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Function and Cytology of the Normal and Autotransplanted Pars Distalis of the Hypophysis in the Toad *Bufo bufo* (L)

W. J. van DONGEN

Biological Laboratory, Medical Faculty, University of Nijmegen, The Netherlands

C. BARKER JØRGENSEN, LIS OLESEN LARSEN, PER ROSENKILDE

Zoophysiological Laboratory A, University of Copenhagen, Denmark

BRIAN LOFTS

Department of Zoology and Comparative Anatomy, St. Bartholomew's Hospital Medical College, University of London, England

AND

P. G. W. J. van OORDT

Zoological Laboratory, University of Utrecht, The Netherlands

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The functional activity of the pars distalis following heterotopic and homoiotopic autotransplantation has been studied in adult toads of both sexes in the spring, about a month after breeding, and in the autumn on toads with mature gonads.

Gonadotropic Function. In males, the homoiotopic grafting of the pars distalis maintained about normal spermatogenesis and interstitial cell activity, but ectopic transplants caused a strong reduction of interstitial cell activity, though spermatogenesis was only slightly reduced. In the spring experiment, there was no significant difference in the ovaries of the two graft-bearing groups, which were intermediate between the normal and the hypophysectomized controls. In autumn females, however, the ovaries of 5 of 8 toads with homoiotopic grafts atrophied, as in the hypophysectomized controls. The 3 remaining toads had ovaries of nearly normal size filled with mature eggs, but there were strong indications that in these 3 toads also the mature eggs that were present at the time of operation degenerated immediately after the operation, and was followed by a phase of extensive growth and maturation of small oocytes during the 2 months of the experiment. All females with ectopic grafts had atrophic ovaries at the end of the experiment.

Adrenocorticotropic Function. This function was normal in the toads with homoiotopic grafts. No corticotropic function could be demonstrated in the ectopic grafts although the cytological study of males in the spring experiment showed that, in general, the adrenocortical cells in the toads bearing ectopic grafts appeared more active than those in the hypophysectomized controls.

Thyrotropic Function. Thyroidal uptake of ^{131}I in the autumn experiment was used to evaluate thyrotropic function. Uptake was normal in toads with homoiotopic grafts, but reduced in the toads with ectopic grafts. In the males with ectopic grafts, the ^{131}I accumulation in the thyroids was not significantly higher than in the thyroids of the hypophysectomized controls.

Fat Bodies. In the autumn experiment, feeding was insufficient to maintain body weight, and the fat bodies were greatly reduced in both normal controls and in the toads with homoiotopic grafts. Hypophysectomized controls, however, possessed large fat bodies despite an even greater loss of body weight. In the toads with ectopic grafts, net mobilization of fat from the fat bodies was intermediate between the normal and hypophysectomized controls. In the spring experiment, feeding was efficient. All groups increased in weight, and all showed fat deposition.

Cytology of Pars Distalis. Four of the five tinctorial cell types, described for the pars distalis of *Bufo bufo,* were observed: carminophils, orangeophils, globular basophils, and violet cells.

In most of the controls the carminophils and orangeophils were storing secretory granules; the globular basophils and the violet cells showed signs of secretory activity.

All four cell types remained largely unchanged in the area of the homoiotopic grafts, bordering the median eminence and pars intermedia.

In the rest of such grafts and in the heterotopic transplants cysts were formed, and degeneration and dedifferentiation of cells took place; carminophils and orangeophils had slightly degranulated, globular basophils had become strongly regressed in number and granulation, and violet cells had almost completely disappeared.

Generally, there existed a close correlation between the state of the target organs and the morphological signs of secretory activity of the pars distalis cells. It is suggested that not only the globular basophils, but also the violet cells have a gonadotropic function, and are responsible for the development and maintenance of the interstitial Leydig cells in the testis.

It is well-established that functions of the pars distalis of the vertebrate hypophysis may be under central nervous control, and for more than 10 years the nature of this control and the identification of the hypothalamic structures that mediate it have been a focus of interest (for literature, see Harris and Donovan, 1965; Reichlin, 1963; Jørgensen, 1965; Jørgensen and Larsen, 1966). Furthermore it is known that the various pars distalis functions are not equally dependent upon central nervous control, and that varying degrees of activity can be demonstrated in the absence of normal connections between hypophysis and central nervous system (see review by Ball *et al.*, 1965).

Nonmammalian vertebrates have been less extensively investigated than mammals, and further comparative studies are necessary to determine how far the various functions of the pars distalis are dependent on central nervous control in these lower groups.

In the present paper the importance of the central nervous control on various pars distalis functions, as exerted by means of the normal hypothalamic-hypophysial connections, has been evaluated in the toad *Bufo bufo* by investigating the effect of ectopic transplantation of the gland. Furthermore, by studying the cytology of the pars distalis it was hoped that correlative changes in pars distalis cell types and target organs might reveal their functional significance. Preliminary reports have already been published (Jørgensen, 1963; Larsen, 1963; Lofts, 1963; van Oordt, 1963b; Rosenkilde, 1963).

MATERIALS AND METHODS

Two series of experiments were performed, one in the autumn of 1962 lasting for 10 weeks and the other in the spring of 1964 lasting for 6½ weeks. In both experiments only sexually mature specimens were used (except one female in the autumn experiment).

Specimens of both sexes were captured in August, 1962 and mid-April, 1964 and transferred to the laboratory where they were kept in groups of 4 in large glass containers with a little water. Only spent females were used (determined by palpation).

In the 1962 experiment force-feeding with minced ox meat mixed with wheat bran and sand was given every fifth day, but the toads consistently lost weight (Table 1). In the 1964 experiments, therefore, the toads were fed thrice weekly on meal worms or a mixture of minced pig liver, wheat bran, wheat flour, and sand; in animals

TABLE 1
DATES OF AUTUMN EXPERIMENT 1962. SURVIVAL AND WEIGHT CHANGES OF TOADS

Exptl. groups	Sex	No. of toads at start	No. of toads at end	Change in body wt. (%)[b]	Date of operation	Date of decapitation
Normal controls	♂	8	—	—	—	August 20
	♀	8	—	—	—	
Normal controls	♂	8	8	−7	—	October 29–30
	♀	8	8[a]	−5	—	
Pars distalis extirpated	♂	8	8	−14	August 21–25	
	♀	8	3	−14		
Pars distalis retranspl.	♂	8	8	−4		
to median eminence	♀	8	8	−9		
Pars distalis transpl.	♂	8	8	−13		
to eye muscle	♀	8	7	−13		

[a] One of these (specimen no. 1142) was infantile and was excluded from judgment of gonadotropic function.
[b] Weight at the start of the experiment: ♂♂ 31–52 gm (mean 41 gm); ♀♀ 52–105 gm (mean 72 gm).

TABLE 2
DATES OF SPRING EXPERIMENT 1964. SURVIVAL AND WEIGHT OF CHANGES TOADS

Exptl. groups	Sex	No. of toads At start	No. of toads At end	Change in body wt. (%)[a]	Date of operation	Date of decapitation
Normal controls	♂	10	—	—	—	May 15
	♀	7	—	—	—	
Normal controls	♂	8	8	+13	—	June 29
	♀	8	8	+11	—	June 23
Pars distalis extirpated	♂	8	8	+11	May 13–14	June 29
	♀	8	8	+8		June 23
Pars distalis retranspl.	♂	8	8	+16		June 29
to median eminence	♀	8	7	+10		June 23
Pars distalis transpl.	♂	8	8	+11		June 29
to eye muscle	♀	8	8	+13		June 23

[a] Weight at the start of the experiment: ♂♂ 23–43 gm (mean 33 gm); ♀♀ 56–144 gm (mean 81 gm).

which lagged behind in weight increase, this diet was supplemented with grasshopper nymphs. The result was that these toads consistently gained in weight (Table 2).

In the spring experiment 10 normal males and 7 females, and in the autumn, 8 males and 8 females, were killed for autopsy at the start. In both years 8 unoperated males and 8 females served as normal controls at the end of the experiments. The remaining specimens were subjected to one of three types of operations: (1) extirpation of pars distalis, (2) extirpation of pars distalis and autografting under the median eminence, or (3) extirpation of pars distalis and autografting to the cut surface of an eye muscle. Each experimental group included 8 males and 8 females.

In the experimental animals an incision was made in the mucosa of the mouth and the retractor muscles of the eyes were sectioned. The parasphenoid was broken off and the hypophysis exposed by removal of cartilage and meninges. Subsequently, the pars distalis was separated from the median eminence and cautiously pulled off from the neurointermediate lobe. The pars distalis was discarded or autotransplanted according to the type of operation intended, and finally the parasphenoid was replaced and the mucosa sewn. In the autumn experiment the pars distalis was randomly oriented when transplanted to the median eminence, whereas it was normally oriented in the spring experiment.

Previous work has shown that the condition of molting and the length of survival in the toad can be used to evaluate the corticotropic function of the pars distalis (Jørgensen and Larsen, 1963). Furthermore, by using these criteria it has been demonstrated that the ectopically transplanted pars distalis exerts no corticotropic function (Jacobsohn and Jørgensen, 1956). In order to study the effect of extirpation or ectopic transplantation on functions other than corticotropic, therefore, the experimental animals must be treated with ACTH or corticosteroids. In the present investigation 10 milliunits of ACTH (Corticotropin, Orthana) was injected 3 times a week into the dorsal lymph sac and was sufficient to maintain most of the toads in a tolerably healthy condition throughout the experiment (Jørgensen and Larsen, 1963).

In the groups in which the pars distalis was retransplanted on the median eminence reestablishment of corticotropic function was followed by observing the condition of molting and general state of health.

The dates on which specimens were killed are given in Tables 1 and 2. After weighing the whole animal, the gonads and fat bodies were dissected out, weighed, and then fixed for histological and histochemical examination, together with the adrenals, thyroids, and pars distalis. The following histological techniques were used:

Testis. The testes were examined from all the males. Bouin-fixed material was wax-embedded and sectioned at 4 μ. Sections were stained with iron hematoxylin and orange G for estimation of the spermatogenetic condition. The degree of spermatogenetic activity was estimated by counting all cell nests in different spermatogenetic stages in 20 cross sections of testis tubules. The mean for each spermatogenetic stage was then estimated and used as representative of the condition in that particular specimen. The different stages were represented as follows:

O = cell nests with one primary spermatogonium
I = cell nests with secondary spermatogonia
II = cell nests with primary spermatocytes
III = cell nests with secondary spermatocytes
IV = cell nests with spermatids
V = sperm bundle attached to a Sertoli cell.

Testicular material was also fixed in formal-calcium (autumn experiment) or in formal-saline (spring experiment) (Baker, 1949), and embedded in gelatin. Thin sections (4 μ) were colored with Sudan Black B to reveal lipids and with carmalum to demonstrate nuclei. Thicker sections (10 μ and 15 μ) were subjected to the Schultz test for cholesterol.

Thumb-Pads. In both series, the thumb-pads of the males were inspected during the experiment. Moreover, in the spring experiment the thumb-pads were fixed in Bouin's solution, sectioned at 6 μ, and stained with hematoxylin and eosin. The thickness of the epithelium was measured in 10 sections from the same pad, and the mean used as an indication of the functional activity of the interstitial tissue.

Ovary. Ovarian differentiation was estimated from histological examination (1962 experiment) or from direct inspection under the Zeiss operation microscope (1964 experiment) of Bouin-fixed material. Special attention was given to the presence or absence of gonadotropin-exacting stages of oocytes in the groups with the pars distalis transplanted.

Adrenal Gland. Only the adrenals of the males in the spring experiment were examined. One kidney + adrenal was fixed in Helly's stock solution, sectioned at 7 μ (and stained with hemalum-eosin). The other was fixed in formal-saline, embedded in gelatine, and frozen sections of 7–10 μ were stained for lipids (Sudan Black B) and cholesterol (Schultz).

^{131}I Uptake. In the autumn experiment the thyrotropic function was evaluated by measuring the ^{131}I uptake in the thyroids. Five weeks after the operation, ^{131}I (carrier free NaI) was injected. The males received 10 μc and the females 16 μc; these doses gave about equal thyroidal counting in normal toads of the two sexes. After 7 days the ^{131}I content of the thyroids was measured by a screened scintillation counter with a narrow opening slit. The radioactivity was determined first in the thyroid zone at the neck with a double counting, and then in a neutral area. The difference between the neck count and the neutral count expressed the thyroidal ^{131}I content.

Pars Distalis. The hypophysial region and the transplants were fixed in sublimated Bouin's fluid and embedded in paraffin wax. The pars distalis from 5 animals in each group were sectioned at 4 μ and stained with Cleveland and Wolfe's trichrome stain (erythrosin, orange G, aniline blue), Herlant's (1960) Alcian blue (AB) at pH 0.2—periodic acid Schiff (PAS)-orange G technique and Herlant's (1960) tetrachrome stain (E. Gurr's Acid Alizarin blue, erythrosin, orange G, aniline blue). The cell nuclei were counterstained with Hansen's iron trioxyhaematin (Romeis, 1948).

The methods enabled the vizualization of the five chromophilic cell types previously reported in the pars distalis of *B. bufo* (Zuber-Vogeli, 1953) and other anuran species (Guardabassi and Grosso, 1959; van Oordt, 1961, 1963a,b). The names used by these authors for the cell types are given in the first three columns of Table 3.

As a result of international discussions upon the nomenclature of the hormone-producing cell types in the adenohypophysis, it has been suggested to abandon the Greek letter system (Benoit and daLage, 1963; van Oordt, 1965). It was, therefore, decided to use the names given in the last column of Table 3. These names are based on the original ones introduced for *Bufo* by Zuber-Vogeli (1953), with the exception that "azanophil" was replaced by "carminophil," as being more characteristic for the cell type of that name.

Experimental evidence seems to justify the use of the functional name "gonadotrop" for the globular basophils (Zuber-Vogeli, 1953). But functional names cannot be used for the other cell types, as their function is either unknown or uncertain (van Oordt, 1963a).

The cell types may be characterized as follows:

Carminophils. Evenly distributed, big elongated or somewhat irregular cells, filled with small granules that are completely AB and PAS negative, but stain with erythrosine and orange G. The rostrally situated carminophils are slightly bigger than the more caudally situated ones; a common feature of the anuran pars distalis (Dierickx, 1963). The nuclei are irregularly shaped; nucleoli are distinct.

Orangeophils. Rounded or somewhat elongated cells; smaller than the carminophils; often in groups and bordering blood sinuses; concentrated in the dorsocaudal part of the pars distalis. The fine granules are orange G positive and stain very faintly with PAS. This gives these cells a slightly more brownish yellow color than the carminophils when the sections are stained with Herlant's AB-PAS-orange G. The best contrast between these two types of acidophilic cells can, however, be obtained with Herlant's tetrachrome that stains the carminophils red and the orangeophils yellowish-orange.

Globular Basophils. These cells are the most prominent in size and number. The staining method of choice is Cleveland and Wolfe's trichrome technique which shows very fine aniline blue-positive material, as well as both finer and coarser orangeophilic granules. Herlant's AB-PAS-orange G technique stains some granules blue and others purple, but there is no clear correlation between the color of individual granules stained by these two methods. Herlant's tetrachrome stains practically all granules in the globular basophils blue; only some very coarse granules or globules may become yellow or red. The cells are evenly distributed, but are bigger and more columnar in shape in the rostroventral than in the dorsocaudal regions of the pars distalis. Similarly the orangeophilic granules are often more abundant in the rostroventral than in the dorsocaudal cells where the aniline-blue positive inclusions tend to predominate. The nuclei are usually ovoid, and often contain one or two distinct nucleoli.

Small Basophils. These are small, containing fine, aniline blue positive granules, and usually situated in the central, lateral, and caudal portion of the pars distalis. With Herlant's AB-PAS-orange G or tetrachrome technique they cannot be distinguished from small globular basophils; in sections stained with Cleveland and Wolfe's trichrome this distinction is only possible if these latter cells contain numerous orangeophilic granules, and are thus not predominately blue in color.

Violet Cells. These are almost completely restricted to the mediorostral and rostroventral parts of the pars distalis, and border the branches of the portal vessels. Only a few violet cells are present in the rostrodorsal and central regions, and they are absent in the dorsocaudal parts. They are elongated cells with ovoid, apical nuclei, containing conspicuous nucleoli. The very fine cytoplasmic granules stain violet with Cleveland and Wolfe's trichrome, and deep blue or purple with Herlant's tetrachrome. A better staining method for these cells, however, is Herlant's AB-PAS-orange G; with this the pink or brick-red cells stand out very clearly against the yellow acidophils and the blue basophils.

Abbreviations. ACTH, adrenocorticotropin; TSH, thyrotropin; FSH, follicle stimulating hormone; LH (ICSH), luteinizing hormone; GH, somatotropin.

TABLE 3
NOMENCLATURE OF CELL TYPES IN PARS DISTALIS OF THE TOAD HYPOPHYSIS

Zuber-Vogeli (1953)	Guardabassi (1959, 1960)	van Oordt (1961, 1963a,b)	Present paper
Cellules azanophiles	cellule acidofile di secondo tipo	carminophils	carminophils
Cellules orangeophiles	cellule acidofile di primo tipo	orangeophils	orangeophils
Grandes cellules basophiles à globules acidophiles	cellule gonadotrope δ	β-cells	globular basophils
Petites cellules basophiles ou cyanophiles	piccole cellule basofile	δ-cells	small basophils
Cellules mauves ou violettes	cellule tireotrope β	γ-cells	violet cells

TABLE 4
WEIGHTS OF TESTES

Exptl. groups	Autumn expt. (mg/100 gm of body wt.)	Spring expt. (mg/100 gm of body wt.)
	S.E.	S.E.
Normal controls at start	470 ± 33	426 ± 24
Normal controls at end	A 416 ± 28	G 407 ± 39
Pars distalis extirpated	B 194 ± 23	H 280 ± 27
Pars distalis retranspl. to median eminence	C 455 ± 18	I 378 ± 37
Pars distalis transpl. to eye muscle	D 399 ± 39	K 347 ± 18

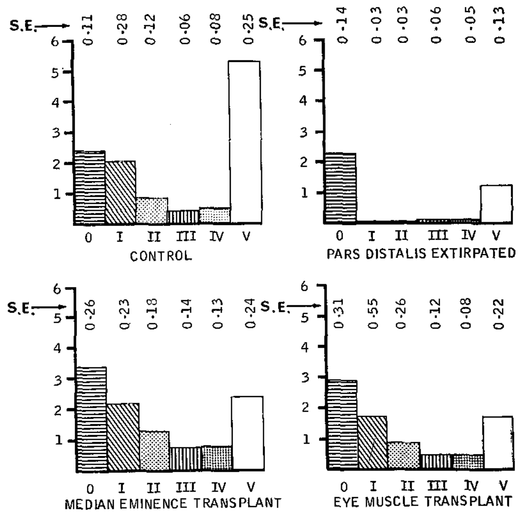
Note. t tests for identity gave the following results: A = B, $p < 0.001$; A = D, $p > 0.7$; B = D, $p < 0.001$; G = H, $p < 0.02$; G = I, $p = 0.6$; G = K, $p \sim 0.2$; H = I, $p < 0.055$; H = K, $p < 0.07$; I = K, $p > 0.4$.

RESULTS

GONADOTROPIC FUNCTION

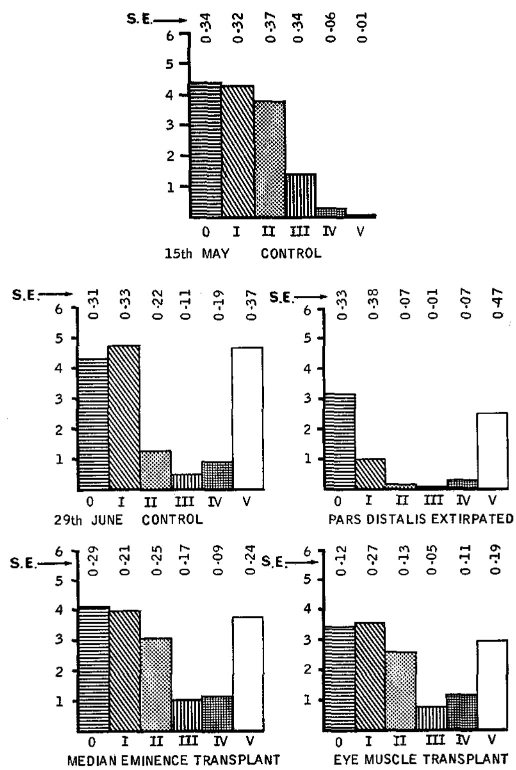
Testes. Extirpation of the pars distalis resulted in a decrease in testicular weight, both in the autumn and spring experiments, whereas the weight of testes in the toads of all groups with pars distalis transplanted did not differ significantly from the normal controls (Table 4).

Seminiferous Tubules. The condition of the seminiferous tubules were in general agreement with the data on testicular weights (Figs. 1 and 2). The tubules of the normal controls showed well marked spermatogenetic activity. In the spring experiment there was a greater production of early stages. The differences between the control animals of May and June show a considerable production of sperm cells, and also indicate that the transformation of primary spermatocytes into secondary spermatocytes was stronger than the transformation of secondary spermatogonia into primary spermatocytes (Figs. 2, 3 and 7). By the autumn many cell nests had matured into sperm bundles so that an increase in the latter paralleled a decrease in cell nests (Fig. 1). The Sertoli elements were without cytoplasmic lipid droplets and gave a negative cholesterol reaction.



AUTUMN EXPERIMENTS 1962

FIG. 1. Effect of extirpation and transplantation of pars distalis of the hypophysis on spermatogenetic activity in the testis of *B. bufo*. For explanation of symbols see text p. 494.



SPRING EXPERIMENTS 1964

FIG. 2. See legend to Fig. 1.

Extirpation of the pars distalis was followed by a regression of the seminiferous tubules. In the autumn experiment no spermatogenetic activity was observed, except in specimen no. 1167, and the tubules contained only inactive primary spermatogonia and degenerating sperm bundles. Many of the Sertoli cells had become detached from the tubule wall and contained small cytoplasmic sudanophilic droplets that reacted faintly to the Schultz test.

In the spring experiment the testes did not regress to the same extent as in the autumn experiments; cell nests were still present at the end of the experiment, although significantly less than in the normal controls, except in specimen no. 2100. This may be due to the shorter postoperative period. Lipoidal Sertoli cells were present in many tubules, however, and no mitotic figures were observed.

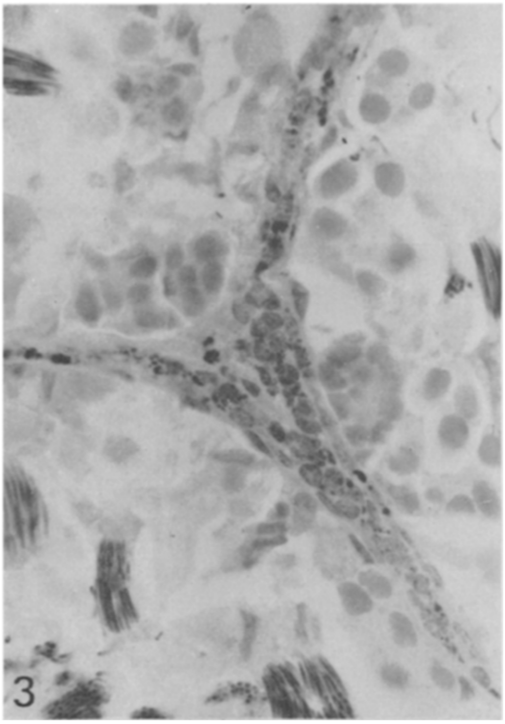
All the groups with the pars distalis transplanted showed pronounced spermatogenetic activity (Figs. 1 and 2). The activity in the toads with pars distalis transplanted to an eye muscle was well marked, but never as much as in the toads with pars distalis retransplanted onto the median eminence. The latter groups did not differ significantly from the normal controls (Figs. 6 and 8). There were relatively few lipoidal Sertoli cells and no Schultz reaction.

Interstitium and Thumb-Pads. The results of the autumn experiments are shown in Table 5 and those of the spring experiments in Table 6. The interstitial tissue of the testes of the normal toads from both series of experiments was extensive, and the cells contained numerous small cytoplasmic lipid droplets that gave a weak cholesterol reaction. The cell nuclei were large and round with fairly coarse, clumped chromatin, but no obvious nucleolus. This is referred to as a secretory type nucleus (Fig. 3). The thumb-pads were well-developed and could be correlated with the condition of the interstitial tissue. The height of the epithelium of the thumb-pads was measured in the spring experiments only and was found to be thick and papillate (Tables 5 and 6).

Extirpation of the pars distalis produced a regression of the thumb-pads, the epithelium becoming thinner and smooth. This was accompanied by parallel changes in the interstitial cells; the nuclei became shrunken and wrinkled in outline, and the chromatin was often fine with a conspicuous nucleolus; they were thus of the nonsecretory type (Fig. 4). The cytoplasmic lipids built up to a dense mass which gave a marked cholesterol-positive reaction (Fig. 5). These regressive changes occurred to a greater extent in the fall experiments, possibly due to the longer postoperative period (Tables 5 and 6).

In the toads with pars distalis transplanted onto the median eminence the activity of the interstitial tissue was maintained in both series of experiments (Tables 5 and 6; Fig. 6). Thumb-pads were well developed and many nuclei of the secretory type were present in the interstitium. The cells contained numerous discrete lipid droplets giving a cholesterol-positive reaction. In the spring series, two specimens were exceptions to this rule. Specimen no. 2113 had poorly developed thumb-pads and relatively few secretory type nuclei were seen. Specimen no. 2107 had a relatively thick epithelium, but it was not papillate, and the interstitial cells were largely of the nonsecretory type.

Transplantation of the pars distalis to an eye muscle produced regression of the thumb-pads; but at a slower rate than in the toads with the pars distalis extirpated. In the fall experiments thumb-pads thus still persisted in the group with the transplants on 4 October (Table 5), whereas they were completely regressed in the hypophysectomized toads. On 14 October, however, the thumb-pads had also completely regressed in all but one of the toads with an eye muscle transplant. In the spring, at the end of the experiment, the thumb-pads were generally less regressed in the group with the transplants than in the group lacking the pars distalis. The mean thickness of the epithelium was greater in the former group, and the thumb-pads were all more or less pronouncedly papillate, whereas papillate thumb-pads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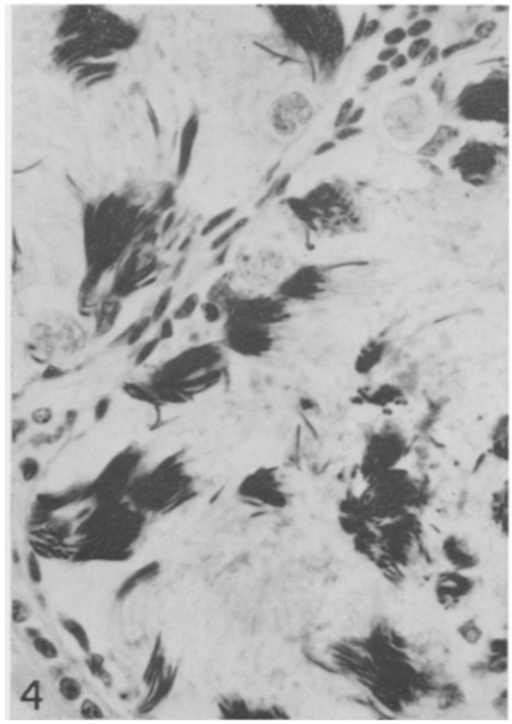
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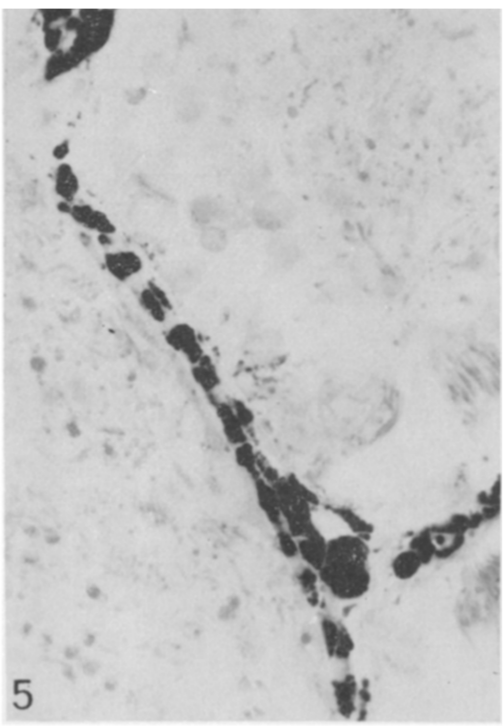
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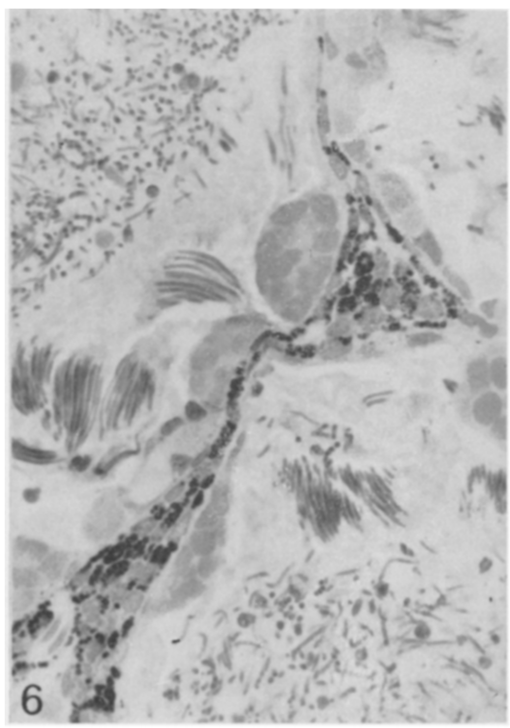
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TABLE 5
EVALUATION OF INTERSTITIAL CELL ACTIVITY IN AUTUMN EXPERIMENT

Exptl. groups	Specimen no.	Thumb-pad color Oct. 4	Oct. 14	Oct. 23	Oct. 27	Nucleus type	Lipid content
Normal controls	B 1149	xx	xx	xx	xx	S	+
	50	xx	xx	xx	xx	S	+
	51	xx	xx	xx	xx	S	+
	52	xx	xx	xx	xx	S	+
	53	xx	xx	xx	xx	S	+
	54	xx	x	xx	xx	S	+
	55	x	xx	x	xx	S	++
	56	xx	x	—	xx	S	+
Pars distalis extirpated	B 1165	—	—	—	—	N.S.	++
	66	—	—	—	—	N.S.	+++
	67	—	—	—	—	N.S.	+++
	68	—	—	—	—	N.S.	+++
	69	—	—	—	—	N.S.	+++
	70	—	—	—	—	N.S.	+++
	71	—	—	—	—	N.S.	++
	72	—	—	—	—	N.S.	+++
Pars distalis transpl. to median eminence	B 1181	xx	xx	xx	xx	S	++
	82	xx	xx	xx	xx	S + few N.S.	++
	83	—	xx	xx	xx	S	++
	84	—	—	x	xx	S	++
	85	x	xx	xx	xx	S + N.S.	+++
	86	xx	xx	xx	xx	S	+
	87	xx	xx	xx	xx	S	++
	88	—	xx	xx	xx	S	+
Pars distalis transpl. to eye muscle	B 1197	x	—	—	—	N.S. + few S	+++
	98	xx	—	—	—	N.S. + few S	+++
	99	xx	x	—	—	S. + N.S.	++
	1200	xx	—	—	—	N.S.	+++
	01	xx	—	—	—	N.S. + few S	++
	02	xx	—	—	—	N.S. + few S	++
	03	xx	—	—	—	N.S.	+++
	04	xx	—	—	—	N.S. + few S	++

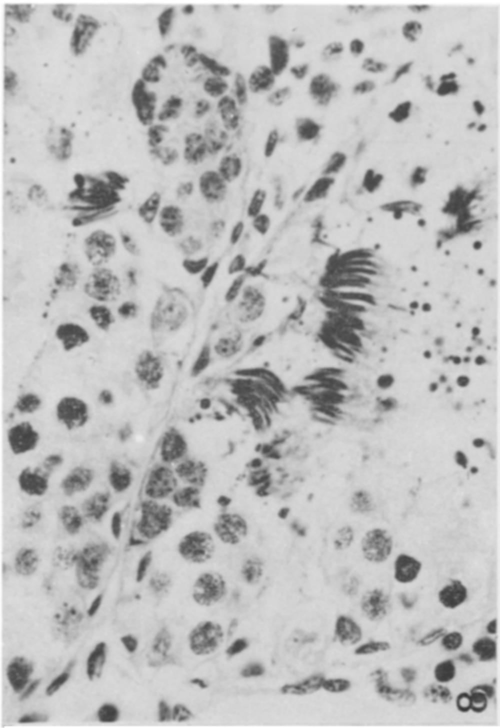
Note. xx, darkbrown; x, light brown; —, colorless or absent; S, secretory; N.S., nonsecretory.

FIG. 3. Testis of control toad (June, 1964). The interstitial tissue contains large rounded nuclei and small lipoidal droplets. The adjacent seminiferous tubules contain several nests of developing germ cells. Formal saline fixation; Sudan Black and carmalum staining. ×480.

FIG. 4. Testis of hypophysectomized toad (1962). The interstitial tissue has regressed and nuclei are shrunken and no longer round. Spermatogenesis has terminated and only primary spermatogonia and loosened sperm bundles are present in the tubules. Bouin fixation; iron hematoxylin and orange G staining. ×480.

FIG. 5. Testis of hypophysectomized toad (1964). A dense mass of cholesterol-positive lipid accumulates in the interstitial cells. Technique as in Fig. 3.

FIG. 6. Testis of toad with pars distalis transplanted onto the median eminence (1964). Spermatogenesis is well established, and nests of developing germ cells can be seen in the seminiferous tubules. The interstitial tissue is well developed with rounded nuclei and numerous small sudanophilic droplets. The technique as in Fig. 3.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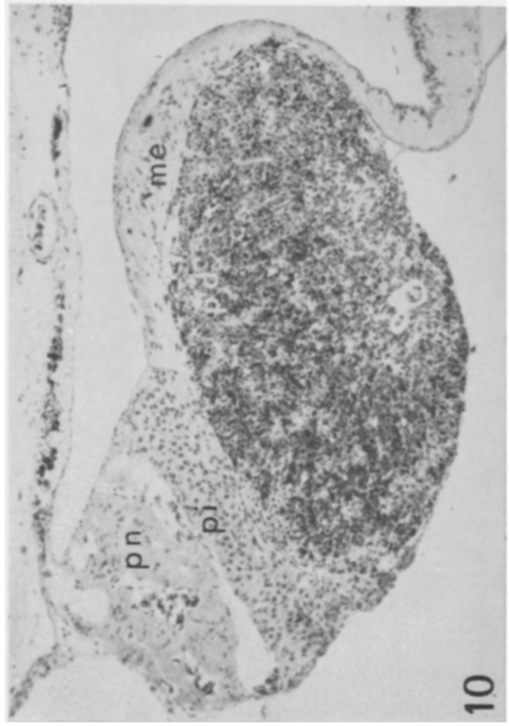
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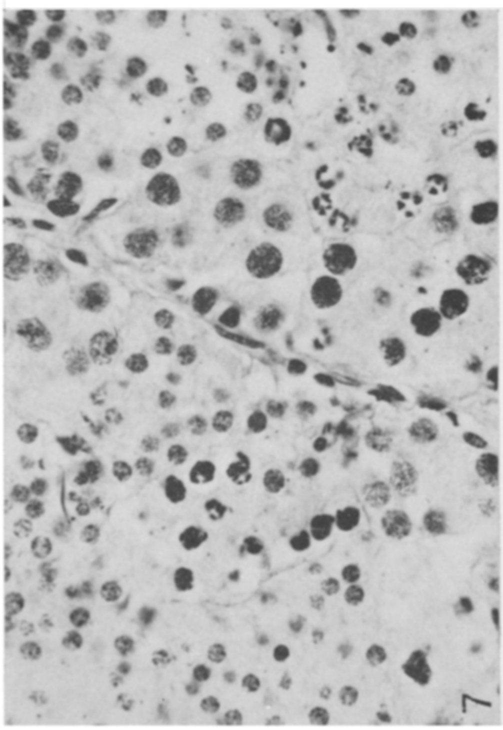
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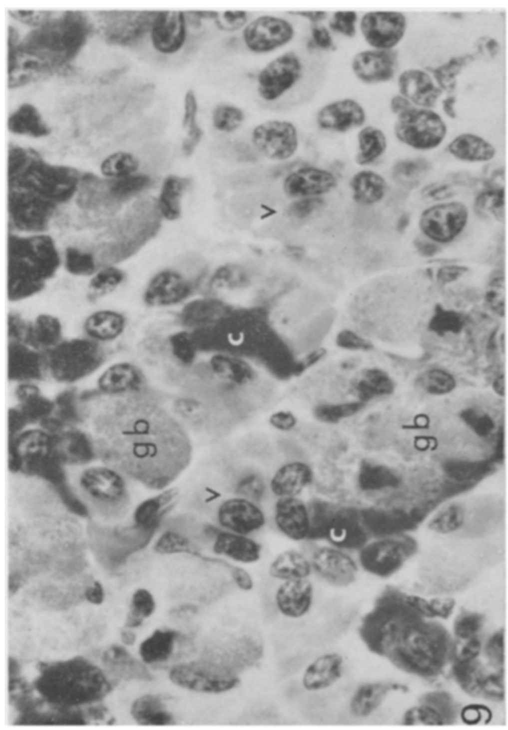
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were only found in one of the hypophysectomized toads.

The cytology of the interstitium is in general agreement with these results (Tables 5 and 6). In the autumn experiment generally few secretory interstitial cells were observed, whereas in the spring experiment, interstitial cells of both the secretory and the nonsecretory type often occurred together.

Ovary. The data on ovarian weight are summarized in Table 7. The effect of transplanting the pars distalis apparently varied with the season.

In the autumn experiment, the ovaries were already mature at the beginning of the experiment but grew somewhat during the experiment (Table 7). The increase in ovarian weight from 9.8 to 13.0% of the body weight in this group does not express the absolute growth of the ovaries in the experimental period because the toads lost 5% in weight. Ovarian weight at the end of the experiment is thus only 12.3% of the initial body weight. The calculated increase in weight of the ovaries during the experiment is therefore about 26%. This increase is significant at the 1% level. The marked reduction in weight after extirpation was mainly due to the disappearance of all mature eggs that were reduced to small atretic follicles, mostly consisting of pigment. The ovaries of the toads with the pars distalis transplanted to an eye muscle were slightly heavier, but histologically similar to those of the hypophysectomized controls with maximum oocyte size of about 600–700 μ. In toads with the pars distalis retransplanted under the median eminence, 5 had similarly reduced ovaries, but 3 had ovaries that were of about the same weight as the normal controls and filled with normally sized mature eggs.

In the spring experiment, the ovaries were in the beginning of the growth phase at the start of the experiment and the oocytes did not reach maximum size during the experiment. At the finish, ovarian weight of normal controls had increased from 3.2% to 5.4% of the body weight, whereas in the group without the pars distalis the weight had decreased to 1.5%. In both groups with transplanted pars distalis ovarian weights were about the same as at the beginning of the experiment (Table 7).

These results are in general agreement with the variation in maximum oocyte size (Fig. 15). After extirpation of the pars distalis the larger oocytes degenerated, and at the end of the experiment only the small gonadotropin-independent stages remained. In the normal controls the oocytes continued to grow, but none developed to the normal maximum of about 1550–1700 μ. In the groups of toads with transplanted pars distallis the maximum oocyte size was about the same as at the start of the experiment.

Adrenocorticotropic and Adrenocortical Function

The efficiency of ACTH treatment in maintaining the toads was assessed by ob-

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Fig. 7. Testis of control toad (May, 1964). Spermatogenesis is very active and the tubules are filled with nests of developing germ cells. Bundles of sperms have not yet been produced. Technique as in Fig. 4.

Fig. 8. Testis of toad with pars distalis transplanted onto an eye muscle (June, 1964). It can be seen that spermatogenesis is occurring and numerous nests of developing germ cells are present in the tubules. The interstitial tissue, however, is regressing and rounded nuclei are relatively rare in this specimen (no. 2120). Technique as in Fig. 4.

Fig. 9. Cells in the rostral part of the distal lobe of female control toad no. 1146 from the autumn experiment, stained with Cleveland and Wolfe's trichrome. ×750; film Agfa 13/10° Din; filter Schott VG9. c = carminophils, gb = globular basophils, v = violet cells.

Fig. 10. Median sagittal section through pituitary of female toad no. 1178 with homoiotopically transplanted pars distalis, from the autumn experiment, stained with Cleveland and Wolfe's trichrome. ×80; film Agfa 13/10° Din, filters Schott BG 23 and VG9. me = median eminence, pd = pars distalis, pi = pars intermedia, pn = pars nervosa. Note normal tissue in dorsal part of distal lobe and dedifferentiated tissue, including small cyst, in ventral part.

TABLE 6
EVALUATION OF INTERSTITIAL CELL ACTIVITY IN SPRING EXPERIMENT

Exptl. groups	Specimen no.	Height of thumb-pad epithelium (μ)	Nucleus[a] type	Lipid content
Normal controls	B 2091	115	S + few N.S.	++
	92	148	S	+
	93	160	S	+
	94	160	S	+
	95	200	S	+
	96	148	S + few N.S.	+
	97	160	S	+
	98	115	S	+
		Mean 150.75		
		SE ± 9.69		
Pars distalis extirpated	B 2099	56	N.S.	+++
	2100	185	S + N.S.	+
	01	115	N.S.	++
	02	56	N.S.	++
	03	111	N.S.	++
	04	56	N.S.	++
	05	75	N.S. + few S	+
	06	68	N.S.	++
		Mean 90.25		
		SE ± 15.97		
Pars distalis retranspl. to median eminence	B 2107	150	N.S. + few S	++
	08	130	S + few N.S.	++
	09	185	S + few N.S.	+
	10	148	S	+
	11	185	S	+
	12	130	S + few N.S.	+
	13	75	N.S. + few S	++
	14	185	S	+
		Mean 148.50		
		SE ± 13.42		
Pars distalis transpl. to eye muscle	B 2115	160	S + N.S.	+
	16	100	S + N.S.	+
	17	75	S + N.S.	++
	18	75	S + N.S.	++
	19	130	S + few N.S.	+
	20	95	N.S. + few S	+++
	21	200	S + N.S.	+
	22	148	S + N.S.	+
		Mean 122.88		
		SE ± 15.75		

[a] S = secretory; N.S. = nonsecretory.

TABLE 7
WEIGHTS OF OVARIES

Exptl. groups	Autumn expt. (as % of body wt.) S.E.	Spring expt. (as % of body wt.) S.E.
Normal controls at start	9.8 ± 0.44	A 3.15 ± 0.24
Normal controls at end	13.0 ± 0.54	B 5.37 ± 0.25
Pars distalis extirpated	3.3	C 1.46 ± 0.14
Pars distalis retranspl. to median eminence	11.1 (av. 3 toads) 3.6 (av. 5 toads)	D 3.03 ± 0.22
Pars distalis transpl. to eye muscle	3.6 ± 0.19	E 3.07 ± 0.46

Note. t tests for identity gave the following results: A = B, $p < 0.001$; A = C, $p < 0.001$; B = E, $p < 0.001$; C = E, $p < 0.01$.

serving their liveliness and agility in turning when placed on their back. The data are summarized in Table 8.

It can be seen that among the toads receiving ACTH the spring animals were generally in a better condition than those from the autumn experiment. This is probably at least partially due to the longer duration of the autumn experiment. In the latter the males were maintained in a better condition than the females of which less than half survived to the end, whereas the spring females remained healthier than the males. On the whole the condition of the toads with an ectopic pars distalis was not superior to that of the toads without the pars distalis.

In the groups of toads with the pars distalis transplanted under the median eminence, adrenocorticotropic function was normal in all but specimens no. 2107, a male which was sluggish and showed abnormal molting, and no. 1180, a female which molted normally for only a short period. Adrenal cytology indicated that the corticotropic activity was reduced in the former (Table 9).

In the adrenocortical tissue of the normal toads the cell nuclei were round and the cytoplasm compact. In some, many of the cells contained a vacuole close to the nucleus (paranuclear vacuole), while in others, such vacuoles were absent. The size and appearance of the cells varied, being small and separate in some toads, but large and closely packed in others.

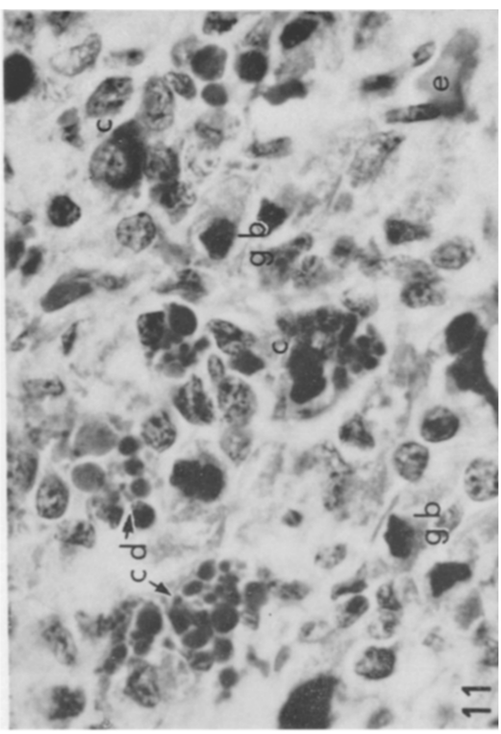
The cytoplasm contained a little lipid which was mostly cholesterol negative (Table 9). The content of the paranuclear vacuoles was always Sudan negative.

In the toads with pars distalis extirpated the adrenocortical cells were shrunken and the nuclei were closely packed together. Moreover, the nuclei were smaller than those of the normal controls and more or less irregular in outline. Paranuclear vacuoles were absent, except in specimen no. 2099. The adrenocortical tissue of this

TABLE 8
GENERAL CONDITION OF TOADS AT END OF EXPERIMENTS

		No. of toads			
		Autumn expt.		Spring expt.	
Exptl. groups	Sex	Normal	Subnormal to bad	Normal	Subnormal to bad
Normal controls	♂	8	0	8	0
	♀	8	0	8	0
Pars distalis transpl. to median eminence	♂	8	0	7	1
	♀	7	1	6	1
Pars distalis transpl. to eye muscle[a]	♂	1	7	3	5
	♀	0	4	6	2
Pars distalis extirpated[a]	♂	0	8	1	7
	♀	0	3	8	0

[a] The toads in these groups were injected with 10 milliunits of ACTH thrice a week.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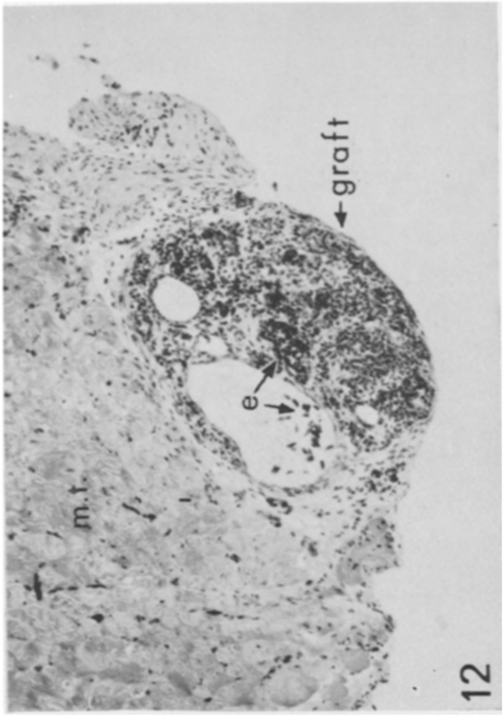
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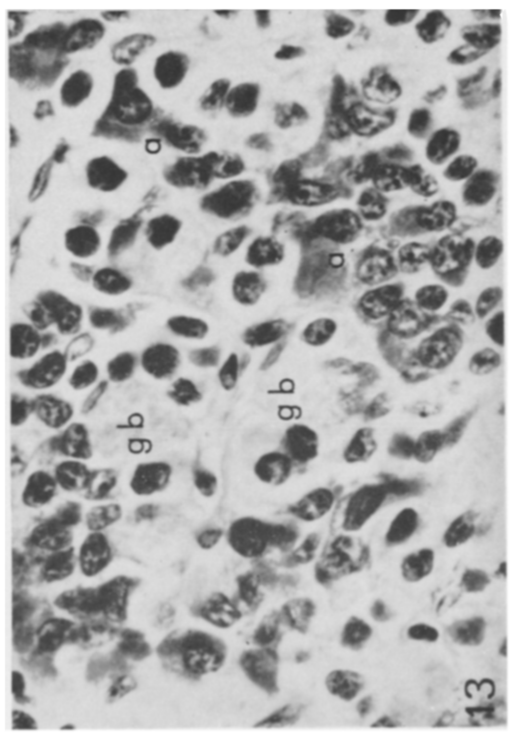
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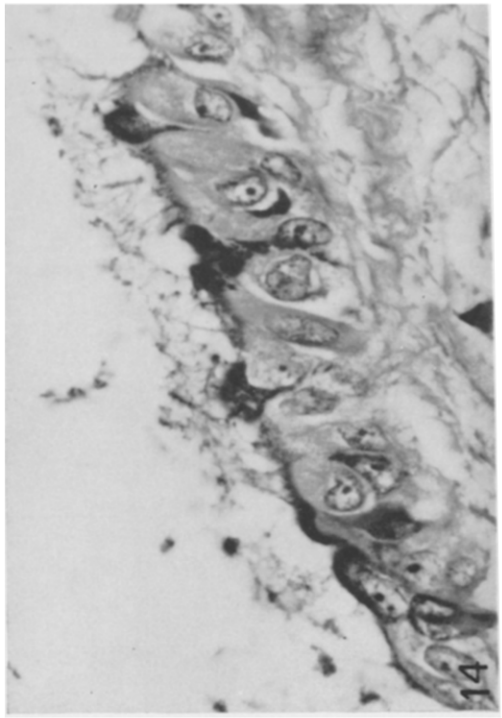
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TABLE 9
EVALUATION OF ADRENOCORTICAL ACTIVITY OF MALES IN SPRING EXPERIMENT

Exptl. groups	Specimen no.	Lipid	Cholesterol	Liveliness	Turning reflex
Normal controls	B 2091	±	–	1	1
	92	±	±	1	1
	93	lost		1	1
	94	±	±	1	1
	95	+	±	1	1
	96	+	–	1	1
	97	±	±	1	1
	98	+	±	1	1
Pars distalis extirpated	B 2099	++++	±	3	3
	2100	+++	+++	2	2
	01	++	–	2	2
	02	++	–	1–2	1
	03	+++	+++	1–2	2
	04	++	–	1–2	2
	05	++	–	1–2	2
	06	++	±	1–2	1
Pars distalis retranspl. to median eminence	B 2107	+++	++	2	3
	08	±	–	1	1
	09	±	±	1	1
	10	±	±	1	1
	11	+	–	1	1
	12	++	+	1	1
	13	±	±	1	1
	14	+	+	1	1
Pars distalis transpl. to eye muscle	B 2115	+++	±	1–2	2
	16	+	±	1–2	2
	17	++	±	1	1
	18	++	++	1	2
	19	+	–	1	1
	20	+	±	1	1
	21	lost		2	1
	22	++++	+++	1–2	2

Note. ± very little lipid, or very weak Schultz reaction for cholesterol; + → ++++ little to very much lipid, or weak to very strong reaction for cholesterol. 1 normal; 2 sluggish; 3 very sluggish.

FIG. 11. Section through ventral area of the homoiotopically transplanted pars distalis of female toad no. 1177 from the autumn experiment. cd = inter- and intracellular colloid droplets, e = erythrocyt. Other abbreviations and technical details as in Fig. 9.

FIG. 12. Section through ectopic pars distalis of male toad no. 1201 from the autumn experiment. Note many erythrocytes—some indicated with the letter e—in the graft and in the muscular tissue (mt). Technical details as in Fig. 10.

FIG. 13. Section through ectopic pars distalis of female toad no. 2088 from the spring experiment. Note partly degranulated acidophils (a) and almost empty globular basophils (gb). Technical details as in Fig. 9.

FIG. 14. Section through epithelial cells bordering cyst in ectopic pars distalis of male toad no. 2121 from the spring experiment, stained with Herlant's AB-PAS-orange G method. Note cilia and dark AB positive material in the apical parts of the cells. Other technical details as in Fig. 9.

specimen was similar to that of the normal controls.

The cytoplasm was very lipoidal but only weakly responsive or negative to the cholesterol test. Specimens no. 2100 and no. 2103 (Table 9), however, were strongly cholesterol positive.

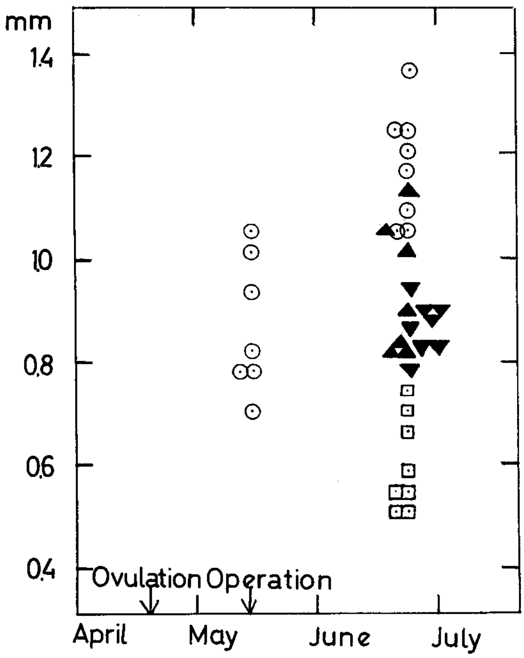


FIG. 15. Effect of extirpation and transplantation of pars distalis on oocyte development. ⊙, diameter of the largest oocytes in the ovaries of normal control toads at start and end of experiment; ⊡, of hypophysectomized toads; ▲, of toads with pars distalis transplanted onto the median eminence; ▼, of toads with pars distalis transplanted to an eye muscle.

In the toads with pars distalis retransplanted under the median eminence, the histological and cytological appearance of the adrenocortical tissue was in general the same as in the normal controls (Fig. 16). However, paranuclear vacuoles appeared to be slightly less common; they were absent in specimens no. 2108, 2112, and 2114, and only a few were detected in the remaining specimens of the group.

Cytoplasmic lipid was sparse as in the normal controls (Fig. 18), except in specimen no. 2107. In 3 members of the group, the lipid showed a pronounced cholesterol positive reaction (Table 9).

In the toads with ectopic transplants the adrenocortical cells were generally only slightly smaller than in the normal controls, and larger than in the group with pars distalis extirpated. Also nuclear size was more comparable with the former group than with the latter (Fig. 17). The cell cytoplasm was generally compact, but in specimens no. 2115 and 2122 the cytoplasm was densely lipoidal and contained numerous very small vacuoles. The nuclei were crenated (Fig. 19). The remainder revealed a sudanophilic condition which was intermediate between that of the normal group and the group lacking a pars distalis. The cholesterol reaction varied from negative to strongly positive (Table 9).

In Table 9 the lipoidal condition of the adrenocortical cells can be compared with the general condition of the toads at the end of the experiment. There appears to be an inverse relationship between lipid condition and the secretory activity as evaluated from the liveliness of the toads. High lipid values correspond to abnormal sluggishness.

THYROTROPIC FUNCTION

Thyrotropic function was only studied in the autumn experiment. Table 10 shows that extirpation of the pars distalis strongly reduced the iodine uptake by the thyroids. Retransplantation of the pars distalis onto the median eminence did not significantly alter the ability of the thyroids to accumulate the ^{131}I, whereas transplantation to an eye muscle resulted in a low retention of the injected ^{131}I. In the males of this group the ^{131}I accumulation in the thyroids was slightly, but not significantly, higher than in the hypophysectomized controls, whereas the thyroids of the females retained significantly larger amounts of ^{131}I than the thyroids of the controls without a pars distalis.

FAT BODIES

Dissection of female toads at the end of the autumn experiments revealed that the fat bodies were very much larger in hy-

TABLE 10
^{131}I UPTAKE IN THYROIDS OF TOADS IN AUTUMN EXPERIMENT

Exptl. groups	^{131}I uptake (counts/min)[a]	
	Females	Males
A Normal controls	3253 ± 338	2936 ± 356
B Pars distalis extirpated	747 ± 118	1291 ± 180
C Pars distalis retranspl. to median eminence	2984 ± 343	3167 ± 451
D Pars distalis transpl. to eye muscle	1437 ± 174	1685 ± 293

[a] ± standard error.

Note. t tests for identity gave the following results: Females—C = D, $p < 0.01$; B = D, $p < 0.01$; C = A, $0.5 < p < 0.6$; A = D, $p < 0.001$. Males—A = D, $0.01 < p < 0.02$; C = D, $0.01 < p < 0.02$; B = D, $0.2 < p < 0.3$; C = A, $0.8 < p < 0.9$.

pophysectomized toads than in normal controls; also the females with pars distalis transplanted to an eye muscle had large fat bodies. The fat bodies in the males were weighed to determine whether the size was influenced by extirpation or transplantation of the pars distalis. It can be seen from Table 11 that the fat bodies were small in the normal controls and in the toads with pars distalis retransplanted onto the median eminence, with no significant difference between these groups. In the toads with pars distalis extirpated the fat bodies were large, whereas the fat bodies were of intermediate size in the toads with pars distalis transplanted to an eye muscle. Unfortunately, data on fat body weights at the beginning of the experiments were not recorded. However, fat bodies are normally large in freshly caught toads in August (unpublished observations). There is, therefore, good reason to believe that the weight of the fat bodies in the hypophysectomized toads had not changed much during the experiment, whereas those of the normal controls had been strongly reduced.

In the spring experiment the fat bodies were small at the beginning of the experiment, as they usually are at this time of the year. Their size increased markedly in all experimental groups (Table 11).

HISTOLOGY AND CYTOLOGY OF PARS DISTALIS

Normal Controls (Fig. 9). In the pars distalis of the normal toads of both series of experiments the acidophils were always densely filled with granules. Signs of a secretory activity were seldom noticed; only in some animals from the spring experiment (specimens no. **2059**, **2091**, **2093**) were cytoplasmic vacuoles seen, and in specimens no. **2059** and **2062** occasional intracellular colloid droplets were found within carminophils.

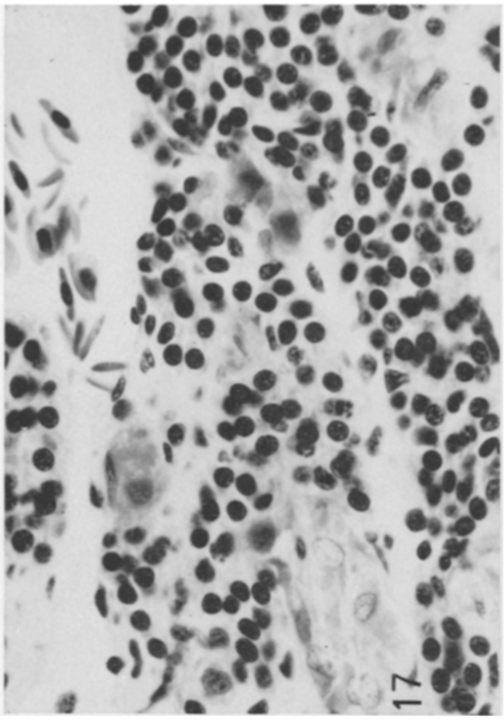
Generally speaking, in the animals au-

TABLE 11
WEIGHTS OF FAT BODIES

Exptl. groups	Sex	Autumn expt. (mg/100 gm of body wt.)[a]	Spring expt. (mg/100 gm of body wt.)[a]
Normal controls at start	♂		106 ± 30
	♀		61 ± 19
Normal controls at end	♂	129 ± 39	1246 ± 62
	♀		323 ± 32
Pars distalis extirpated	♂	1287 ± 206	975 ± 135
	♀		734 ± 129
Pars distalis retranspl. to median eminence	♂	173 ± 61	825 ± 197
	♀		367 ± 111
Pars distalis transpl. to eye muscle	♂	739 ± 106	1373 ± 124
	♀		673 ± 99

[a] ± standard error.

t test for probability in the autumn expt. group with extirpated pars distalis is identical with group with pars distalis transplanted to eye muscle: $0.02 < p < 0.05$.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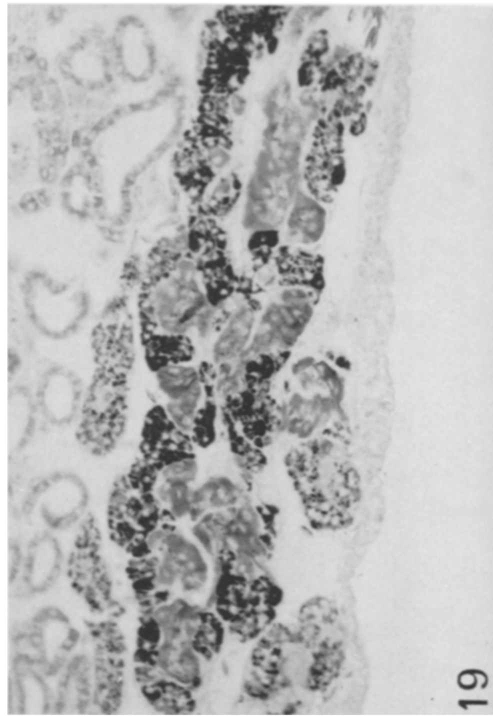
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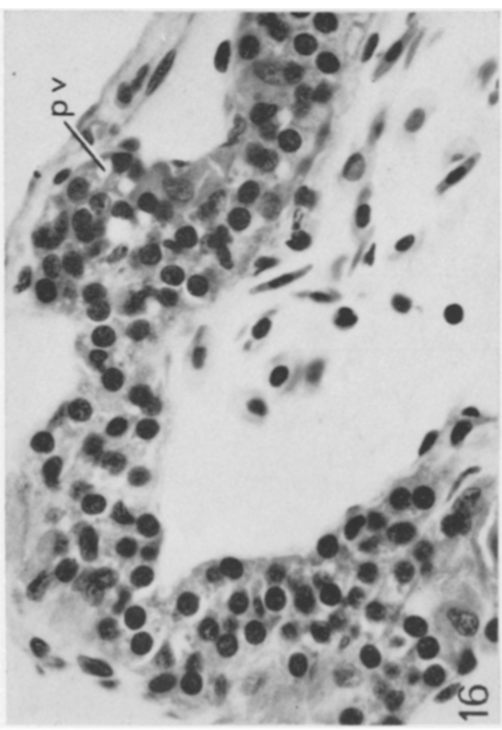
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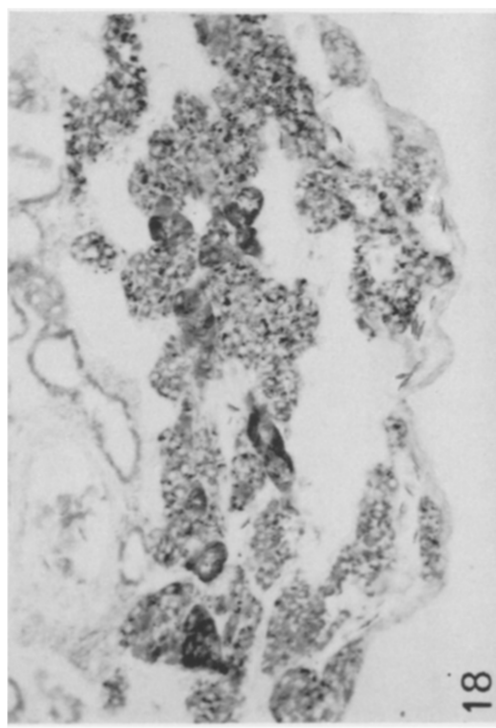
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topsied in October the globular basophils were large, but only sparsely filled with orange granules. Many rostrally situated cells were highly vacuolated; in the more caudally situated globular basophils cyanophilic granules predominated. Extremely big, intracellular orangeophilic globules were found at random, particularly in specimens no. 1148 and 1156. Hyperchromatic, amorphous material filling up most of the cytoplasm was found in dorsally situated cells of specimens no. 1145 and 1156. As a rule the nuclei were small and irregular in shape; big, vesicular nuclei, containing conspicuous nucleoli, were not found in the globular basophils of this group.

In the controls autopsied in June the globular basophils were relatively small, and many contained numerous orange granules. Others, and again particularly those in the caudal part of the pars distalis, were almost purely cyanophilic. Many of the latter showed small cytoplasmic vacuoles. In the more orangeophilic globular basophils very big globules were no exception. Hyperchromatic cells were met with in specimen no. 2095. The nuclei of the globular basophils were ovoid or irregular, and often contained a conspicuous nucleolus.

In the present material small basophils could never be identified with certainty. This does not imply that they were absent, but only means that the staining methods were insufficient to distinguish these cells from smaller, largely cyanophilic globular basophils.

Violet cells were well represented in both groups of normal controls. In the toads, autopsied in October, they were big and sparsely but evenly granulated; in the spring controls they were somewhat smaller and often more densely granulated. In the latter group many violet cells contained some small cytoplasmic vacuoles, usually situated at the base of the cell. The nuclei were always big and vesicular, and contained conspicuous nucleoli.

Median Eminence Groups (Figs. 10 and 11). Sagittal sections showed that in practically all animals the pars distalis had been placed with its dorsal side against the median eminence and pars intermedia. All grafts were well vascularized by blood vessels coming from the median eminence, and entering at various places in the original dorsal side instead of the rostral pole of the pars distalis, as is the normal situation.

Notwithstanding this ample blood supply smaller or larger parts of the rostro-ventral or ventrocentral areas of the distal lobe had lost their normal appearance in all specimens except no. 2111. Numerous cells had degenerated or dedifferentiated to fibroblast-like elements. In such parts violet cells had disappeared completely. Many globular basophils had lost their orangeophilic granules and had turned into weakly cyanophilic cells. Others retained some orangeophilic granules. Prior to degeneration, the globular basophils often formed colloid droplets. These droplets were AB and PAS positive. In sections stained with Cleveland and Wolfe's trichrome they were generally orange and seldom blue; and in sections stained with Herlant's tetrachrome they were predominantly blue, only very large droplets having an orange center. The acidophilic cells had an almost normal appearance, although many were somewhat smaller and less densely granulated than those in the control pituitaries.

In between these basophils and acidophils, remains of disintegrated cells as well as colloid droplets had accumulated. In some of the pituitaries belonging to the autumn group (specimens no. 1173, 1178, 1179), the disintegration of cells had led to the formation of cysts or pseudofollicles,

FIG. 16. Adrenal gland after homoiotopic transplantation of pars distalis. Cortical cells with round nuclei and compact cytoplasm. Paranuclear vacuoles (pv). (From specimen no. 2112). ×290.

FIG. 17. Adrenal gland after ectopic transplantation of pars distalis. Cortical cells with small nuclei. No paranuclear vacuoles. (From specimen no 2116). ×290.

FIG. 18. As Fig. 16. Little lipid. (From specimen no. 2111). ×80.

FIG. 19. As Fig. 17. Very much lipid (From specimen no. 2115). ×80.

containing some degenerating cells, cell debris, and often some erythrocytes. As a rule, such cysts were bordered by pituitary cells that had transformed into chromophobic epithelial elements. These cysts were also very common among the toads of the spring experiment, being absent only in specimens no. 2107 and 2111.

The area of the grafts immediately bordering the median eminence and pars intermedia always consisted of normal pars distalis tissue, in which numerous acidophils, globular basophils and violet cells could be recognized. These cells differed little from those found in the corresponding control groups. The acidophils had become slightly smaller and sometimes stained less clearly, so that it was more difficult to distinguish between the carminophils and the orangeophils. The globular basophils contained few orangeophilic granules and globules and had irregular or ovoid nuclei. Many of the more rostrally situated globular basophils were larger and contained cytoplasmic vacuoles; the more dorsocaudally situated cells were smaller and more cyanophilic. Violet cells were as usual seen in contact with blood vessels, and—due to changes in the vascularization—could be found in more dorsal areas of the pars distalis as well as in those rostral parts where the cells had not dedifferentiated. They tended to be somewhat smaller and less chromophilic than in the corresponding group of normal controls, but had vesicular nuclei and conspicuous nucleoli. Some had lost their cytoplasmic vacuoles.

Eye Muscle Groups (Figs. 12, 13 and 14). The most important feature of the grafts transplanted onto an eye muscle was the presence of large, irregularly shaped cysts or pseudofollicles. Only in specimens no. 1196, 2090, 2120, and 2122 did these cysts remained inconspicuous. The cavities of the cysts were usually lined by flat, chromophobic epithelial cells. In very large cysts cubic and columnar epithelial cells were present, not seldom provided with well-developed cilia, and containing AB positive secretory granules. Their nuclei were very large and vesicular, and showed prominent nucleoli. The contents of the cysts were similar to those found in the homoiotopic grafts, but apart from the cell remnants and erythrocytes, some AB positive residue of what was probably a mucous substance secreted by the ciliated epithelial cells, was found in connection with these cells. Erythrocytes were also present in the numerous smaller and wider blood vessels that had entered the graft from the surrounding muscular tissue.

In between and around the cysts numerous dedifferentiated, fibroblast-like cells, degenerating cells containing orangeophilic globules, and extracellular colloid droplets, as well as cell debris were observed. Apart from these, smaller or larger areas made up of recognizable pars distalis cells were always present, and, generally speaking, were no smaller than in the homoiotopic grafts. Only in specimens no. 2090 and 2121 were relatively few recognizable pars distalis cells observed.

These strands of normal tissue contained considerable numbers of small acidophilic cells. Most of these were partly degranulated, staining brownish red or orange with Cleveland and Wolfe's trichrome and blueish red or orange with Herlant's tetrachrome, thus making it difficult to distinguish between the carminophils and orangeophils. There can be little doubt, however, that both types had survived the heterotopic transplantation. The globular basophils had changed more thoroughly; very few contained granular material, the majority being small and void of chromophilic granules. Globular basophils with more than very few orange staining granules were only observed in specimens no. 1192, 1201, 2083, and 2122. The nuclei of the basophils, as well as those of the acidophils, were ovoid or irregular; nucleoli were present, but seldom conspicuous. Contrary to the acidophils and globular basophils, violet cells had practically disappeared from the heterotopic transplants. A few small violet cells were noticed in three specimens of the spring experiment (no. 2085, 2088, 2122), and one or two remnants of such cells in a section of the graft from specimen no. 1202 of the autumn experiment.

DISCUSSION

Gonadotropic function of the transplanted pars distalis was studied at two different stages in the gonadal cycles, viz. in the spring at a time when the gonads had just begun the production of new gametes after the extrusion and degeneration of the old population of mature sperm and eggs; and in the autumn at a time when the gonads are in a more or less static phase, filled with mature sexual products.

Males. Even in the autumn experiment, the testes of the normal controls showed a pronounced spermatogenetic activity. This high activity is probably not typical at this time of the year, but may be a result of the high temperature at which the toads were kept (van Oordt, 1960). In the frog *R. temporaria* van Oordt and Lofts (1963) have shown that in autumn the spermatogenetic rate is low under natural conditions, but is sometimes highly stimulated in frogs brought from their outdoor quarter to the high temperature of the laboratory.

Irrespective of the time of the year, the pars distalis transplanted under the median eminence differed only little from the pars distalis of the nonoperated control with respect to gonadotropin secretion. In most of the toads, normal, or close to normal, spermatogenetic activity was maintained, as well as a secretory interstitial tissue.

After transplantation of the pars distalis to an eye muscle, gonadotropin secretion proceeded at a level high enough to secure an only slightly reduced spermatogenetic activity. In contrast, the activity of the interstitial tissue was depressed, and to a greater extent in the autumn experiment than in the spring experiment. In the autumn experiment the activity level was close to that of the hypophysectomized controls. It may be that if the toads in the spring experiment had been maintained for the same post-operative period as the toads in the autumn experiment, interstitial activity might have become further depressed.

In the frog *R. temporaria* it has been shown that the interstitial tissue can be stimulated by purified ICSH (LH) of mammalian origin, and spermatogenesis by purified FSH (Lofts, 1961). Furthermore, the gonadotropin responsible for spermatogenesis and interstitial cell activity in the toad and other amphibians appear to be produced by separate cell types (see below). It therefore seems likely that two gonadotropins are secreted by the amphibian pars distalis. The present experiments thus indicate that in the male toad the ectopically transplanted pars distalis secretes sufficient FSH to maintain a distinct spermatogenetic activity, whereas the secretion of ICSH decreases strongly after the operation.

In the spring experiment a significant production of sperm bundles had taken place in the toads with extirpated pars distalis, whereas the intermediate stages in the spermatogenetic cycle were scarce or lacking. The situation confirms the finding of van Oordt (1956) in the frog *R. temporaria* that the transformation of spermatocytes into spermatids and sperm cells goes on in the absence of gonadtropins.

Females. In the preliminary report (Jørgensen, 1963) it was assumed that the mature eggs in the 3 toads of the median eminence group with normal sized ovaries, represented eggs present at the beginning of the experiment which had been maintained. Recently, however, unpublished observations have indicated that mature eggs do not survive even passing fluctuations in gonadotropin levels such as result from retransplantation of the pars distalis onto the median eminence; or from a single injection of chorionic gonadotropin (Physex Leo), even in amounts smaller than those needed to cause ovulation. Some few days after such treatment mature eggs degenerate. Young oocytes may or may not start developing after degeneration or elimination of mature eggs by artificially induced ovulation. If they do, a normally sized ovary filled with mature eggs develops within a few months. The response of immature oocytes is therefore an all-or-none response, perhaps mainly depending upon their degree of sensitivity towards circulating gonadotropin (unpublished experiments).

Thus, it is possible that in the 3 median eminence group toads with large ovaries, degeneration of old mature eggs and a subsequent growth of a new generation of mature eggs had taken place, and gonadotropin production had been sufficiently large to support rapid development and maturation of oocytes. The 5 remaining members of the group may have secreted gonadotropin at equally high rates (see below), but without the oocytes responding. In the 8 toads with ectopic transplants, growth of young oocytes did not occur simultaneously with the degeneration of the mature eggs, but whether this was due to low gonadotropic secretion or unresponsive oocytes, could not be determined.

In the spring experiments there was no significant difference between ovarian weights and degree of maturation of the growing oocytes in the two graft-bearing groups at the end of the experiment. In both, ovarian development was intermediate between that of the hypophysectomized and normal controls; the largest oocytes being about the same size as at the start of the experiment.

This result may be interpreted in two ways. Either the large oocytes present at the end of the experiment were the same as at the start and had consequently been maintained but not grown, or the transplantation was followed by degeneration of all pars distalis dependent oocyte stages, so that the large oocytes at the end of the experiment represent regrowth of a new generation of oocytes.

If the first interpretation is correct, transplantation depressed gonadotropic secretion to about the same extent in both groups, but if the second interpretation is true gonadotropin secretion in these specimens was similar to normal controls, since the oocyte size reached during the experimental period is comparable to that reached in the ovaries of normal toads 6 weeks after spawning (see Fig. 15) (assuming that oocyte growth started immediately after spawning).

Irrespective of which of the two interpretations is correct, however, in these spring experiments a dependence on normal hypothalamic connections for gonadotropic production could not be demonstrated, as evaluated from the state of the ovary.

Adrenocorticotropic and Adrenocortical Functions. The present experiments have confirmed previous findings that generally a significant adrenocorticotropic function can be demonstrated in homoiotopically, but not in ectopically transplanted pars distalis tissue, when the functional state is judged from length of survival, molting, and condition of the toads.

In males of the spring experiment the lipid content of the adrenocortical cells showed a correlation with the general condition of the toads, and thus with the adrenocortical function; a high lipid content representing a low secretory activity and vice versa. The correlation between the reaction for cholesterol and the functional state of the adrenocortical cells was less pronounced. Higher cholesterol values were, however, found in toads with symptoms of deficient adrenocortical function (Table 9).

Lipid content of adrenocortical cells as well as general condition suggest that in the spring experiment some of the male toads with an ectopically transplanted pars distalis had more active adrenocortical tissue than corresponding animals without a pars distalis. This might be explained by assuming that the heterotopic graft secreted a hormone acting synergistically on the adrenocortical cells with the ACTH injected to keep the animals alive. Lostroh (1958), for example, has demonstrated such a synergistic effect of somatotropin with a corticotropin on the adrenals in rats. It is also possible, however, that the ectopically transplanted pars distalis secreted small amounts of ACTH which acted supplementary to the injected dose of ACTH.

In this respect it is of interest that in the adrenocortical tissue of the toads with an ectopically transplanted pars distalis, cytological characteristics such as size and shape of the cells and nuclei, and the presence of cytoplasmic vacuoles, more closely resembled those in the normal control animals than those in the hypophysectomized toads. This may mean that in animals with an ectopic graft the adrenocortical tissue was more active than in the toads without pars distalis. It cannot mean, however, that in the former the adrenocortical tissue was near to normal, as was concluded by Vivien (1959) from the cytological aspects of adrenocortical cells of *R. temporaria* with ectopic pars distalis graft.

Thyrotropic Function. Ectopic transplantation of the pars distalis strongly reduced its thyrotropic activity, evaluated from the iodine uptake by the thyroids. In the females the level of activity of the thyroids in the group with pars distalis transplanted to an eye muscle was significantly higher than in the hypophysectomized controls; but not so in the males.

In a further experiment on males, ^{131}I uptake in a group of 11 toads with the pars distalis transplanted to an eye muscle was likewise slightly, but statistically insignificantly, higher than in a group of 11 toads with extirpated pars distalis (Per Rosenkilde, unpublished experiments). Further experiments are planned to see whether the different results obtained in males and females represent a consistent difference in the thyrotropic function of the ectopic pars distalis transplant in the toad.

Fat Bodies. The size of the fat bodies is a result of a complicated interplay between fat-depositing and fat-mobilizing factors.

In the autumn experiment the situation favored fat mobilization: fat was mobilized from the big fat bodies because of insufficient food supply. The ability of the hypophysectomized toads to mobilize fat was impaired; the males with pars distalis transplanted to an eye muscle had fat bodies of sizes between normal and hypophysectomized toads. It thus appears that some pars distalis hormone(s) is necessary for the net mobilization of fat during hunger, and that mobilization is reduced when pars distalis is removed from its hypothalamic contact. Further experiments are needed to determine whether the change in function of pars distalis resulting from ectopic transplantation is due to changes in secretory rates of fat-mobilizing or fat-depositing principles, or both.

In the spring experiment the conditions favored fat deposition, and it can be concluded that pars distalis hormones (other than ACTH) are not of decisive importance in this process.

Histology and Cytology of Pars Distalis. A description of the cyclic changes in the pars distalis of normal *B. bufo* has been given by Zuber-Vogeli (1953). She wrote that in the autumn the cells increase in size and granulation. This regranulation was particularly clear within the globular basophils. Also in our autumn controls the chromophilic cells were well developed, but except in the acidophils, there were no signs of a storage of secretory granules. It, therefore, seems justified to infer that because of laboratory conditions the globular basophils of the autumn controls showed out of season signs of secretory activity, as observed by van Oordt and Lofts (1963) in similar cells of *R. temporaria* males kept at high temperatures during autumn.

Likewise, in the controls of the spring experiment, there were indications of a release of secretory products, not only in the globular basophils and violet cells, but also in the acidophils of some animals. This corresponds to summer conditions rather than to the appearance seen under natural conditions in spring (Zuber-Vogeli, 1953). This may mean that the secretory activity of the pars distalis was speeded up by the laboratory conditions. The advanced spermatogenetic activity in the testes seems to indicate that this was indeed the case with the release of the FSH. The state of the ovary, was, however, not abnormal for the time of year (unpublished results). This means that any increase in FSH output had been insufficient to stimulate the ovaries.

The presence of a broad zone of healthy pars distalis tissue containing all chromophilic cell types in the grafts retransplanted under the median eminence leaves no doubt as to the functional potentialities of these grafts.

As in the controls, the normal cells in the transplants under the median eminence showed signs of secretory activity, but the overall slight dinimution in stainability indicates that the storage of secretory products had decreased. Since cytological differences were not great and quantitative measurements of the hormone release could not be carried out, it is of no value to speculate on any small individual differences in secretory activity among the homoiotopic grafts.

Cell necrosis and dedifferentiation always took place in the rostroventral or central parts of the transplanted pars distalis, i.e. the area normally vascularized by the sinusoids immediately branching off from the portal vessels. It must, however, be admitted that the very same area was seldom in direct contact with the median eminence and pars intermedia after transplantation.

The reason for the constancy in distribution of normal and dedifferentiated tissue may thus be caused by the fact that the normal dorsocaudal areas, being in contact with other tissues were sooner revascularized and suffered less from ischemia than the dedifferentiated rostroventral parts. It may also be, however, that the cells in the latter parts were more sensitive to disturbances in the blood supply via the portal vessels than dorsocaudally situated cells. The absence of recognizable violet cells in the dedifferentiated parts and their reappearance along the blood vessels in the dorsal zone point in that direction. Such behavior of the violet cells was also observed by Pasteels (1960) in homoiotopic transplants of the pars distalis in *Pleurodeles waltlii*. Their complete or nearly complete absence in the heterotopic transplants of the present experiments may serve as an extra proof for the great importance of the portal circulation for this cell type. It is perhaps safer not to speak of a complete absence of the violet cells, for it is not unthinkable that they had only lost their chromophilia and had thus become unidentifiable. This means that the possibility cannot be excluded of a secretion of hormones formed by 'violet cells' in the dedifferentiated parts of the homiotopic grafts, or in the heterotopic grafts (Petrovic, 1963).

Contrary to the situation in the violet cells, the acidophils did not seem to suffer from a disruption of the portal vessels, but again it cannot be concluded from the cytological picture that these partly degranulated carminophils and orangeophils kept up the same rate of production and release as they did before the operation. It seems certain, however, that transplantation did not lead to a distinct increase in number of the acidophils as was found by Etkin and Ortman (1960) in heterotopically transplanted adenohypophyses of *R. pipiens* larvae. Neither were the acidophils the only recognizable cell types in the ectopic grafts, as was observed by Pasteels (1957, 1960) in *P. waltlii* and by Etkin and Ortman (1960) in *R. pipiens*. This may, however, have been a matter of time. At any rate the globular basophils had regressed in number and granulation in the dedifferentiated areas of the homoiotopic transplants and in the heteroptopic grafts. Still, they remained in sufficient number to suppose some production and release of the hormone normally produced by this cell type. Moreover, the orangeophilic colloid droplets appearing within the globular basophils prior to their disintegration and found in the intercellular spaces after disruption of the cell wall, so much resembled the orangeophilic globules in form and stainability that they not only seemed to have a function in the disintegration of the cell, but also in the release of secretory products and thus indicated holocrine, or in less severe cases merocrine secretion (Doerr-Schott, 1964, 1965).

Functional Cytology of the Pars Distalis. When looking for the function of the normal and autotransplanted pars distalis as a whole, and for the function of the different cell types in particular, the absence of a direct measurement of the hormone release by the pars distalis makes it difficult to draw conclusions from a comparison of the pars distalis cell types and their target organs. Only very striking qualitative features of the pars distalis cell types can be taken into account; smaller and individual variations in the morphological aspects of the cell types cannot be used for the interpretation of nature and measure of the cell activity.

If the arguments of Zuber-Vogeli (1953) in favor of a gonadotropic nature of the globular basophils are accepted, the cytological aspects of these cells indicate a distinct gonadotropic activity of the homoiotopic grafts. The strong spermatogenetic activity observed in the testes of the males, and the normal appearance of the ovaries in three females belonging to the median eminence group of the autumn experiment support this assumption.

In the ectopic transplants the regression of the cell types was strongest in the violet cells. The disappearance of these cells in the heterotopic grafts of the autumn experiment has been connected with the poor state of the interstitium of the testes (van Oordt, 1963b). The slow regression of the thumb-pads indicated that this poor development was reached only a few weeks before the end of that experiment. It could thus be expected that in the shorter spring experiment neither the violet cells in the pars distalis nor the interstitial cells and the thumb-pads would have totally regressed. This was indeed the case; the interstitial cells and thumb-pads were better developed than in the hypophysectomized frogs, and violet cells were seen in three animals, showing that their regression had not reached its maximum. It, therefore, seems justified to ascribe ICSH production to the violet cells. This would be in agreement with a similar function of the corresponding cell type, the so-called γ-cell, in *P. waltlii* (Pasteels, 1960) and *R. temporaria* (van Oordt 1961; Lofts and van Oordt, 1962; van Oordt and Lofts, 1963). On the other hand, Guardabassi and Grosso (1959) as well as Mazzi and Guardabassi (1959) expressed the opinion that in *B. bufo* the violet cells produce thyrotropic hormone. The absence of a correlation between the moderate activity of the thyroids in the female toads with heterotopically transplanted pars distalis of the autumn experiment and the maximal regression of the violet cells in these grafts does not, however, favor that opinion.

Cyst Formation in the Grafts. The presence of smaller or larger cysts within the grafts is a very common result of transplantation of the adenohypophysis, and may even be found in normal glands (Zuber-Vogeli, 1953; Girod, 1963). Likewise, the mucus-secreting, ciliated cells have been observed before (Pasteels, 1957, 1960; Girod, 1963, Massimello, 1963). It was Pasteels who rightly compared these cells with the epithelial cells lining the roof of the pharynx.

General Evaluation of the Importance of Normal Structural Relations between Pars Distalis and Central Nervous System for Pars Distalis Function in the Toad. A complete assessment of the functional consequences of disrupting the normal connections between central nervous system and pars distalis would necessitate studies at several levels of activity of the various pars distalis functions. Theoretically, structural disconnection of pars distalis from the central nervous system may exhibit deleterious effects on a pars distalis function only under special conditions, namely, in situations where the secretion of pars distalis hormones is variably or discontinuously stimulated or inhibited by the central nervous system, e.g., as a consequence of rhythmic activity of the central nervous system or initiated by external or internal stimuli. The deleterious effects may also reveal themselves at a more basic level of pars distalis function, for instance when the central nervous system normally exerts a more constant stimulatory or inhibitory action on the secretion of a pars distalis hormone. When we are dealing with the tropic hormones among the pars distalis hormones it may also be useful to distinguish between the activity of the hormones as it can be evaluated from size, histology and cytology of the dependent endocrine glands, and their effect on the secretory activity of the glands.

The present study is far from being a complete analysis of the effects of eliminating normal structural connections with the hypothalamus on the various pars distalis functions in the toad. However, the analysis, as far as it goes, has provided examples of effects at the various levels of pars distalis activity mentioned above (Table 12). Thus with respect to the gonadotropic function it is indicated that in the males the FSH production is diminished but remains sufficient for a considerable spermatogenetic activity, when the connections between central nervous system

TABLE 12
EFFECT OF ECTOPIC TRANSPLANTATION ON PARS DISTALIS FUNCTIONS IN THE TOAD

Hormone	Functional and morphological criteria	Degree of function	
		Spring expt.	Autumn expt.
FSH	Globular basophils	++	++
	Spermatogenesis	++	++
	Oocyte growth	+++	0
ICSH	Violet cells	+	+
	Interstitial cells of testis	+	0
TSH	^{131}I uptake in thyroids	n.m.	++ (females) + (males)
ACTH	General conditions of toads	0 (females) + or 0 (males)	0
	Cytology of adrenocortical tissue	n.m. (females) + or ++ (males)	n.m.

Note. +++, as in controls; ++, reduced but still significant; +, strongly reduced or doubtful; 0, reduced to hypophysectomy level; n.m., not measured.

and pars distalis have been disrupted. Also in the females disconnection of the pars distalis leads to a gradual decrease in FSH production, as indicated by the state of the globular basophils, but only in the autumn experiment to a level which prevented growth of oocytes.

Judged from the morphological aspects of the violet cells in the ectopic pars distalis, their function seems generally strongly reduced. In connection with this the interstitial tissue of the testis had become very inactive. This effect was stronger in the autumn than in the spring experiment.

The normal level of TSH secretion is significantly reduced in the ectopic pars distalis. The present experiments suggest a difference in dependency upon normal hypothalamic connections between the sexes.

As evaluated from molting, liveliness, and survival of the toads, ACTH secretion almost entirely depends upon normal hypothalamic connections. Only in some animals with an ectopically transplanted pars distalis had the general condition not come down to the level found in hypophysectomized animals. Cytochemical criteria, likewise, indicate a strong reduction of adrenocortical activity after displacement of the pars distalis. Other morphological criteria seem to indicate that the ectopic pars distalis exerts some stimulatory effect upon the adrenocortical cells. It has, however, not been possible to label this with certainty as an effect of ACTH.

On the Interpretation of Function of the Ectopically Transplanted Pars Distalis. Large parts of pars distalis tissue survived transplantation. The significant reduction that was observed of some of the functions in the ectopically transplanted pars distalis is probably not caused by lack of sufficient amounts of living pars distalis tissue (Dierickx, 1964), but by the absence of normal morphological connections to the hypothalamus, specifically the hypophysial portal circulation through which the hypothalamic transmitter substances are normally carried to pars distalis cells.

When functional activity is observed in an ectopically transplanted pars distalis the question presents itself whether this activity proceeds independently of central nervous influences; or whether chemical transmitters normally carried in the hypophysial portal vessels reach the graft in concentrations sufficient to maintain its function. Experimental evidence to answer this question is not available in amphibians and conclusive evidence as to the importance of circulating transmitter substances in maintaining pars distalis function in the ectopic pars distalis is difficult to obtain.

None of the functions of the pars distalis, studied in the present experiments, i.e., the gonadotropic, thyrotropic, and corticotropic activity, remained unchanged after displacement of the pars distalis. The FSH output, however, generally remained sufficient for a distinct gametogenesis. This does not mean that the FSH secretion was maintained by an FSH-releasing factor circulating in the blood. Neither does it imply that the differentiation of a hypophysial portal circulation has not been a preliminary for a normal FSH secretion. It is more likely that the pars distalis cells in general show some independence of hypothalamic stimulation (the FSH-cells to a larger extent, the ICSH-, TSH-, and particularly the ACTH-cells to a smaller extent) but that this hypothalamic stimulation is necessary for a normal functioning of the cells and especially for cyclic variations in their activity.

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