EFFECTS OF SEASONS, CASTRATION AND CRYSTALLINE SEX HORMONES UPON THE UROGENITAL SYSTEM AND SEXUAL BEHAVIOR OF THE LIZARD (ANOLIS CAROLINENSIS)

I. THE ADULT FEMALE [1]

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THREE PLATES (TWENTY-THREE FIGURES)

Histological descriptions of the urogenital system of the female lizard are rare in the literature. Although Leydig (1872), Hoffmann (1890), Giacomini (1893), Krause ('22) Giersberg ('23), Regamey ('35) and Jacobi ('36) contribute to a fairly complete account of several species of Lacerta, no other genus has been comparably treated. The mating behavior of female reptiles is even less well known. In fact, estrus, or sexual receptivity correlated with the ovarian cycle, has not hitherto been attributed to any member of this class of vertebrates.

The social behavior of a common lizard of the American tropics (Anolis carolinensis) has been extensively studied at the Laboratory of Experimental Biology during the past

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6 years (Noble and Greenberg, '39). As a result of this work, it was discovered that the female chameleon exhibits a specific pattern of sexual receptivity during the breeding season. Consequently, it became possible, for the first time, to investigate the endocrine basis of the sexual behavior of a female reptile, as related to the structure of the reproductive system.

Since reptiles have been shown to react markedly to crystalline sex hormones, it was decided to use these agents as a means of eliciting or accentuating normal, seasonal growth changes and behavior patterns, in the presence and absence of the gonads.

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MATERIALS AND METHODS

Thirty-two adult female lizards (Anolis carolinensis) were selected for this experiment from a stock of several hundred animals. They were secured from Thibodaux, Louisiana, during January, 1940. The experiment was begun on February 12, and terminated April 8 to 11, 1940.

The females were separated into groups of eight, occupying four large cages. The cages were located in a sunlit greenhouse with a controlled temperature of about 28°C. Table 1 presents the distribution of all animals and their operative and hormonal treatment.

Sixteen lizards were subjected to bilateral ovariectomy by means of a ventral incision. After sufficient time for full recovery (several weeks), pellets of crystalline testosterone propionate or estradiol dipropionate [3] were implanted into half of each group. Estradiol pellets were not available at the beginning of the experiment, and therefore loose crystals were packed together and implanted into four intact females.

[3] We are indebted to the Ciba Pharmaceutical Products, Inc., Summit, N. J., for the testosterone propionate (Perandren) and the alpha-estradiol-dipropionate (Di-ovocylin) utilized in this study.

Several weeks later, a group of spayed females received compact estradiol dipropionate pellets. In all cases, the hormone was introduced under the skin of the mid-dorsolateral region of the body.

TABLE 1

Treatment of adult female Anolis carolinensis with androgen and estrogen pellets

NO. OF INDIVIDUALS Exp.	Controls	DATE GONADECTOMIZED	DATE IMPLANTED	KIND OF HORMONE	WT. OF PELLET AT INTRODUCTION IN MG.	WT. OF PELLET AT REMOVAL IN MG.	DIFFERENCE IN MG.	INTERVAL IN CALENDAR DAYS
					4.60	2.00	2.60	36
4	4	—	3/3/40	Testosterone	6.00	2.88	3.12	36
				propionate	5.25	2.86	2.39	36
					5.25	1.18	4.07	36
					4.40	1.36	3.04	36
4	4	2/13/40	3/3/40	Testosterone	5.40	2.77	2.63	36
				propionate	4.25	1.47	2.78	36
					6.40	Missing	—	36
					9.20	8.71	.49	17
4	4	3/12/40	3/25/40	Estradiol	9.30	8.00	1.30	17
				dipropionate	8.85	8.12	.79	17
					7.30	6.35	.95	17
					6.4	—	—	24
4	4	—	3/11/40	Estradiol *	4.6	—	—	20
				dipropionate	5.3	—	—	30
					5.2	—	—	21

* Loose crystals packed to resemble pellets.

The lizards were fed blowflies (Callophora) and mealworms (Tenebrio) three times weekly and the cages were sprinkled several times a day.[4] Continuous behavior records were taken from 9 A.M. through 4 P.M. on week-days and occasionally on Saturdays and Sundays. The entire observation period extended from March 5 to April 10, 1940.

Experimental and control animals were sacrificed at approximately the same time and immediately fixed in Zenker-

[4] The personnel of Works Progress Administration, Official Project No. 265-1-97-16 WP 10 aided in animal maintenance and preparation of materials.

formol. The gross structure of the urogenital tracts was studied in situ. Ovaries and oviducts were then weighed from 70% alcohol by drying for a few seconds with absorbent paper and dropping them into a weighed bottle of 70% alcohol. Two representative specimens from each group of four (table 1) were cross-sectioned at 10 μ throughout the length of the tract and stained with haematoxylin and eosin or Mallory's triple stain. The remaining cases were checked by sectioning comparable regions.

RESULTS

A. Macroscopic observations

1. Control females. Unoperated controls were sacrificed at the height of the usual breeding season. Nevertheless, the ovaries and oviducts of these females varied widely in size and weight. Three of seven controls possessed bilaterally undeveloped ovaries (fig. 1), ranging in weight from 1.29 to 5.88 mg. The left and right ovaries of the remaining four were unequal, one weighing 3 to 8 times as much as the other (table 2). Figures 2, 3 and 4 illustrate this condition, which can be seen to be correlated with the greater hypertrophy of a single ovum in one of the ovaries.

Although two of the control females had already ovulated, as indicated by the presence of an egg within an oviduct, the ovaries of these showed similar weight differences and the ovary on the same side as the oviduct containing the egg was the smaller and lighter one (fig. 4).

The weights of the oviducts of intact controls were directly related to the presence of a large maturing ovum or an egg within the opposite oviduct (table 2). The females with undeveloped ovaries possessed oviducts which weighed little more than those of the spayed controls.

2. Testosterone-treated females. Testosterone strongly stimulated the ovaries (table 2). Those of treated females weighed from 31.29 to 309.0 mg., while the control ovaries ranged from 1.29 to 65.63 mg. In all four cases, one ovary weighed much more than the other. Two of the treated females had an egg within an oviduct, and the ovary on that side was the smaller; the opposite ovary contained two mature ova instead of the usual one (fig. 5).

The oviducts of the treated intact females (except those with eggs) weighed an average of 37.68 mg., whereas the

TABLE 2

Effect of testosterone propionate on weights of ovaries and oviducts in adult female Anolis (weights in mg.)

	OVARIES				OVIDUCTS			
	Pellet ♀♀		Control ♀♀		Pellet ♀♀		Control ♀♀	
	Left	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
	45.65	135.41	53.00	7.00	34.29	36.14	19.76	15.35
Intact	309.00	98.47	5.88	5.23	45.47	Egg	8.53	9.47
females	31.29	108.71	5.65	3.35	31.47	40.47	8.00	9.00
	259.03	127.35	6.52	18.52	—	Egg	Egg	24.47
			1.29	1.53			6.71	6.88
			53.53	5.82			12.29	12.29
			65.63	8.00			32.29	Egg
Average					37.08	38.30	12.51	10.07
Ovariec-					36.23	23.35	7.84	7.47
tomized					27.41	21.59	7.94	7.88
females					46.94	50.41	6.00	6.83
					41.88	42.88	5.35	5.00
Average					38.12	34.56	6.78	6.79

weight of the heaviest control oviduct was 32.29 mg. Therefore, testosterone treatment effected a greater hypertrophy than that observed in any of the seasonal controls.

The oviducts of treated spayed females also showed a pronounced hypertrophy (fig. 7). Treated oviducts ranged in weight from 21.59 to 50.41 mg. with an average of 36.34; control oviducts weighed from 5.00 to 7.94 mg. with an average of 6.79 mg. The treated oviducts were thicker and much more folded.

3. Estradiol-treated females. Prolonged treatment was fatal to three of the four intact females which had been implanted with loose crystals of estradiol dipropionate. These died within a month after implantation while the fourth appeared ill at termination, as did all of the treated spayed females. Postmortem examination revealed a general, hemorrhagic condition of the ovaries, and, in some cases, of other organs, such as liver or intestines.

The ovarian condition appeared to consist of resorption of large ova, if present, and stasis of follicular development (fig. 6). The oviducts of the treated females, both intact and spayed, were uniformly enlarged in similar fashion as with testosterone (fig. 9).

B. Microscopic observations

The ovary of Anolis is similar to that of oviparous Lacerta and other genera described by a large number of the earlier writers. Therefore, the present report will be restricted to seasonal and experimental changes in the oviduct, cloaca and metanephros.

The lizard oviduct consists of three general regions: (1) infundibulum and tube, (2) uterus and (3) vagina. For purposes of description, the vagina of Anolis is divided into proximal, middle and distal portions as reflected in a true histological difference.

1. Intact control females. Histological examination confirmed the gross correlation between size of ovaries and growth of the oviducts. Figures 1–4 illustrate this correlation. Three of the eight control females are represented by figure 1, two by figure 2, one by figure 3 and two by figure 4. To facilitate comparisons, figures 1–4 will be referred to as typical stages of an indicated progressive development.

Infundibulum. The infundibulum of the first type of control female (fig. 1) is seen to be closed through practically its entire length. Two straight rows of columnar cells with basal nuclei are pressed closely together, obliterating the lumen.

In the second type (fig. 2) the duct is patent and the lining is thrown into folds. The columnar cells are more distinctly ciliated in the third and fourth females (figs. 3 and 4), and their eosinophilic, homogeneous cytoplasm is lighter.

Tube. This portion lies open in all of the females. Its columnar epithelium does not appear to differ from that of the infundibulum and is continuous with the inner lining of the uterus. Folding progresses, through the series, from fairly straight to low waves and folds superficially resembling the glands of the uterus. However, the infundibulum and tube have no acinar glands.

Uterus. Between the inner epithelial lining and the outer tunica propria of the uterus there are present acinar glands which are not numerous in those females with undeveloped ovaries (fig. 1). Loose connective tissue is abundant between them. The glands are oval, composed of broad columnar, granular cells arranged around a very small lumen. Invaginations from the inner lining of the oviduct can be traced toward them but the terminations are not clear.

In females with maturing ova, these shell glands become progressively larger, more discrete and granular. They pack the uterine wall of the estrous female (fig. 10). When an egg is present, the inner epithelium of the uterus is seen to be closely applied to the developing shell. The glands become smaller and are distributed within the thinned out uterine wall in two or three layers. Below the egg, the glands are again large and ducts invaginate among them from the inner epithelium.

Vagina. Below the uterus, the inner lining is much folded. Between this and the thin tunica there is loose connective tissue into which shell glands do not penetrate (fig. 11). In the females with maturing ova, this proximal part of the vagina becomes wider and the folds more numerous.

Thick smooth muscle characterizes the middle portion of the vagina. A cross-section of this region reveals three chambers (fig. 12). The center one is continuous with the proximal portion and its epithelium is also cuboidal. The outer chambers are lined by mucoid columnar cells. Toward the distal vagina, all three merge together, and the epithelium changes gradually to the multilayered condition of the terminal region. The middle vagina shares in the general growth of the oviduct, mainly by increased diameter and thickening of the musculature.

The mucosa of the distal portion of the vagina is composed of layers of stratified squamous cells. The deepest layer contains a row of peculiar, clear cells, irregularly shaped, with large nuclei. Above this, the cells are typically squamous, flattening from the middle layers to the surface, with progressively pycnotic nuclei. An outer border of cuboidal or columnar cells is present and is apparently a continuation of the common lining of the oviduct.

This stratification becomes thicker as the ovaries hypertrophy (fig. 13) and the cells of the middle layers appear more sharply defined, with nuclear degeneration further advanced than in the first female. The outer cuboidal layer tends to be stripped off. In the female with egg in oviduct, the epithelium is thickest and the nuclear pycnosis is furthest advanced, especially within the upper third. The outer cuboidal layer is still present in some places.

Urodeal cloaca. Just above the entrance of the oviducts, the cloacal epithelium of the first type of female (fig. 1) is of identical nature and merges with that of the distal vagina. The dorsal wall is stratified, but the ventral wall is covered with a bilaminar epithelium. However, at entrance of the oviducts, the entire urodeum is lined by stratified cells except for a small ventral piece.

In the females with developing ova, the urodeal cloaca is multi-layered for a considerable distance above and below the juncture with the oviducts (fig. 14). One can distinguish an upper cross-section of the cloaca of the estrous female from the distal vaginae only by their relative positions.

2. Spayed controls. The changes following ovariectomy are conspicuous in Anolis, especially (1) atrophy of the shell glands and (2) involution of the stratified epithelium of both terminal vaginae and urodeal cloaca (fig. 15). All regions of the oviduct can be seen to have regressed, as indicated by the general decrease in diameter. The inner lining, continuous throughout the specialized regions, uniformly diminishes in length and height, with resultant reduced folding. Connective tissue and smooth muscle appear less affected and due to the shortening of the lining, occupy a relatively greater part of the oviduct wall.

3. *Testosterone-treated females.* Hypertrophy of the ovaries, as a result of testosterone treatment, was not accompanied by detectable abnormalities of ovarian structure. The enlarged ova did not differ appreciably from those of controls. Oviducts and urodeal cloacas of treated intact females closely resembled those of the estrous control female.

The oviducts of treated spayed females were stimulated to a similar degree. The contrast between treated and control spayed females was the more striking in view of the rather complete involution of the control oviducts. Testosterone induced full breeding development of the infundibulum, tube, uterus and vagina. Stratification of the distal vagina and urodeal cloaca, although definitely elicited (fig. 16), differed somewhat from the estrous type in that cellular elements were less distinct. Since this epithelium resembles the condition observed in females having smaller ovaries than those of the estrous females, it is probable that testosterone produced a lesser effect here than in other regions of the oviduct.

The metanephric kidneys of both intact and spayed testosterone-treated females underwent an interesting transformation. The collecting ducts (Forbes, '41) which in untreated females are uniformly as small as the other tubules of the kidney (fig. 22) became, with testosterone treatment, fully as hypertrophic and secretory as the corresponding tubules of sexually active males. They enlarged in diameter and the columnar cells became tall, thin and highly granular, with secretion material present in the lumen (fig. 23).

4. *Estradiol-treated females.* Three of four intact females, which had received large doses of estradiol dipropionate in

the form of loose crystals (table 1), died before termination of the experiment. A widespread hyperemia was found: almost every blood vessel in every tissue and organ was distended with thick, colloid-like material, which penetrated into the smallest capillaries and sinusoids (figs. 20, 21). Erythrocytes dotted this thickened plasma and formed large aggregations in the arteries.

The estradiol-treated spayed females, having been implanted with compact pellets for a shorter period of time (table 1), all survived to termination. They exhibited the identical circulatory disturbance, but to a lesser degree.

The oviducts and urodeal cloacas of treated spayed females were stimulated in similar manner as those treated with testosterone (fig. 17). In estradiol-treated intact females, the shell glands of the uterus were packed densely (fig. 18) and the epithelium of the terminal vagina and urodeal cloaca was considerably thicker than that of treated spayed females (fig. 19).

Metanephric collecting tubules were not hypertrophied by estradiol treatment.

C. Reproductive behavior

1. *Normal.* Several distinctive kinds of reaction pattern are observed in Anolis carolinensis during the breeding season. The fight display of the male differs from courtship in respect to (1) posture and (2) rate of movement; the fighting male nods stiffly, with sides flattened and body tense, while the courting male, body relaxed, bobs rapidly, jerkily, his movements resulting in a strutting advance toward the female. When the male approaches closely, the female may run, usually escaping with ease, or she may remain in place and submit to copulation. Often she responds with a downward flex of the neck as the male mounts. This gesture is interesting, for the male must grip her dorsal neck skin with his jaws in order to proceed further. Submission to copulation with the characteristic neck bend have been found to be criteria of full estrus or sexual receptivity. After securing a grip,

the male moves his partner to a secure place. Then he shifts his tail and hind legs into copulatory position: his jaws hold fast to a fold of the neck skin, his body is arched over one side of the female, the tail is thrust underneath, while one hind leg securely grips the female's tail base. Copulation usually lasts from 4 to 30 minutes.

Female Anolis also fight, both during and out of season, and their fight pattern is similar to that of the males. No definite relationships appear to exist between the ovarian condition and frequency of fight responses.

Untreated females have not been observed to display the male courtship pattern except in certain rare cases of abnormality which will be fully reported elsewhere.

2. *Intact controls.* Only one of the intact controls (female 7, table 3) was observed to display spontaneous estrus. The genital system of this female has been described above. She displayed full estrus repeatedly and was "copulated" six times by testosterone-treated females. Two of the other three controls in this cage were found to have bilaterally undeveloped ovaries while the third showed a smaller developing egg than that of the estrous female (compare figs. 2 and 3). This is suggestive of a direct correlation between maturation of an egg and sexual receptivity but further evidence is necessary to demonstrate such a relationship.

The remaining four intact control females were kept with estradiol-treated females. They were courted only by occasional, introduced males and were not observed to react sexually.

Five of the eight control females displayed the fight pattern at least once, three being actual victors in forty-six, thirteen and five encounters, respectively. The female winning thirteen encounters exhibited bilaterally small ovaries (fig. 1), while the two other females showed a developing egg (fig. 2). The sole estrous female was not observed to fight, but little significance may be attached to this since in other experiments estrous females have frequently been observed to fight and dominate other females.

None of the control females dominated their cages and none were ever observed to exhibit the male courtship pattern.

3. Spayed controls. The eight spayed controls (two cages) never displayed overt signs of estrus, but seven of the eight showed fight reactions at least once. For 3 weeks after the start of observation, one of these females dominated her cage, which also contained four testosterone-treated spayed females. This control won 183 combats (table 4), more than any other in any of the cages, but eventually gave ground to an aggressive, treated female. The other controls fought much less, taking part in thirty-one, fourteen, and one encounters, respectively.

4. Testosterone-treated females. All of the eight treated females exhibited typical male courtship behavior and all, except one, performed the complete male copulatory pattern. In addition, six of the females showed estrus and underwent copulation by other treated females or introduced males (tables 3 and 4). Fight aggressiveness appeared important in regulating the sexual relationships among both intact and spayed treated females. In each cage, a single treated female dominated the group and also courted most frequently. Intact female 3 (table 3) courted in typical male manner a total of ninety-five times, and in eight of these instances went through the complete male copulatory pattern. She won 159 of a total of 243 fights, clearly dominating this group throughout the observation period.

The situation in the cage of spayed females was complicated by the aggressive behavior of one of the controls, who was the most effective fighter during the first 3 weeks of observation. A treated female finally succeeded in defeating her and was dominant thereafter. This female courted and mated most frequently as the masculine partner (female 1, table 4).

These two dominant treated females submitted to copulation only by introduced males. In such instances, the male was first attacked by the dominant female in a manner resembling that of a territory-holding male. The newcomer, being larger and sexually active, quickly won, and in three instances copulated with the aggressive treated female.

Three of four treated intact females showed typical estrus, while the sexual behavior of spayed females treated with testosterone propionate (table 4) included not only submission

TABLE 3

Behavior of intact females implanted with pellets of testosterone propionate

		PELLET FEMALES					CONTROL FEMALES				
		♀1	♀2	♀3	♀4	Totals	♀5	♀6	♀7	♀8	Totals
Male Behavior	Courtships	43	15	95	1	154	—	—	—	—	—
	Copulations	9	3	8	1	21	—	—	—	—	—
Estrous Behavior	Copulation by males	1	0	2	0	3	—	—	—	—	—
	Copulation by females	4	4	0	7	15	—	—	6	—	6
	Other estrous signs shown	7	—	1	4	12	—	—	5	—	5
Aggressive Behavior	Fights	12	7	159	6	184	46	13	—	—	59
	Fight mixed with courtship	2	0	27	1	30	—	—	—	—	—
	Totals	14	7	186	7	214	46	13	—	—	59

to copulation, in three of the four cases, but also the neck reflex (♀2 and ♀4, table 4).

5. *Estradiol-treated females.* The estradiol-implanted females became sluggish and most of them either died or appeared ill at termination of the experiment. Treated females rarely fought and never displayed the male type of courtship

or copulatory pattern. However, three of the four estradiol-treated spayed females were mated by introduced males, and one of these, after 12 days of treatment, showed full estrus, including the neck bend and submission to copulation.

TABLE 4

Behavior of spayed females implanted with pellets of testosterone propionate

		PELLET FEMALES					CONTROL FEMALES				
		♀1	♀2	♀3	♀4	Totals	♀5	♀6	♀7	♀8	Totals
Male Behavior	Courtships	163	25	21	12	221	—	—	—	—	—
	Copulations	10	—	1	1	12	—	—	—	—	—
Estrous Behavior	Copulation by males	1	—	—	2	3	—	—	—	—	—
	Copulation by females	—	3	—	9	12	—	—	—	—	—
	Other estrous signs shown	—	1	—	5	6	—	—	—	—	—
Aggressive Behavior	Fights	142	40	21	9	212	28	183	1	—	212
	Fight mixed with courtship	26	1	4	—	31	—	—	—	—	—
	Totals	168	41	25	9	243	28	183	1	—	212

DISCUSSION

A. The ovaries

Evans and Clapp ('40) state that they could observe no differences between left and right ovaries of Anolis carolinensis. However, they did not weigh the ovaries of "summer" females. Hartman ('39), in a comprehensive review of the literature, finds that regular, alternate ovulation has not been observed in any species concerning which facts are available, namely, cow, sheep, monkey and man.

Our data are suggestive of a regular swing in function between left and right ovaries, resulting in ovulation into alternate oviducts. The fact that female Anolis carolinensis have never been found to have more than one egg in either oviduct and lay a single egg at a time, would seem to argue for such a mechanism.

Additional evidence bearing on this point is presented by the striking ovarian hypertrophy with testosterone treatment. In two of the four cases, not only were the ovaries greatly enlarged, but ovulation had actually taken place (fig. 5). Nevertheless, the basic alternation still appeared present, for the ovary opposite the oviduct containing the egg was the larger and retained two mature ova instead of the usual one. It seems likely, from the similar results with mammals, that the anterior lobe of the pituitary of Anolis was stimulated by testosterone propionate to secrete an abnormal amount of gonadotropic hormone. The possibility of a direct effect should be tested with hypophysectomized subjects.

The nature of the effect of estradiol upon the ovaries is not clear from our results. Certainly no such great hypertrophy took place as with testosterone treatment. The use of smaller doses to avoid extensive hemorrhage and exploratory laparotomy at start of treatment might reveal whether this hormone exerts a stimulative or inhibitory effect.

B. The oviducts and cloaca

Lereboullet (1851) and Giacomini (1893) distinguished four parts of the oviduct of lizards: (1) infundibulum, (2) tube, (3) uterus and (4) vagina. The most detailed histological descriptions of the oviduct and cloaca available are those by Giersberg ('23), Regamey ('35) and Jacobi ('36). The oviduct of Lacerta, as reported by these authors, differs from that of Anolis mainly in respect to the terminal portion or vagina.

Giersberg and Regamey describe the vagina of lizards as consisting of high, narrow folds clothed by cylindrical, ciliated cells. This may correspond to our description of the proximal portion of the vagina of Anolis. In Sphenodon punctatus, according to Osawa (1898), these folds merge into a region with thickened musculature and then enter into the cloaca. A middle, muscular part of the vagina, like that of Anolis, is nowhere else mentioned. Jacobi ('36) pictures a "glandular end-piece" in Lacerta vivipara (abbs. 13, 14, pp. 421–422) which, he believes, probably secretes something of importance for the introduced spermatozoa. His illustrations closely resemble the pluri-stratified squamous epithelium of the Anolis distal vagina, which is non-glandular. Regamey mentions no such structure in Lacerta agilis but rather finds that the extremities of the oviduct are lost in the "glands" of the urogenital hollow (p. 121) and probably do not open until the moment of egg-laying. This is not the case in Anolis, where the oviducts open directly into the cloaca at all seasons of the year, unless kept closed by sphincter action.

Regamey '35) was the first to report the seasonal transformations which take place in the urogenital sinus of female lizards. He makes a distinction between the cloaca, which resembles that of the male, and its annexes, two of which are described as urogenital hollows, indented in the dorsal wall of the urodeum. In females killed from May to September, the epithelium of the anterior wall of these hollows was found to be composed of a very dense cellular mass resembling the pluri-stratified squamous of the Anolis distal vagina and urodeum. Regamey observed tubular glands within the upper layers of this mass which he compared to the uterine glands of mammals, considering them to play a role at the time of egg-laying. In August and September, regression of both epithelium and glands took place; castration likewise brought about complete involution.

There appears to be little doubt that the "urogenital hollow" described by Regamey corresponds to the Anolis urogenital sinus. Our description differs from that of Regamey in the absence of tubular glands and the fact that the entire urodeum of Anolis becomes stratified both above and below the entrance of the oviducts. Furthermore, the terminal ends of the vaginae of Anolis are similarly clothed and are preceded by a muscular region. A possible function of the stratified squamous layers may therefore be suggested: to serve as protection against the stress of passage of the egg at oviposition. If this were the function also in Lacerta, Jacobi's "end-piece" might be interpreted as the stratified distal vagina and Regamey's "urogenital hollow" as the point of entrance of the vagina into the urodeum.

It appears, therefore, from an analysis of the literature and comparison with the Anolis picture, that the differences in the available histological descriptions of the Lacerta oviduct may be more apparent than real. The oviducts and urodeal cloacas of Anolis and Lacerta are apparently very closely comparable.

Testosterone propionate elicits growth changes in the oviduct of immature female alligators (Forbes, '39) and adult female Sceloporus (Gorbman, '39). Kehl ('38) secured this effect with androsterone benzoate in adult female Uromastix. Since we have shown that testosterone propionate hypertrophies the ovaries of Anolis, the possibility exists that the growth of the oviducts observed by Kehl, Forbes and Gorbman was the result of the secretion of estrogens by androgen-stimulated ovaries. However, we have demonstrated that the action of testosterone propionate is independent of the presence of the ovary. All regions of the oviduct and the urodeal cloaca of spayed females react to this hormone with unmistakable growth changes. In the intact females, synergistic action of testosterone and secreted estrogens probably occurred, since the oviducts of treated females exceeded in weight the heaviest oviducts of the controls.

As in the case of testosterone, estrogens have also been used entirely upon intact subjects. Kehl ('30) stimulated the oviducts of female turtles with folliculin. Dantchakoff and Kinderis ('37) injected folliculin into lizard eggs, securing enlargement of the oviducts at hatching. Forbes ('38) treated immature alligators with estrone and induced well-formed oviducal glands, smooth-muscle layers and ciliation. Gorbman ('39) and Evans and Clapp ('40) stimulated the oviducts of Sceloporus and Anolis carolinensis with theelin. In the latter, small doses (11.5 R.U.) caused growth of shell glands in winter oviducts.

In the present experiment, relatively large doses of estradiol dipropionate induced seasonal development of all regions of the oviduct and urodeal cloaca of spayed female Anolis carolinensis. Testosterone and estradiol are thus seen to produce similar effects upon the oviduct and urodeum of spayed Anolis.

C. Metanephros

The "sexual segment" of the reptile metanephros (Regaud and Policard, '03) has recently been studied by Forbes ('41) in male Sceloporus. The latter concludes that the hypertrophic tubules are actually the collecting ducts. In the male lizard, these tubules undergo a striking seasonal change which does not take place in the female. However, Kehl ('38) showed that the potency for such transformation is latent in the female, since it could be evoked by treatment with androsterone.

Corresponding tubules of the metanephros of both intact and spayed adult female Anolis are strikingly stimulated by testosterone. Full secretory hypertrophy becomes evident in the granular aspect of the tall, thin columnar cells and the presence of secretion material in the lumen. Strong doses of estradiol dipropionate have no such effect and the columnar ducts remain small in diameter.

D. Sexual behavior

The estrous behavior of Anolis has been found to include: (1) submission to copulation and (2) a distinctive neck reflex when mounted by the male. Evans ('38) also reports that "cooperation" by the female may be observed during the mating of Anolis, but does not give objective criteria for its determination. Neither "mere flight" (Evans, '35) nor the head-nod gesture (Evans, '38) are acceptable as evidence of receptivity, since spayed females and immature males nod and run when courted but never submit to copulation. Our criteria have been subjected to experimental analysis and have been found to be reliable (Noble and Greenberg, in preparation).

We have used the term estrus to describe the sexual behavior of the female lizard (Noble and Greenberg, '40, '41), with full recognition of the phylogenetic comparisons that such usage implies. As will be more extensively discussed elsewhere, we believe that the female lizard's submission to copulation with characteristic neck bend is comparable to (1) the sexual receptivity exhibited by female mammals, (2) the squatting of the hen for the cock, (3) the female Hyla's approach to the calling male (Noble and Noble, '23), and, in Rana pipiens, the ovulating female's failure to give the warning croak when clasped, followed by back-shuffling movements and oviposition (Noble and Aronson, in preparation), and (4) the courtship behavior of the gravid female jewel fish (Noble, '38).

The common basis for all of these behavior patterns probably is the ovarian condition and ovarian hormones. Young ('37) and his co-workers (Blandau, Boling and Young, '41) have demonstrated a definite correlation between sexual receptivity and time of ovulation in the guinea pig and rat. Our data suggest that such a correlation may be present in the female lizard. The estrous behavior of the lizard is dependent upon the ovary, for ovariectomy eliminates estrus completely while administration of crystalline sex hormones to spayed or intact females or frog anterior pituitaries to intact females (Noble and Greenberg, in preparation) induces full sexual receptivity.

Pellets of crystalline testosterone propionate readily activate the complete male type of courtship and copulatory pattern in adult female Anolis. Although this effect has not previously been reported in reptiles, similar results have been secured with birds and mammals. Injection of testosterone propionate elicits some phases of male courtship in female canaries (Leonard, '39; Shoemaker, '39; and others). Adult hens crow and become more aggressive (Allee, Collias and Lutherman, '39), when so treated. Noble and Wurm ('40) injected adult female night herons with testosterone propionate and secured various elements of the male courtship pattern. Ball ('40) found that although testosterone propionate, injected into young female rats, inhibited vaginal estrous cycles, it stimulated masculine behavior. Ball noted that in those cases where vaginal estrus was completely inhibited, heat behavior continued at a very low level of intensity. The histological picture of the ovaries of two such females made it seem likely that they were secreting small amounts of estrin.

Pellets of testosterone propionate, implanted into spayed female Anolis, not only elicit the complete male copulatory pattern but also induce estrous responses. Three of the four treated spayed females submitted to copulation and two of these exhibited a high degree of receptivity (table 4). Immature castrate male Anolis have also been observed to respond to testosterone treatment in this dual manner (Noble and Greenberg, '40, '41). It appears possible, therefore, to postulate a direct ambisexual effect of testosterone propionate upon the sexual behavior of Anolis. Similar treatment of castrate subjects may well reveal the same action in other animals.

E. Estradiol and circulatory disturbance

Prolonged treatment of male Sceloporus with estrone pellets was fatal to most of the animals (Forbes, '41). Burns ('40) observed that estradiol dipropionate injected into opossum pouch young of both sexes induced an extensive cornification of the urogenital sinus, leading to fatal urinary retention. We secured stratification and cornification of the urodeum with estradiol dipropionate pellets implanted into female

Anolis but cannot consider this as harmful, since such stratification is the normal, estrous condition. However, the extensive nature of the hyperemia present in our treated females, and the peculiar colloid-like thickness of the contents of the blood vessels, appear to be strong indication of deleterious effects.

Poris ('41) described dilated sinusoids in the anterior lobe of the pituitary of Anolis carolinensis following continued estradiol injections. A "colloid-like" material filled the sinusoids and was suggested to be a secretion of the basophils. If this substance is the product of hyperactive secretory cells, it cannot be considered as a specific secretion of the basophils, since similar "colloid" filled the blood vessels in other body regions of our estradiol-treated animals.

On the other hand, the vascular changes observed in estradiol-treated Anolis resemble certain phases of adrenal insufficiency in mammals. Swingle, Parkins, Taylor and Hays ('38) found that adrenalectomized dogs maintained on cortical extract and subjected to trauma promptly develop a circulatory collapse which they attributed to general capillary atony, dilatation and stasis. Adrenal cortical hormone, when administered in adequate amounts, restores the circulation to normal. Although the effects of adrenalectomy have not been described in Anolis, it appears likely that the vasodilation and hemoconcentration associated with estradiol treatment are adrenal cortical insufficiency symptoms.

SUMMARY

1. Evidence is presented suggesting that in Anolis carolinensis there is an alternate, unilateral ovulation.

2. Pellets of testosterone propionate effect a striking hypertrophy of the ovaries but apparently do not change the sequence of ovulation.

3. Testosterone propionate and estradiol dipropionate, administered separately, produce similar growth changes in the oviducts and urodeal cloacas of spayed Anolis.

4. Testosterone also elicits a typical male growth reaction of the metanephric collecting tubules of spayed and intact females.

5. Prolonged treatment with estradiol dipropionate pellets leads to widespread dilation of blood vessels, which become filled with thick, "colloid-like" material. It is suggested that this effect involves the adrenals.

6. Estradiol induces estrous behavior in spayed female Anolis. Testosterone elicits the complete male type of courtship and copulatory pattern in spayed and intact females; the same treated females may likewise give estrous responses and submit to copulation. Dominance of the male or female pattern in any individual is correlated with fight aggressiveness.

7. It is concluded that testosterone propionate has a direct ambisexual effect upon the mating behavior of Anolis as well as a dual action upon the urogenital system.

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PLATE 1

EXPLANATION OF FIGURES

1 Ventral view of the ovaries and oviducts of a control female. The ovaries are bilaterally undeveloped, the oviducts correspondingly thin. × 2.66.

2 Ventral view of a second control. Note the unilateral growth of a single follicle and the thickening of the oviducts. × 2.66.

3 Ventral view of a third control. The large follicle in the left ovary is further advanced than the one in the right ovary. The oviducts are thick and folded. × 2.66.

4 Ventral view of a fourth control female. An egg is present in the right oviduct and the ovary on that side is the smaller, while the left ovary contains a maturing ovum. × 2.66.

5 Ventral view of the ovaries and oviducts of a testosterone-treated female. Both ovaries are hypertrophied, the left containing two large eggs and the right ovary one. An egg is present in the right oviduct. × 2.66.

6 Ventral view of the ovaries and oviducts of an estradiol-treated female. Note the hemorrhagic condition of the ovaries and the resorption of the largest follicles. × 2.66.

7 Ventral view of the oviducts of a spayed female, implanted with a pellet of testosterone propionate. × 2.66.

8 Ventral view of the oviducts of a spayed control. × 2.66.

9 Ventral view of the oviducts of a spayed female, implanted with a pellet of estradiol dipropionate. × 2.66.

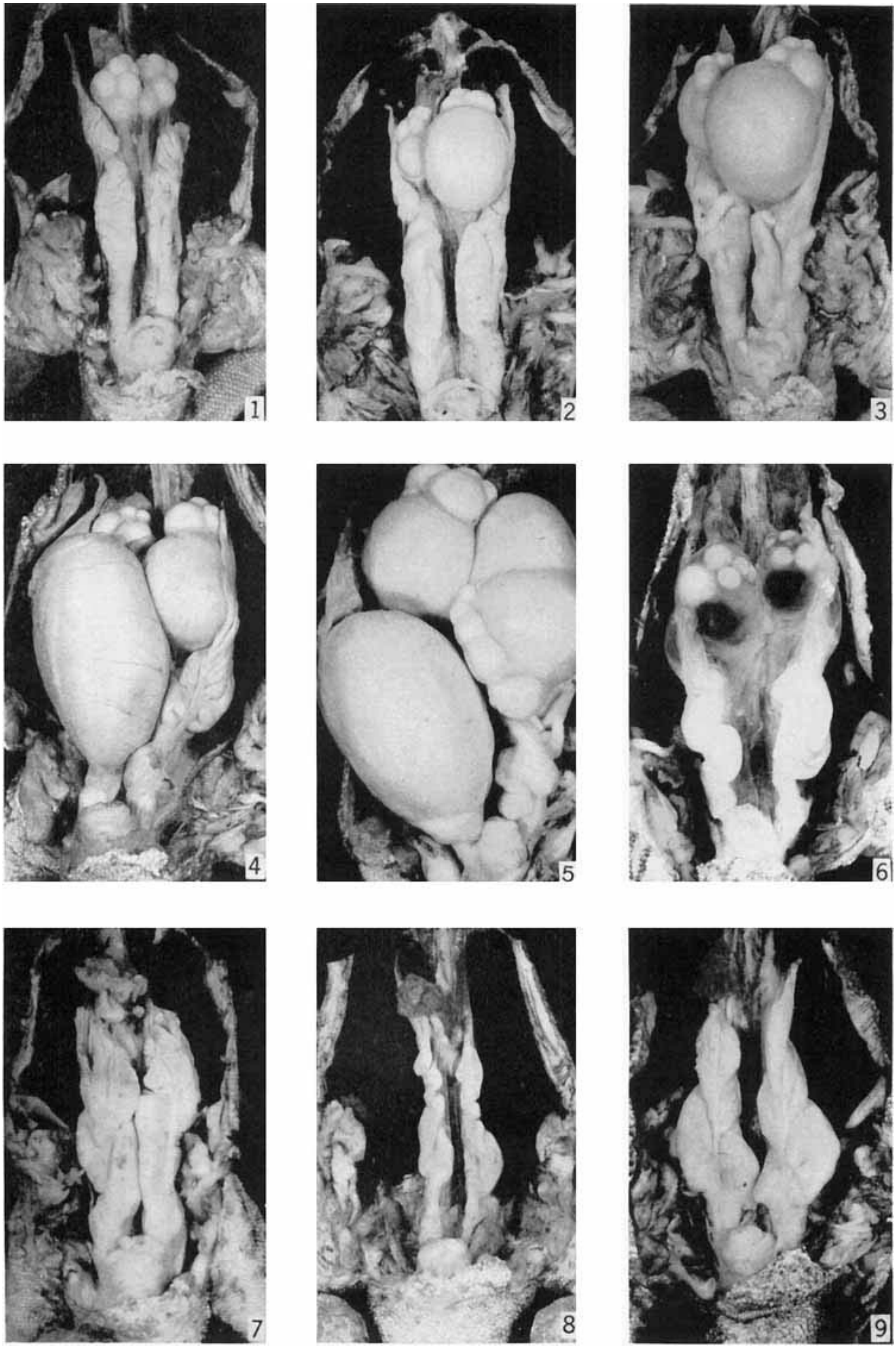
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PLATE 2

EXPLANATION OF FIGURES

10 Cross-section through the uterus of the intact control female represented in figure 3. Shell glands fill the space between inner lining and propria. × 50.

11 Cross-section through the proximal portion of the vagina of this female. Note the greatly folded lining and absence of shell glands. × 50.

12 Cross-section through the middle, muscular portion of the vagina of this female. Note the inner and two outer chambers, separated by smooth muscle and connective tissue. × 50.

13 Cross-section through the distal portion of the vagina of this female. Note the thick stratification. × 50.

14 Cross-section through the juncture of oviduct and urodeal cloaca of this female. The stratification of the distal vagina extends into the cloaca, which is similarly lined above and below the entrance point. × 50.

15 Cross-section through the entrance of oviduct into urodeal cloaca of a spayed control. One of the ureters is at upper left. × 50.

16 Cross-section through the juncture of oviduct and cloaca of a testosterone-treated, spayed female. The cloaca is at lower left. Note identical stratification of both. × 50.

17 Cross-section through the juncture of oviduct and cloaca of a spayed female treated with estradiol dipropionate. × 50.

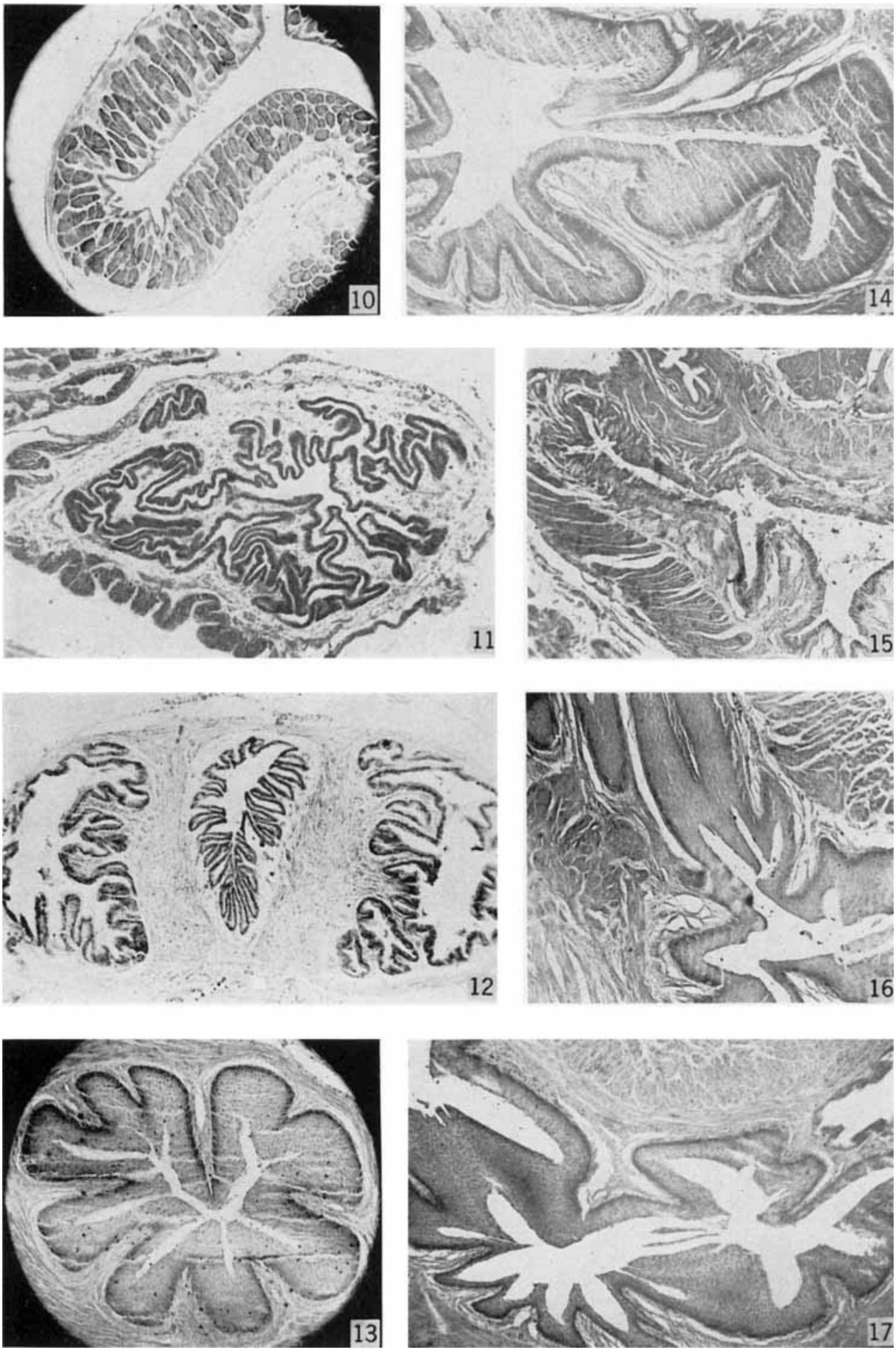
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PLATE 3

EXPLANATION OF FIGURES

18 Cross-section through the uterus of an intact female, implanted with loose crystals of estradiol dipropionate. Note the hyperplastic appearance of the shell glands which are packed together, with indistinct outlines. × 50.

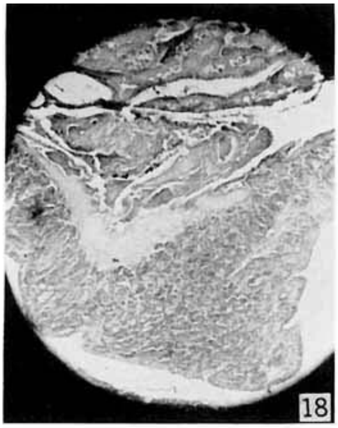
19 Cross-section through one fold of the urodeal cloaca of this female, showing the thick layers of stratified cells. Note the pycnotic nuclei. × 440.

20 Cross-section of the ovary of an intact female implanted with loose estradiol crystals. Note abundance of ''colloid''. × 440.

21 Cross-section of the adrenal gland and adjacent blood vessels of a spayed female, implanted with an estradiol pellet. Note the presence of ''colloid-like'' material in the sinusoids and adjacent blood vessels. × 100.

22 Cross-section through the metanephros of the intact control female represented in figure 3. Note the fairly uniform, small diameter of the tubules. × 100.

23 Cross-section through the metanephros of the testosterone-treated, spayed female (fig. 7). The collecting ducts are greatly hypertrophied. Note the tall, thin, columnar cells, with basal nuclei, and secretion material in the lumen. × 100.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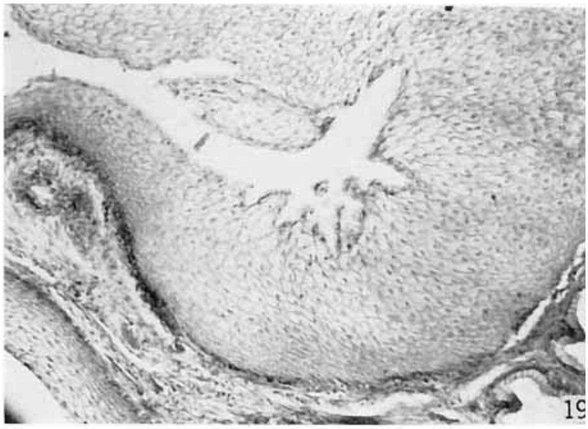
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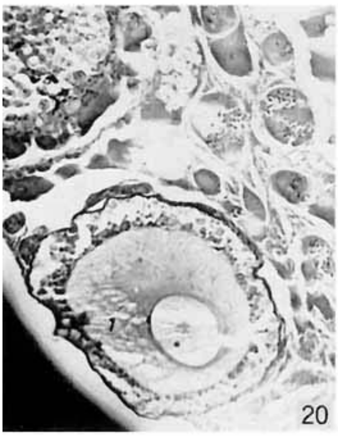
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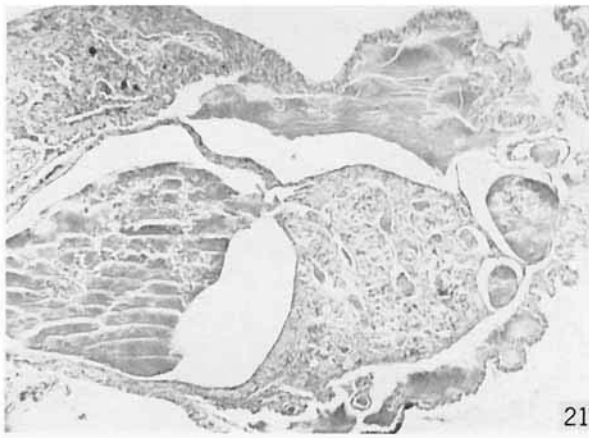
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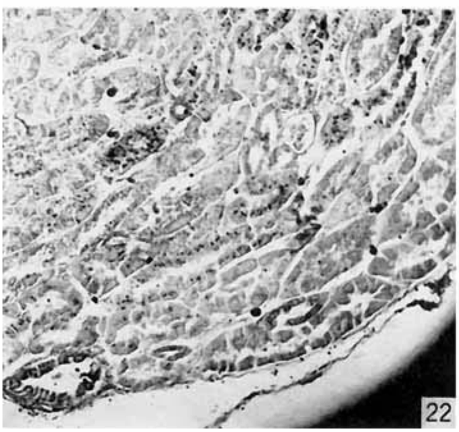
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