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The Gonadotropin-Producing and Other Cell Types in the Distal Lobe of the Pituitary of the Common Frog, *Rana temporaria*

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Pituitaries of male *Rana temporaria* were fixed in Bouin-sublimate, sagittally sectioned at 4 μ, and stained with different trichrome methods, with PAS-orange G, Gabe's AF or Herlant's Alcian blue-PAS-orange G.

Four chromophilic cell types have been described in the distal lobe of the pituitary, i.e. α-cells or acidophils, β-cells or amphiphils type I, γ-cells or amphiphils type II, and δ-cells or cyanophils.

The same cell types were observed in sagittal sections of the pars distalis of *Bufo bufo* and *Xenopus laevis*.

β-Cells were absent from the pituitaries of juvenile *Rana temporaria*. One and three years after castration hyperactivity was observed in the β-cells. Secretory activity of these cells was reduced after the administration of testosterone, and was stimulated in frogs which were kept at high temperatures during the hibernation period. Moreover, in two out of four frogs the γ-cells had increased in number three years after gonadectomy, and a correlation was found between the atrophy of the secondary sex characters and the regression of the γ-cells in frogs which were kept at high temperatures. It is inferred that the β-cells produce a gonadotropic hormone, probably FSH, and that the γ-cells may secrete ICSH.

INTRODUCTION

Results of investigations into the regulation of the spermatogenetic cycle in the common frog, *Rana temporaria*, have shown the importance of gonadotropic hormones, secreted by the distal lobe of the pituitary, and have led to the conclusion that these hormones are not secreted continuously, but only at certain times of the year. This cyclical process appears to be influenced by several internal and external factors (cf. van Oordt, 1960).

In order to increase the knowledge of sexual endocrinology in male common frogs, we have extended our study of the regulation of the spermatogenetic cycle by histological investigations into the production and secretion of gonadotropic hormones. The results of some introductory experiments concerning the identification of the gonadotropin-producing cells are dealt with in this paper.

MATERIALS AND METHODS

Pituitaries of adult male *Rana temporaria*, collected during the resting period of the spermatogenetic cycle (September–March), were fixed in Bouin's fluid, or in Bouin's to which 10% of a saturated sublimate solution had been added (cf. Herlant, 1956). After paraffin embedding 4 μ sagittal sections were prepared, and these were stained according to the following methods:

1. Trichrome stain of Cleveland and Wolfe (erythrosin, orange G and aniline blue);
2. Heidenhain's Azan;
3. PAS-orange G (Herlant, 1956);
4. Aldehyde-fuchsin (Gabe, 1953) after oxidation in Gomori's oxidation mixture (permanganate-sulfuric acid); combined with Halmi's counterstain (Halmi, 1950);

5. Aldehyde-fuchsin without previous oxidation;
6. Alcian blue at pH 0.2 and pH 3.0, following oxidation in Gomori's oxidation mixture (Herlant, 1960); combined with PAS and orange G.
7. Alcian blue at pH 0.2 and pH 3.0, without previous oxidation.

In most sections the nuclei were stained with Hansen's iron trioxyhaematin (Romeis, 1948).

The results of the various staining methods were compared with each other by careful examination of contiguous sections stained in different ways, and by comparing color photographs of stained sections with other color photographs of the same sections, after these had been treated with acid alcohol and restained by another method.

Juvenile. In order to identify the gonadotropin-producing cells in the distal lobe of the pituitary, hypophyses of adult male frogs were compared with those of nine juvenile animals, measuring about 30 mm, and autopsied in September and October 1959. The pituitaries were placed in Bouin-sublimate, sectioned sagittally at 4 μ, and treated with Cleveland and Wolfe's trichrome staining method, PAS-orange G, and aldehyde-fuchsin (AF) according to Gabe (1953). In addition, the following experiments were carried out:

Castration. A number of adult male frogs were castrated in November 1954. The animals were kept in an outdoor vivarium under almost natural conditions and fed with mealworms and other insects. Four experimental and four control frogs were autopsied three months, six months, one year, and three years after the operation; the pituitaries were fixed in Bouin's fluid, and 4 μ sagittal sections were stained with Cleveland and Wolfe's trichrome stain and with PAS-orange G.

Testosterone. In the course of 1958 several experiments were carried out to study the effect of testosterone, administered as small, hypodermic pellets, upon spermatogenesis in common frogs. A detailed description of these experiments has been published elsewhere (van Oordt and Basu, 1959, 1960). The pituitaries of five of these experimental frogs, treated with testosterone between May 6 and July 1, and those of five control frogs, autopsied on the same day, were collected in order to ascertain whether or not the male sex hormone had affected the glandular cells of the pars distalis. After fixation in Bouin-sublimate the pituitaries were sectioned sagittally at 4 μ, and the sections stained with Cleveland and Wolfe's trichrome stain, PAS-orange G, and AF according to Gabe (staining method 4).

High temperature. From February 12, 1960, a number of male common frogs were kept in a constant temperature room at about 23°C and were fed with mealworms. Control frogs were kept in an outdoor vivarium. Six weeks after the beginning of the experiment autopsies were carried out on four high-temperature and four control frogs, and three weeks later six experimental and six control frogs were autopsied. The pituitaries were fixed in Bouin-sublimate, sectioned sagittally at 4 μ and stained with Cleveland and Wolfe's trichrome stain, PAS-orange G, and AF according to Gabe (staining method 4). The testes, thyroids, and seminal vesicles were placed in Bouin's fluid, sectioned at 7 μ, and stained with hemalum-eosin.

In order to facilitate comparison of our results with those of similar experiments, carried out with other Anura (Zuber-Vogeli, 1953; Cordier, 1953; Cordier and Herlant, 1957), hypophyses of adult male *Bufo bufo* and *Xenopus laevis* were fixed in Bouin-sublimate. Sections of these pituitaries were stained with Heidenhain's Azan, Cleveland and Wolfe's trichrome stain, and with PAS-orange G. The *Xenopus* pituitaries were also treated with Herlant's Alcian blue-PAS-orange G (staining method 6).

RESULTS

Cell Types in *Rana temporaria* (Plate I, Figs. 1 and 2)

α-Cells or acidophils. In the pituitary of *Rana temporaria* only one type of acidophilic cell was observed. These cells are distributed throughout the distal lobe, but are particularly numerous in the central, lateral, and caudal areas. They are usually rectangular in shape and are filled with small granules. The colors of these granules, following the different staining reactions, are given in Table 1. The granules appear to be PAS- and AF-negative, and they also lack any affinity for Alcian blue, even after oxidation.

One of the narrow ends of each cell is usually attached to a blood vessel. Sometimes the cells possess cytoplasmic protrusions that may envelop other cells or connect the cell with the wall of a blood sinus. The nuclei are often lobed and contain a distinct, acidophilic nucleolus.

β-Cells, or amphiphils type I. These large columnar cells are present in all parts of the distal lobe, but show a somewhat

higher concentration in the central portion of the gland. One pole of the β-cells is always attached to the wall of a blood vessel, the nucleus lying at the opposite pole. Sometimes both poles are attached to a capillary wall, in which case the nucleus is central in position. The nuclei are usually ovoid and vesicular, and contain one or two definite, acidophilic nucleoli.

The cytoplasm of the β-cells stains blueish-grey with aniline blue and pink with PAS. It contains extremely small cyanophilic and acidophilic granules, which take the colors mentioned in Table 1. The β-cells, moreover, very often contain orangeophilic globules of different sizes.

they become deep red; 3. Those packed with numerous acidophilic globules, among which hardly any cyanophilic or acidophilic granules are visible. After application of Cleveland and Wolfe's trichrome stain or Azan these cells are brownish-orange, and after PAS-orange G staining they are scarlet-orange.

When Gabe's AF and Halmi's counterstain are applied all forms of β-cells stain deep purple. The globules can sometimes be seen as chromophobic droplets in the darkly stained mass of small granules, but following this staining method there is no clear distinction between the different forms of β-cells. Similarly, with Herlant's

TABLE 1

STAINING REACTIONS OF CYTOPLASMIC GRANULES IN THE PARS DISTALIS OF *Rana temporaria*

Cell types	Staining methods					
	Cleveland and Wolfe	Heidenhain's Azan	PAS-orange G	Gabe's AF + Halmi's counterstain	Herlant's Alcian blue (pH 0.2) – PAS-orange G	Herlant's Alcian blue (pH 3.0) – PAS-orange G
α	red	scarlet-orange	yellowish-orange	orange	orange	orange
cyanophilic granules:	blue	blue	magenta	purple	purple or blue	blue
β acidophilic granules:	red	red	orange	- -	- -	
acidophilic globules:	orange	orange	orange	- -	—	- -
γ	lilac	lavender blue	brick red	greenish-grey	deep pink with purplish or brownish shade	deep pink with purplish or brownish shade
δ	blue	blue	purple	purple	blue	blue

The cytoplasmic inclusions are not always present in the same proportion. Roughly speaking, three characteristic forms of β-cells can be distinguished: 1. Those filled with cyanophilic granules, among which very few acidophilic elements can be seen. With Cleveland and Wolfe's trichrome stain and Heidenhain's Azan these cells stain blue; with PAS-orange G they are purple; 2. Those containing both many cyanophilic and many acidophilic inclusions. They stain in a somewhat blueish-brownish or violet shade with the trichrome stains, while with PAS-orange G Alcian blue at pH 0.2 in combination with PAS and orange G all varieties of this cell type stain blue, purplish-blue, or purple, and when the same method is used with Alcian blue at pH 3.0 all β-cells turn deep blue.

γ-*Cells, or amphiphils type II.* Of all cells present in the pars distalis, the γ-cells have the most distinct restriction in their distribution; they are always found in intimate association with the vessels of the secondary capillary net immediately derived from the portal vessels, in the medio-rostral and the rostro-ventral parts

of the distal lobe. The γ-cells are smaller than the α- and β-cells and are elongated in form. The nuclei are usually basal in position, ovoid in shape, and they contain a distinct nucleolus. The granulation of the γ-cells is exceedingly fine, and is amphiphilic, the staining reactions being shown in Table 1.

δ-*Cells or cyanophils.* In most pituitaries there are found some scattered cells which contain strongly cyanophilic granules but no acidophilic inclusions. With few exceptions these cells are small and of a slightly elongated or somewhat irregular shape, while their nuclei are usually irregularly lobed and contain a distinct, acidophilic nucleolus.

As may be seen in Table 1, the cytoplasmic granules are aniline blue-, PAS-, AF- and Alcian Blue-positive. If, however, Gomori's oxydation mixture is omitted, they do not react with AF and Alcian Blue. In fact, these basic dyes do not stain any cell type in the pituitary of the common frog without previous oxidation.

Apart from these purely cyanophilic cells, other cells occur that are almost identical with the δ-cells; at high magnification, however, it can be seen that they contain some orangeophilic granules or globules. These are particularly evident when the cyanophilic granules are stained light blue with aniline blue. It is difficult to decide whether such cells should be reckoned among the δ-cells or whether they belong to the first form of the β-cells. If the latter were the case, the cyanophilic cells might be young β-cells that do not yet contain acidophilic inclusions. Indeed, Metuzals (1951) comes to the conclusion that in *Rana temporaria* cyanophils can develop into amphiphilic cells. Moreover, Mazzi (1949), describing the cytology of the pituitary of *Triturus cristatus carnifex*, considered the cyanophils and the cells containing both cyanophilic granules and acidophilic globules to be different forms of one cell type (Mazzi's δ-cells). Zuber-Vogeli (1953) and Cordier (1953), however, are of the opinion that cyanophilic cells with or without a few acidophilic inclusions belong to one cell type, and differ from the amphiphils that contain numerous acidophilic globules.

Chromophobes: In *Rana temporaria* chromophobes do not constitute a distinct, functional cell type in the pars distalis of the pituitary. Many such cells may be observed, however, both singly or in small groups. The nuclei are usually somewhat irregularly shaped, while only very little cytoplasm is present, and this does not contain any chromophilic inclusions. The relation of chromophobes to acidophils, amphiphils, and cyanophils follows from the fact that some stainable material is often present in the cytoplasm of small cells which closely resemble the chromophobes.

Bufo bufo

In the pituitaries of *Bufo bufo* we found the same cell types as did Zuber-Vogeli (1953). Comparison of stained sections of pituitaries of *Bufo bufo* and *Rana temporaria*, mounted on the same slide, showed that the β-cells in the common frog gave the same staining reactions as "les grandes cellules basophiles à globules acidophiles" in the toad. Moreover, the γ-cells appeared to be comparable with "les cellules mauves ou violettes," and the δ-cells with "les petites cellules basophiles ou cyanophiles." Contrary to the situation in *Rana temporaria*, however, in *Bufo bufo* cells which are purely cyanophilic are quite numerous in the central, lateral, and posterior parts of the distal lobe. These cells stain intensely with aniline blue after application of Heidenhain's Azan while they often possess a typical bilobed or folded nucleus, not seen in other cell types. It is, therefore, less difficult to identify the cyanophils in *Bufo bufo* than it is in *Rana temporaria*.

Zuber-Vogeli described two different types of acidophilic cells, namely the "cellules azanophiles" (carminophils) and the "cellules orangeophiles." The former were found in all parts of the distal lobe in *Bufo bufo*, and in our material they stained scarlet-red with Cleveland and Wolfe's trichrome stain. There is no doubt that these cells are comparable with the acidophils present in the pituitary of *Rana tempo-*

raria. The orangeophils, on the other hand, are localized in the dorso-caudal portions of the pars distalis and appear orange with Azan and with Cleveland and Wolfe's method. It has been impossible to find identical cells in the pituitary of the common frog. Both types of acidophils are PAS-negative, and thus stain orange or yellowish-orange with PAS-orange G.

Xenopus laevis

In agreement with the results of Cordier (1953), only one type of acidophilic cell was met with in the pituitary of *Xenopus laevis*. This showed all the characteristics of the α-cells or acidophils in *Rana temporaria* and of the carminophils in *Bufo bufo*. Likewise the β-cells or amphiphils type I, present in *Rana temporaria*, appeared to be almost identical with "les amphophiles" (Cordier, 1953) or "les cellules basophiles de type II ou cellules gonadotropes ou cellules bêta" (Cordier and Herlant, 1957) of *Xenopus laevis*. On the other hand, there is little similarity between the δ-cells or cyanophils in the common frog and "les cellules cyanophiles" (Cordier) or "les cellules basophiles de type I ou cellules thyrotropes ou cellules delta" (Cordier and Herlant, 1957) in *Xenopus*. The latter are quite big cells situated in the central, lateral, and caudal parts of the distal lobe. They are strongly aniline blue-, PAS-, AF- (Gabe's method) and Alcian blue-positive (Herlant's method) and always contain some hyaline vacuoles. The nuclei are ovoid or folded as in the cyanophils of *Bufo*. The cyanophils in the pituitary of *Xenopus* are even larger and more numerous than those in the pituitary of the latter, and there is no doubt that they represent a distinct cell type.

Apart from these cell types, other chromophilic cells were observed in the pituitary of *Xenopus laevis* that have not been mentioned by Cordier (1953) and Cordier and Herlant (1957). These are localized in the anterior process of the distal lobe and along the capillaries immediately derived from the portal vessels. They are probably the same as the basophilic cells found in the anterior process by Charipper and Martorano (1948), and since they closely resemble the γ-cells or amphiphils type II of *Rana temporaria* it is suggested that they also be called γ-cells. On the whole, however, these γ-cells in *Xenopus* are smaller, less elongated and less stainable than those in *Rana temporaria*. After application of Heidenhain's Azan or Cleveland-Wolfe's trichrome stain the former appear almost chromophobic. Following the PAS-orange G staining they are light brick red or light reddish brown, and they become purplish or brownish pink after Herlant's Alcian blue-PAS-orange G.

Juvenile

The ovaries of four juvenile female frogs contained oögonia and small oöcytes.

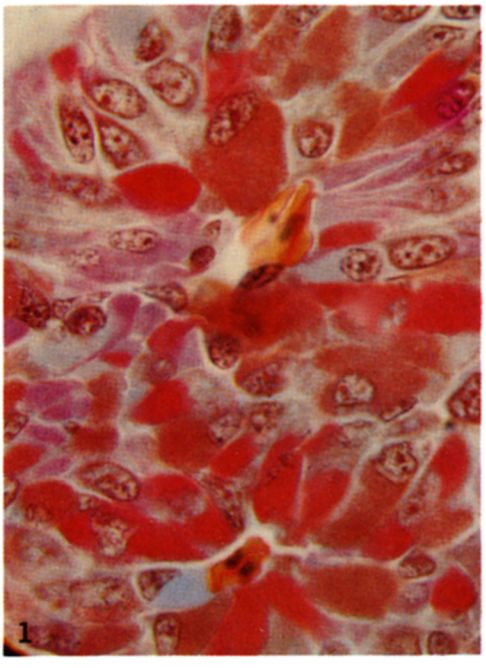
Plate I. Pituitaries of adult male *Rana temporaria*. Parts of 4 μ, sagittal sections of the pars distalis. Fixation: Bouin-sublimate. Magnification: $\times$900, Anscochrome Daylight Film, Ilford Daylight Filter.

Fig. 1. Normal pituitary. Cleveland and Wolfe's trichrome stain. α-Cells: red; β-cells: brownish-orange; γ-cells: lilac; δ-cells: sky blue.

Fig. 2. Normal pituitary. Herlant's Alcian blue-PAS-orange G staining method. α-Cells: orange; β-cells: numerous blue and some purple granules; γ-cells: purplish-pink; δ-cells: cannot be distinguished from the β-cells.

Fig. 3. Pituitary of frog autopsied three years after castration. Cleveland and Wolfe's trichrome stain. α-Cells: red, irregularly shaped, pressed between enlarged β-cells; β-cells: hypertrophied, chromophilic granules have almost completely disappeared, cytoplasm vacuolated, large vesicular nuclei with conspicuous nucleoli; γ-cells: lilac, increased in number.

Fig. 4. Pituitary of frog treated with testosterone for six weeks. Cleveland and Wolfe's trichrome stain. α-Cells: red, as in controls; β-cells: filled with coarse orangeophilic globules, small irregularly shaped nuclei; γ-cells: poorly developed, some small faintly purplish cells in the lower left corner of the photograph; δ-cells: not in this photograph.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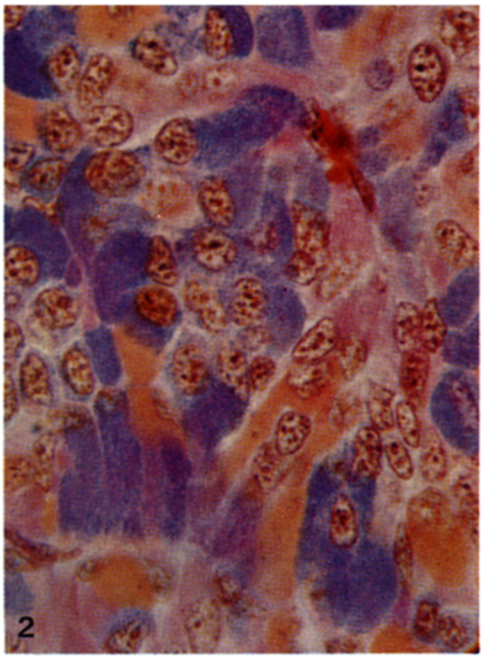
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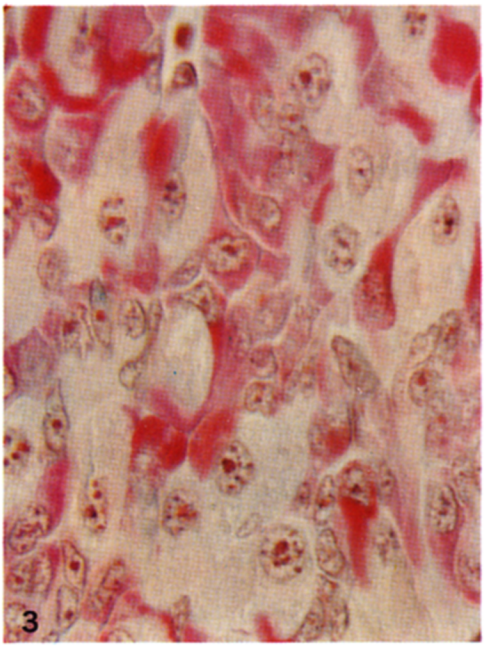
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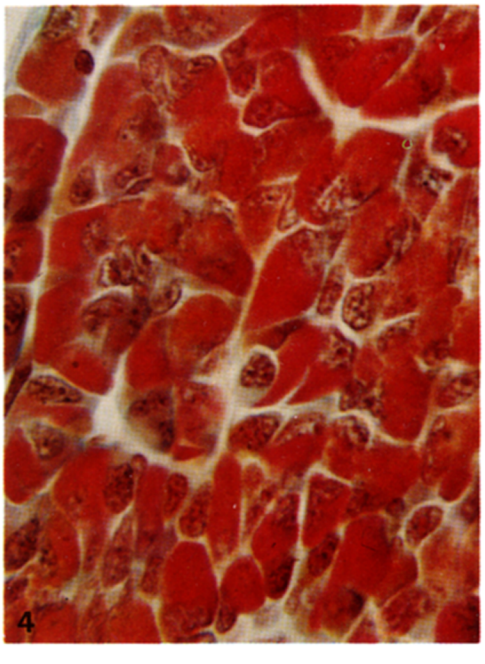
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and in the testes of five juvenile males only quiescent primary spermatogonia were present. This absence of active gametogenesis was accompanied by a complete absence of β-cells from the distal lobe of the pituitaries.

On the other hand, α-, γ-, and δ-cells were observed in all animals. The γ-cells were numerous, almost filling the entire centro-rostral part of the distal lobe, and were the only one of these cell types to show signs of activity, their nuclei being often vesicular and containing a conspicuous nucleolus, while the cytoplasm was filled with a varying quantity of fine granules.

The α-cells were also present in great number, particularly in the central, lateral and caudal portions of the pars distalis. They were smaller than in the adults, contained small, darkly stained nuclei, and were packed with acidophilic granules.

Contrary to the situation in adult *Rana temporaria*, some δ-cells could easily be distinguished as oval or slightly elongated, purely cyanophilic cells. They appeared to be present throughout the distal lobe, but showed some concentration in the lateral and caudal areas. The oval nuclei of the δ-cells were even smaller and more darkly staining than those of the α-cells. They did not contain a distinct nucleolus, and were surrounded by a small layer of cytoplasm, filled with extremely small cyanophilic granules.

Castrated Frogs

No clear differences were found between the pituitaries of control frogs and those autopsied three or six months after castration. One year after extirpation of the testes, however, a strong decrease in number of acidophilic granules and globules was observed in the β-cells. These acidophilic inclusions had partly been replaced by fine cyanophilic granules, while hyaline cytoplasmic vacuoles or droplets of secretion were also visible. Colloidal material was seen in the blood vessels, and this stained brick red with PAS-orange G. This secretory activity was accompanied by signs of an increased formation of secretory products, such as large vesicular nuclei, which contained one or more conspicuous acidophilic nucleoli, and not seldom a chromophobic vacuole, and the transformation of many chromophobes into β-cells.

Three years after gonadectomy (Plate I, Fig. 3) the β-cells had increased in size and number, and showed a strong vacuolization and degranulation of the cytoplasm. Not only had most acidophilic inclusions disappeared, but very often the cyanophilic granules had also decreased in number. The cytoplasmic vacuoles were sometimes quite large and gave the impression of holocrine secretion.

In sharp contrast to this situation in the β-cells, no alterations were observed in the α- and γ-cells of the animals sacrificed one year after castration, and even three years after the operation no increase in secretory activity and production was detectable. However, in two out of four castrated frogs, belonging to the latter group, some hyperplasia of the γ-cells was noted; these cells were seen in groups along the blood vessels in the rostral, ventral, and even in the central portions of the pars distalis. Most α-cells were irregularly shaped and gave the impression that they had been pressed between the enlarged β-cells.

Typical δ-cells were difficult to discern in pituitaries collected one and three years after castration of the animals. This must be ascribed to the fact that many β-cells had become cyanophilic as a result of the increase in amount of cyanophilic granules and the disappearance of the acidophilic inclusions.

Testosterone Treatment

Examination of the pars distalis of the control frogs, sacrificed on July 1, showed that all cell types often lacked a distinct nucleolus. The majority of the β-cells, contained some cytoplasmic vacuoles or droplets of secretion, and this was accompanied by the presence of colloid in the capillaries, reacting both with PAS and orange G. Very often the β-cells contained few acidophilic globules and many cyanophilic granules.

The α-, γ- and δ-cells of the testosterone-treated animals closely resembled those in the control frogs, but the cytoplasm of almost all of the β-cells, was filled with numerous acidophilic globules, many of which were extraordinarily large (Plate I, Fig. 4). The nuclei of these cells were very small and irregularly shaped, nucleoli were seldom seen, cytoplasmic vacuoles were absent, and the blood vessels lacked any colloidal material. These phenomena seem to indicate that in the testosterone-treated frogs the β-cells were storing their secretory products instead of discharging them into the circulatory system.

High Temperature Treatment

In agreement with the results of Galgano and Lanza (1951) and of van Oordt 1956a,b), it was observed that high temperature treatment during the period preceding the onset of spermatogenesis, induced a strong spermatogenetic activity in *Rana temporaria*. In the first group of experimental frogs, sacrificed after they had been in a high temperature room for six weeks, the testis tubules contained numerous cell nests with secondary spermatogonia, and some cell nests with primary spermatocytes. In the animals autopsied three weeks later, most of these had developed into cell nests with spermatocytes and spermatids. In the testes of the control frogs no spermatogenetic activity was observed.

Owing to the high temperature treatment the experimental frogs had lost the dark, rough skin of the thumb pads, normally present at this time of the year. In addition, the epithelium of the Wolffian ducts and of the seminal vesicles was less folded and lower than in the control frogs.

No changes were seen in the thyroids; both in the experimental and in the control animals the follicular epithelium was very low, and the follicles were filled with colloid.

In the control frogs the cells of the distal lobe of the pituitary answered with few exceptions to the description given on pages 365–367. Most of the β-cells contained numerous acidophilic inclusions in addition to cyanophilic granules.

In the frogs submitted to high temperature treatment, a decrease in size of all cells was noted, and big, vesicular nuclei were almost completely absent. Generally speaking, no signs of activity were met with in the α-cells, and their cytoplasm was filled with acidophilic granules. However, in one animal, kept at high temperatures for nine weeks, some small hyaline vacuoles or droplets of secretion were present in these cells.

The β-cells invariably showed signs of degranulation, i.e. either the basal regions or the entire cells were deprived of acidophilic inclusions. On the other hand, the cyanophilic granules had significantly increased in quantity. As a result, many of these β-cells had become almost pure cyanophils, and this made it difficult to distinguish the δ-cells.

A marked effect of the high temperatures was also observed in the γ-cells. After six weeks' treatment they had become smaller in size and number, and contained little stainable material, and this effect was even more pronounced after nine weeks' treatment. In three animals of the latter group hardly any γ-cells were met with in the pars distalis of the pituitary.

DISCUSSION

It is a well known fact that efforts to introduce a generally acceptable terminology for the cell types in the pars distalis of the pituitary have not yet met with success. As a result, several names have been used for one cell type, and one name for different cell types, a confusion which must be ascribed to the absence of a reference technique for the demonstration of the cells which produce the different hormones.

We have tried to overcome this difficulty by applying various techniques that have been used by others to describe pituitary histology in other Amphibia, and also by staining sections of the pituitary of *Rana temporaria* together with those of *Bufo*

bufo and *Xenopus laevis*, of which excellent descriptions have been given by Zuber-Vogeli (1953) and by Cordier (1953; cf. also Cordier and Herlant, 1957) respectively.

Our observations point towards a striking similarity in the histology of the pars distalis of these species, but also towards important differences. Carminophils occur in all species under investigation, but another type of acidophilic cell, the orangeophil, was only found in *Bufo bufo*. The amphiphils type I, or β-cells, showed the same characteristics in frog and toads, but the amphiphils type II, or γ-cells, were smaller and less chromophilic in *Xenopus* than in *Bufo* and *Rana*. The δ-cells, or cyanophils, on the other hand, were more difficult to distinguish in the frog than in *Xenopus* and *Bufo*.

As regards other Amphibia, Joly (1959) and Pasteels Jr. (1960), using the same techniques as ours, found identical cell types in *Salamandra sal. taeniata* and in *Pleurodeles Waltlii* respectively, while essentially similar ones have been observed in *Rana esculenta* (van Oordt, unpublished results).

In the hypophysis of *Triturus c. carnifex* Mazzi (1949) discovered two types of acidophils, i.e. "cellule acidofile del I tipo (orangiofile)" and "cellule acidofile del II tipo (carminofile)." Later, Mazzi (1952) found that the carminophils were PAS-positive, so that they most likely belong to the "cellule basofile del tipo δ," which were described as basophils, containing acidophilic inclusions, and which were supposed to be derived from small, purely cyanophilic δ-cells. In other words, the carminophils together with the δ-basophils in *Triturus c. carnifex* are probably identical with the β-cells plus the δ-cells in *Rana temporaria*. Finally, Mazzi (1952) described "cellule basofile del tipo β," situated in the most rostral part of the distal lobe of *Triturus c. carnifex*, and these closely resemble the γ-cells in the pituitary of the common frog. Thus, it can be said that in both species the same cell types occur in the distal lobe of the pituitary, and that, as in the pars distalis of *Rana temporaria*, purely cyanophilic cells are difficult to distinguish in the hypophysis of *Triturus c. carnifex*.

The cell types in the distal lobe of the pituitary of another Urodele, *Taricha torosa* (=*Triturus torosus*), have recently been described by Miller and Robbins (1955). Apart from acidophilic cells two types of cyanophils were observed, i.e. purely cyanophilic cells, and those containing not only cyanophilic granules but also some orangeophilic inclusions. These cell types were called "β basophils" and "δ basophils" respectively, according to the terminology of Halmi (1950), but it has been pointed out by Herlant (1956) that this nomenclature is based on a misinterpretation of the original one, introduced by Romeis (1940); the β-cells of Halmi are actually identical with the δ-cells of Romeis, and *mutatis mutandis* the δ-cells of Halmi with the β-cells of Romeis. However, in *Taricha torosa* cyanophils as well as amphiphils type I appear to be present, but Miller and Robbins do not mention cells comparable with the γ-cells in *Rana temporaria* and other Amphibia.

Dawson (1957) described cells lying against the capillaries that arise from the portal vessels in the medio-rostral part of the distal lobe of *Rana pipiens*. The distribution of these cells, together with their color following Azan staining, makes them comparable with the γ-cells or amphiphils type II in other Amphibia, but since we have not used Dawson's modification of Halmi's AF method it is difficult to explain why in *Rana pipiens* these γ-cells were AF-positive, whereas in *Rana temporaria* and other species they appeared to be AF-negative.

In addition to the above, Dawson mentioned two acidophilic cell types and two more cyanophilic cell types, but his short description of the cell types in the distal lobe does not allow a comparison with the results obtained in other Amphibia. In 1956, however, Ortman observed in the pars distalis of *Rana pipiens* three types of chromophilic cells which, according to

their reactions to the Azan or the PAS techniques, can be compared respectively with the α-cells, the β-cells and the δ-cells in *Rana temporaria*.

It is not the purpose of this paper to give a complete account of what is known concerning the histology of the pars distalis in Amphibia. However, the above discussion shows that, apart from certain differences between species, the chromophilic cells of the distal lobes of Anura and of Urodela can be divided into four different cell types: the α-cells or acidophils, the β-cells or amphiphils type I, the γ-cells or amphiphils type II, and the δ-cells or cyanophils. We thus come to the question of the relationship between these types and the hormones of this lobe. Since little or nothing is known about the chemical composition of adenohypophysial hormones in Amphibia, histochemical methods do not suffice to answer this question, and biological methods have therefore been used to identify the gonadotropin-producing cells in the pituitary of *Rana temporaria*.

In our experiments both castration and testosterone administration led to profound changes of the β-cells. These results, and the fact that the β-cells were not present in the pituitaries of juvenile common frogs (cf. also Metuzals, 1951), indicate that in *Rana temporaria* these cells are the source of gonadotropic hormone. These experiments have, moreover, shown the existence of a feedback mechanism between pituitary and testes, castration leading to a hyperactivity of the gonadotrops whereas testosterone impedes the secretion of the gonadotropic hormone. It follows from the latter that testosterone acts via the pituitary when inhibiting spermatogenesis in *Rana temporaria* (cf. van Oordt and Basu, 1959, 1960; van Oordt, 1960, 1961).

Similar results have been recorded by Cordier (1953), who concluded from castration experiments that gonadotropin is produced in the β-cells in *Xenopus muelleri*, and by Zuber-Vogeli (1953), who found hyperactivity of these cells in *Bufo bufo* following gonadectomy. Likewise, Joly (1959) did not observe β-cells in larval and juvenile *Salamandra sal. taeniata*.

It is of interest that the gonadotropin-producing cells in *Rana temporaria, Bufo bufo, Xenopus laevis* (cf. Cordier, 1953; Cordier and Herlant, 1957), *Triturus c. carnifex* (Mazzi, 1952, 1958), *Salamandra sal. taeniata* (Joly, 1959) and *Pleurodeles Waltlii* (Pasteels Jr., 1960) are PAS-positive. This may indicate that in Amphibia the gonadotropic hormone is a glycoprotein as in other vertebrates (cf. Ortman, 1956). Moreover, the β-cells appear to be AF- and Alcian blue-positive if pretreated with Gomori's oxidation mixture. This shows that after oxidation the cytoplasmic granules contain acid groups, but further histochemical investigations will be needed to elucidate the exact nature of such groups.

Pasteels Jr. (1960) provided experimental evidence that in *Pleurodeles Waltlii* the β-cells only produce a follicle and spermatogenesis stimulating hormone (FSH) and that a luteinizing or interstitial cell stimulating hormone (LH or ICSH) is formed by the γ-cells. In agreement with this opinion, and with the results of Mazzi's experiments (Mazzi, 1958) with *Triturus c. carnifex*, a correlation was found between secretory activity of the β-cells and spermatogenesis in *Rana temporaria*, when the animals were kept at high temperatures during the hibernation period. Moreover, the γ-cells atrophied as a result of the high temperature, and this atrophy appeared to be correlated with a regression of the secondary sex characters. It is, therefore, not impossible that in *Rana temporaria* also the β-cells should be regarded as the origin of FSH, and the γ-cells as the source of ICSH. The latter is also indicated by the fact that in some animals an increase in number of γ-cells was found three years after castration, for also in gonadectomized mammals the cells producing ICSH are affected later than those producing FSH (cf. Purves and Griesbach, 1955).

On the other hand, it has been suggested that in *Rana pipiens* (Dawson, 1957) and *Triturus c. carnifex* (Mazzi, 1958) cells comparable with the γ-cells in the common frog produce the thyrotropic hormone (TSH). Gasche (1946), however, believes that in *Xenopus* TSH is secreted

by the δ-cells, and the results of Cordier (1953) and of Saxén *et al.* (1957) point in the same direction. Also in *Salamandra sal. taeniata* (Joly, 1959) and in *Pleurodeles Waltlii* (Pasteels Jr., 1960) TSH appears to be formed in the δ-cells.

Experiments have been planned to determine the source of TSH in the pituitary of *Rana temporaria*. We hope also to investigate the cyclical changes in the gonadotropic cells of male common frogs in order to increase our knowledge concerning the regulation of the spermatogenetic cycle by pituitary hormones.

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REFERENCES

Charipper, H. A., and Martorano, J. J. (1948). The morphology of the pituitary gland of the South African clawed toad, *Xenopus laevis* Daudin. *Zoologica* **33,** 157–163.

Cordier, R. (1953). L'hypophyse de *Xenopus*. Interprétation histophysiologique. *Ann. soc. roy. zool. belg.* **84,** 5–16.

Cordier, R., and Herlant, M. (1957). Etudes histochimiques sur les cellules du lobe antérieur de l'hypophyse chez *Xenopus laevis*. *Ann. histochimie* **2,** 349–359.

Dawson, A. B. (1957). Morphological evidence of a possible functional interrelationship between the median eminence and the pars distalis of the anuran hypophysis. *Anat. Record* **128,** 77–90.

Gabe, M. (1953). Sur quelques applications de la coloration par la fuchsine-paraldehyde. *Bull. microscop. appl.* **3,** 153–162.

Galgano, M., and Lanza, B. (1951). Contributi intorno all'azione della temperatura e dell'ormone folliculostimolante sulla stasi spermatogenetica in *"Rana temporaria"* L. *Rend. accad. nazl. Lincei Ser. 8* **11,** 105–111.

Gasche, P. (1946). Basophile Zellen des Hypophysenvorderlappens als Bildungsstätte des thyreotropen Hormons (Untersuchungen an *Xenopus*-Larven). *Rev. suisse zool.* **53,** 546–553.

Halmi, N. S. (1950). Two types of basophils in the anterior pituitary of the rat and their respective cytophysiological significance. *Endocrinology* **47,** 289–299.

Herlant, M. (1956). Corrélations hypophysogénitales chez la femelle de la Chauve-Souris, *Myotis myotis* (Borkhausen). *Arch. biol.* (Liége) **67,** 89–180.

Herlant, M. (1960). Etude critique de deux techniques nouvelles destinées a mettre en évidence les différentes catégories cellulaires présentes dans la glande pituitaire. *Bull. microscop. appl.* **10,** 37–44.

Joly, J. (1959). Données histologiques sur l'adénohypophyse de *Salamandra salamandra taeniata* Düringen (1897). *Compt. rend. acad. sci. Paris* **249,** 312–314.

Mazzi, V. (1949). La citologia dell'ipofiso del Tritone crestato. *Arch. ital. anat. embriol.* **54,** 1–68.

Mazzi, V. (1952). Nuove osservazione intorno alle cellule basofile nell'ipofisi del Tritone crestato *Rend. accad. nazl. Lincei* **12,** 116–122.

Mazzi, V. (1958). Effetti di lesioni alla eminenza mediale sul sistema ipotalamo-ipofisario, adenoipofisi, tiroide e testicolo del Tritone crestato. *Z. Zellforsch.* **48,** 332–348.

Metuzals, J. (1951). Cytologische studien über die *Hypophysis cerebri* des normalen und in dunkelheit gehaltenen Grasforsches (*Rana temporaria* L.) *Z. Zellforsch.* **35,** 550–578.

Miller, M. R., and Robbins, M. E. (1955) Cyclic changes in the pituitary gland of *Taricha torosa* (*Triturus torosus*). *Anat. Record* **122,** 105–114.

van Oordt, P. G. W. J. (1956a). Regulation of the spermatogenetic cycle in the common frog (*Rana temporaria*). Thesis, Utrecht.

van Oordt, P. G. W. J. (1956b). The role of temperature in regulating the spermatogenetic cycle in the common frog (*Rana temporaria*). *Acta Endocrinol.* **23,** 251–264.

van Oordt, P. G. W. J. (1960). The influence of internal and external factors in the regulation of the spermatogenetic cycle in Amphibia. *Symp. Zool. Soc. London* **2,** 29–52.

van Oordt, P. G. W. J. (1961). Experimental and comparative aspects of the pituitary-gonad in-

terrelationship in male Amphibia. *In* "Endocrinologiae Progressus" (J. M. Cañadell, ed.), pp. 145–160. Comité Médico España-Francia, Playa de Aro (Spain).

VAN OORDT, P. G. W. J., AND BASU, S. L. (1959). Spermatogenesis in common frogs (*Rana temporaria*) treated with testosterone. *Acta Physiol. et Pharmacol. Neerl.* **8,** 281.

VAN OORDT, P. G. W. J., AND BASU, S. L. (1960). The effect of testosterone on the spermatogenesis of the common frog, *Rana temporaria*. *Acta Endocrinol.* **33,** 103–110.

ORTMAN, R. (1956). Two types of acidophils in the anterior pituitary of the frog and their relation to the periodic acid Schiff reaction. *J. Histochem. and Cytochem.* **4,** 471–475.

PASTEELS, J. L., JR. (1960). Etude expérimentale des différentes catégories d'éléments chromophiles de l'hypophyse adulte de *Pleurodeles Waltlii*, de leur fonction et de leur contrôle par l'hypothalamus. *Arch. biol.* (Liége) **71,** 409–471.

PURVES, H. D., AND GRIESBACH, W. E. (1955). Changes in the gonadotrophs of the rat pituitary after gonadectomy. *Endocrinology* **56,** 374–386.

ROMEIS, B. (1940). "Handbuch der mikroskopischen Anatomie," (W. von Möllendorf, ed.), Vol. VI, Part 3. Springer, Berlin.

ROMEIS, B. (1948). Mikroskopische Technik. 15th ed. Leibniz, München.

SAXÉN, L., SAXÉN, E., TOIVONEN, S., AND SALIMÄKI, K. (1957). Quantitative investigations on the anterior pituitary-thyroid mechanism during frog metamorphosis. *Endocrinology* **61,** 35–44.

ZUBER-VOGELI, M. (1953). L'histophysiologie de l'hypophyse de *Bufo vulgaris* L. *Arch. anat. histol. embriol.* **35,** 77–180.