 52 gms. Metamorphosis began twelve to fourteen days after the meal and was complete.

* Only Blackfaced and Blackfaced crosses were used.

† The ages are approximate as before and the breeds are not the same in each instance.

No. 2 Male.—Six months from fecundation. Weight 360 oz., weight of thyroid 4.3 gms. Two grams were given to a large black axolotl weighing 43 gms. Metamorphosis began fourteen to sixteen days after the meal. Shortly after shedding its larval skin the transforming axolotl died.

No. 3 Male.—Five months from fecundation. Weight 544 oz., weight of thyroid 4.2 gms. The whole gland was given to a single large axolotl (albino) weighing 67 gms. Metamorphosis began ten to twelve days after the meal and was complete.

No. 4 Male.—Four months after fecundation. Weight 108 oz., weight of thyroid 1.25 gms. After a single meal of the whole gland a black axolotl weighing 29 gms. shed its larval skin on the eleventh day and transformed.

No. 5 Female.—Three months from fecundation. Weight 84 oz., weight of thyroid 0.9 gm. The whole gland administered to a black axolotl weighing 28 gms. produced no metamorphic changes.

No. 6 Female.—Two months from fecundation. Weight 6 oz., weight of thyroid 0.25 gm. Administered to a small black axolotl weighing 10 gms. this produced no visible alteration of the larval structures.

(*c*) **Histology of the Fœtal Thyroid.**—By the axolotl test it was not possible to obtain evidence of active secretion in the fœtal thyroid earlier than three months from fecundation in the sheep and four months in the ox. The structural changes about this period are instructive. In the case of the ox fœtus at one month the thyroid has not as yet assumed its characteristic macroscopic dimensions and texture. In outward appearance it is perfectly normal at two months from conception. Microscopic examination, however, shows a very different appearance (fig. 1, *a*, *b*, *c*). At three months the vesicles are beginning to differentiate, minute colloid masses are seen here and there; but the picture presented shows no striking similarity to the adult condition. At four months typical vesicles with well-defined and large colloid masses are found throughout the gland. By the sixth month the appearance is perfectly characteristic. The histological differentia-

tion of the gland is thus comparatively late in fœtal life—considerably after the differentiation of the organs including the genitalia may be regarded as complete. Moreover, it corresponds in a very striking manner with the period at which by the methods we employed, the gland can be shown

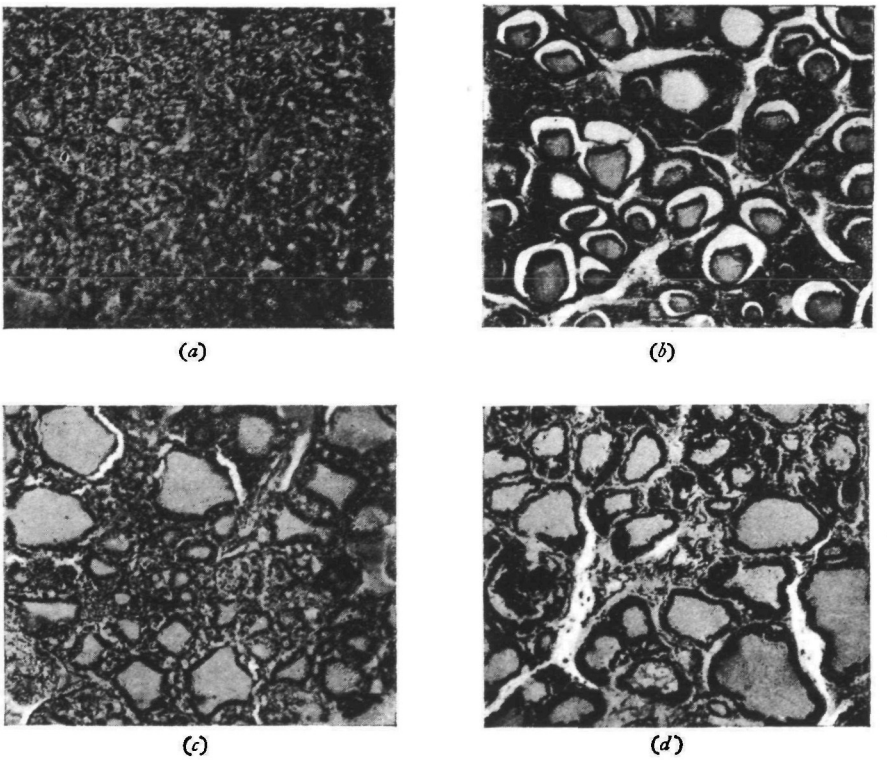


FIG. 1.—Fœtal thyroid of the Ox, T.S. Microphotographs.

(*a*) Three months from fecundation.
(*b*) Four months from fecundation.
(*c*) Six months from fecundation.
(*d'*) From a Dexter monster about the same age as (*c*).

to contain its active secretory product. It is to be noted that Fenger records a great increase in thyroid iodine during the fourth month of fœtal life in the ox. We should not therefore be encouraged to look to thyroid deficiency as a primary factor in the ætiology of developmental disturbances originating in the earlier part of fœtal life, as in the case of the Dexter monster.

3. The Thyroid of the Monstrous Calf of the Dexter Breed.

As is well known to stockbreeders and veterinarians, there appears as the result of inbreeding in herds of Dexter cattle a proportion of abortive calves described as "bulldogs." In

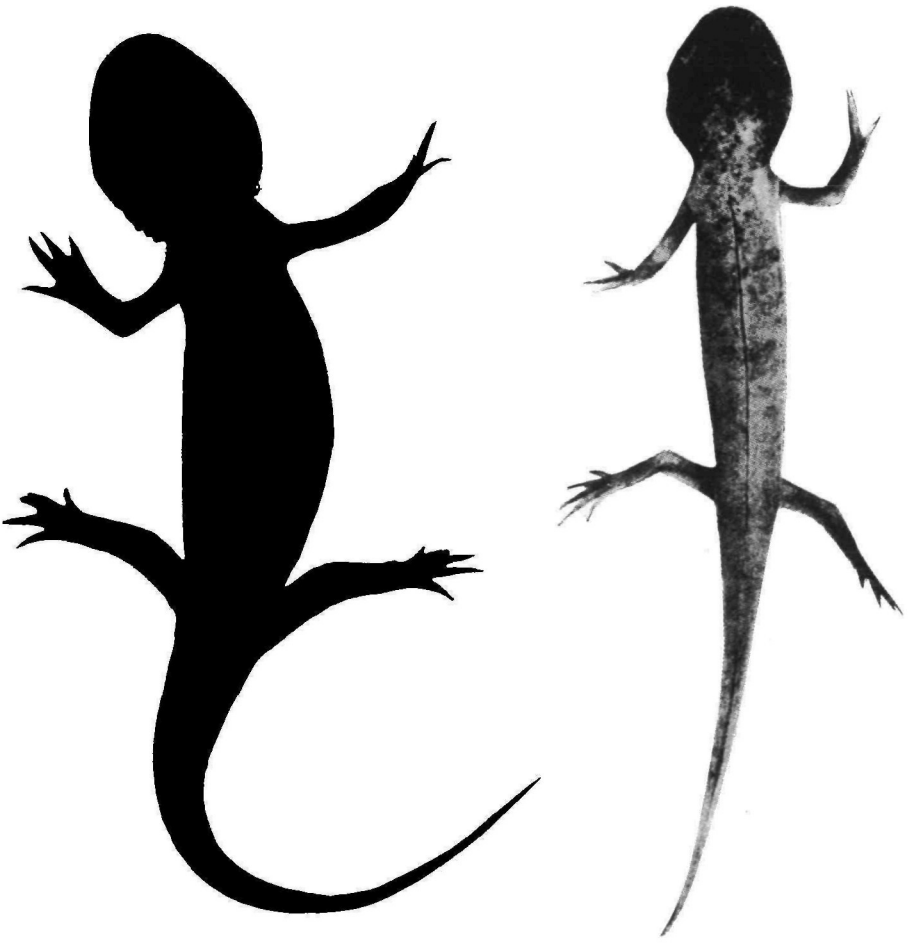


FIG. 2.—Two transformed axolotls (*Amblystoma tigrinum*): on the right, six weeks after a single meal of thyroid gland from a Dexter monstrous calf five months from fecundation; on the left, six weeks after one meal of thyroid from a bulldog fœtus nearing full time.

several particulars the characteristic features of this monster recall some of the more prominent aspects of achondroplasia in the human subject, a resemblance noted by Peloquin as early as the eighteenth century. Other writers have compared

the condition to cretinism, a view urged by Seligmann (1904) who described the microscopic structure of the thyroid in the "bulldog" fœtus as indicative of functional deficiency. This conclusion was contested by Sheather (1911) and one of the present writers (Crew and Glass, 1922). The gland of the Dexter monster shows large well-filled colloid vesicles (fig. 1, *d*). To test whether the gland actually contains its secretory product, two axolotls were fed with glands from "bulldogs" as follows:—

No. 1.—"Bulldog" fœtus, age seven to eight months from fecundation (nearing full time). 2.1 gms. thyroid was given to a large black axolotl weighing 64 gms. Complete metamorphosis occurred, the larval skin being shed twelve to fourteen days after the meal (fig. 2).

No. 2.—"Bulldog" fœtus, age five months from fecundation. 1 gm. thyroid was given to a medium-sized albino axolotl weighing 23 gms. Complete metamorphosis occurred, the larval skin being shed ten to twelve days after the meal (fig. 2).

The photographs in text (fig. 2) show the axolotls a month after treatment when the gill stumps in the small albino larva have been completely resorbed. Taken in conjunction with observations on the microscopic structure of the gland these data afford no confirmation to Seligmann's interpretation of the ætiology of the "bulldog" deformity.

4. Pituitary Activity in Fœtal Sheep and Oxen.

M'Cord's observations on the presence of active secretion in the posterior lobe of the fœtal pituitary were based on the oxytocic test of Dale and his associates. We have employed an entirely different procedure in our investigations on the same problem. The specific action of pituitary extract to induce expansion in the contracted melanophores of the amphibia was first shown by one of the authors (Hogben and Winton, 1922) and has since been noted by Uyeno (1922) and Krogh (1922). As has been shown elsewhere this test is highly specific for pituitary extracts both as contrasted with the action of other tissue extracts and the action of those

drugs which most nearly resemble pituitary extracts in their physiological effect. In particular it commends itself for use in the present enquiry (in the nature of which it was not possible to test the gland until several hours had elapsed since death), because amphibian melanophores have been shown not to react by expansion either to histamine or to the pressor substance in putrid meat. Besides this advantage it commends itself still further by its extreme facility. It is also comparatively delicate, since an injection equivalent to 5×10^{-5} gms. fresh (ox) posterior lobe suffices to transform a pale yellow frog to a dull grey or coal-black aspect (fig. 3) within an

Animal.	Approx. Age of Fœtus.	Test employed.	Result.
Ox	7 months	Two frogs	Full response
„	6 „	„	„ „
„	5 „	„	„ „
„	4 „	„	„ „
„	3 „	„	No response
		Two hypophysectomised axolotls	Full response
„	2 „	„ „ „	No response
Sheep	*5 „	Two frogs	Full response
„	4 „	„	„ „
„	3 „	„	„ „
„	2 „	Two hypophysectomised axolotls	No response
„	1 month	„ „ „	„ „

* Fig. 4 shows one of the frogs and its control.

hour. A refinement of the melanophore test capable of detecting very much smaller amounts has recently been developed by one of the authors,* and was employed where the frog failed to react to extracts employed. In such cases the extracts were tested by their power to induce melanophore expansion (fig. 4) in the hypophysectomised axolotl. Though the removal of the pituitary in Urodela has not been recorded before, it is not a difficult matter if attacked by the method advocated for frogs and toads (Hogben, 1923). Black axolotls after removal of the whole gland live indefinitely in a condition of pale grey aspect never witnessed in normal life. They are extremely sensitive to pituitary extract and resume the black hue of the normal larva after injection of more minute

* Hogben, *The Mechanism of Amphibian Color Response* (in the press).

quantities of pituitrine than are required to induce colour response in the frog. For further particulars relative to the test the papers cited for reference (Hogben and Winton, 1922-23) may be consulted. In the experiments which follow the whole posterior lobe of a single fœtus was ground up with sand and 10 c.c. of frog's Ringer. In each case 0.5 c.c. was injected into the experimental animal, controls being injected with Ringer's solution alone. The results obtained are summarised in the table on page 9.

FIG. 3.—On the right, a frog after injection of post-pituitary extract from ox fœtus five months from fecundation; on the left, control. Photograph taken six hours after injection.

We were able to detect pituitary secretion (posterior lobe) in the fœtal gland of the ox and sheep at three months from fecundation. M'Cord claims that by using a large number of glands for his extracts he detected pituitrine from the eighth week onwards. In view of the dimensions of the gland at this age, it would seem that it is relatively much less active than later; but at present we are not in a position to publish actual quantitative data which will, it is hoped, be obtained later on. The histological differentiation of the pituitary is much earlier than that of the thyroid, since viewed microscopically the pituitary of a two months' ox fœtus presents a quite typical picture.

5. The Adrenals of the Embryo Chick.

We have not made a detailed study of the adrenals in embryonic or fœtal life, because it seemed desirable to investigate correlatively the time at which the chrome-staining reaction of Henle first appears in the medulla. As fresh tissue is essential for this purpose, if critical information is desired, we

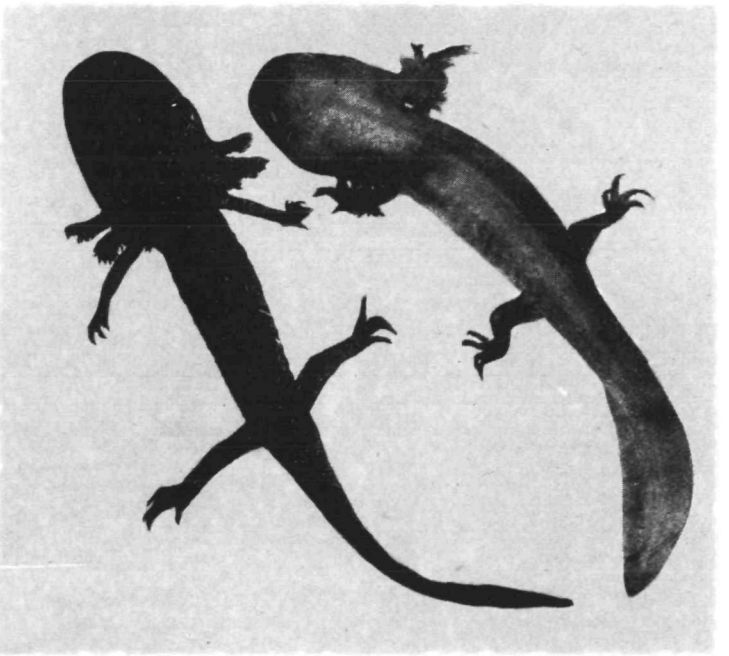
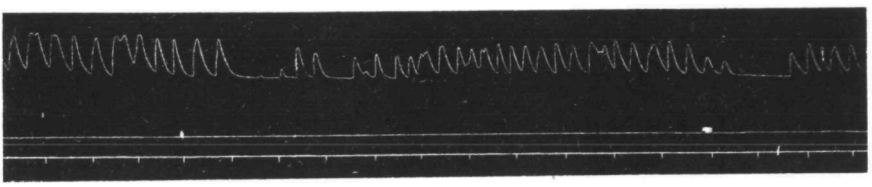


FIG. 4.—Two hypophysectomised axolotls (year-old) of the black variety. On the right, control showing the characteristic pallor which results from pituitary removal in amphibia; on the left, resumption of normal colour after injection of post-pituitary extract from a fœtal sheep three months from fecundation. Photograph twelve hours after injection and six weeks after removal of the pituitary gland.

carried out a few tentative experiments with the chick embryo that it is hoped merit brief mention as suggestive of further enquiry.

We have found that fixation with Regaud's bichromate formol fluid gives most satisfactory demonstration of chromaphil tissue after post-mordanting in bichromate for about ten

days. In material so treated chromaphil tissue is found in the adrenals of the chick embryo at least as early as the fifteenth day from the beginning of incubation. We have tested extracts of adrenal tissue from embryos at the fourteenth, sixteenth, nineteenth, and twentieth days of incubation with the method of Magnus Levy, *i.e.* by their power to inhibit rhythmical contraction and tonus in the isolated gut. A strip of cat's ileum (outer coat) is used for the purpose. As there is little increase in bulk in the case of the adrenal glands of the



1. c.c. 5 chicks' adrenals in 5 c.c. Locke. 16th day.

1 c.c. ditto. 16th-day chick.

FIG. 5.—Inhibitory Action of Adrenal Extract from 16th-day Chick Embryo on isolated gut of the Cat.

chick embryo between the fourteenth day and the end of the period of incubation, the extracts were all made up in the same way, viz., five pairs of carefully dissected fresh adrenals per 5 c.c. Locke-Ringer solution. Extracts of the sixteenth, nineteenth, and twenty-first days gave unquestionably positive indication of the presence of active secretion in the embryonic gland. Several trials of fourteenth-day extracts gave slight and doubtful results.

6. Summary.

The time at which the fœtal thyroid and pituitary glands in sheep and oxen can be shown to contain secretory products as inferred by their action upon amphibian development and colour response conforms to what might be anticipated on histological grounds: neither experimental nor microscopic data provide any justification for attributing the ætiological significance of the monstrous abortive Dexter calf to thyroid deficiency. Preliminary experiments suggest the desirability

of using the embryo chick for studying the correspondence between secretory activity and histological structure in the case of the adrenals.

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