

Zoology. — *Effects of hypophysectomy on the male toad (Bufo bufo L.), with special reference to Bidder's organ.* By A. P. DE GROOT, G. A. DE VRIES and J. C. A. MIGHORST. (Zoological Laboratory, Dept. of Endocrinology, University of Utrecht.) (Communicated by Prof. CHR. P. RAVEN.)

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As in the toad Bidder's organ may develop after gonadectomy into a functioning ovary (HARMS, 1921, 1923; PONSE, 1924), it is likely that the gonads exercise an inhibiting effect on Bidder's organ. It is, however, still unknown in which way this influence is effected; it may be a direct influence, but it is also probable that the gonads act through the pituitary. In this connection it is interesting that in *Bufo arenarum* HOUSSAY and LASCANO-GONZALEZ (1931) found an increase in weight of Bidder's organ, following implantation of pituitaries and a decrease after hypophysectomy.

In 1941 the development and structure of the s.c. corpora lutea pre-ovulationis in the ovary of the Bitterling (*Rhodeus amarus*) were described in detail by BRETSCHNEIDER and DUYVENÉ DE WIT. As the development of these preovulation corpora lutea seemed to be influenced by the pituitary and very similar structures are found in Bidder's organ in which they were already described by HARMS (1921) and EGGERT (1926), who attribute an endocrine function to them, it seemed important to investigate the influence of the pituitary on Bidder's organ by way of hypophysectomy.

In April 1947 a great number of specimens of *Bufo bufo* were treated as follows: 1. One group was hypophysectomized, 2. another group was hypophysectomized and, in addition, castrated, 3. a third group consisting of 4 specimens was castrated only. Mortality was high in groups 1 and 2 and only 14 and 18 specimens respectively could be used for the present investigation. The experimental toads, belonging to the first group, were autopsied 19 to 67 days, those of the second group 4 to 29 days, and those of the third group 48 to 62 days after having been operated upon. Bidder's organs were studied histologically after fixation in Romeis-solution and staining its sections ($7\frac{1}{2}$ μ) with haemalum-eosin. For detailed studies of the organs of Bidder the sections with the largest diameter were always used. The hypophysectomies were performed by MIGHORST, the castrations by DE GROOT. As in groups 2 and 3 no essential changes were observed as a result of castration, only the effects of hypophysectomy will be related here.

1. Hypophyseal influence on the skin.

a. Some days following hypophysectomy the superficial layer of the epidermis assumes a dark brown colour; it is cast off in large sheets,

showing a normal but paler epidermis underneath; a couple of days later moulting is repeated and this occurs afterwards several times though in a less degree. GIUSTI and HOUSSAY (1921, 1922, 1923 and 1924) describing the same phenomenon in hypophysectomized *Bufo marinus* (= *arenarum*), found that after removal of the pituitary the superficial layer of the epidermis increases enormously in thickness and that sheets of it are cast off periodically. GIUSTI and HOUSSAY (1923, 1924) by whom this phenomenon is called *hyperkeratosis* were unable to prevent it by the injection of hypophyseal extracts or by implantation of *Bufo*-pituitaries; but UNGAR (1933) succeeded in preventing it by the injection of extracts of bovine anterior pituitary lobes.

According to GIUSTI and HOUSSAY hyperkeratosis appears also after puncturing the brain just in front of the pituitary. As its pars tuberalis is found here, we think it probable that in the toad this part of the pituitary plays a role in causing this phenomenon.

b. After hypophysectomy another effect on the skin is distinct: its colour changes from dark green into greenish yellow. This phenomenon, also observed in hypophysectomized frogs (*Rana esculenta*) is caused, as TRENDELENBURG (1929) and HOUSSAY and GIUSTI (1929) have already demonstrated, by the absence of the intermediate portion of the pituitary and is the result of melanophore contraction.

2. Effect of hypophysectomy on musculature.

Hypophysectomized toads are very inert and weak and spasms occur frequently. They move with extended legs and body lifted high above the ground. As deficiency of the parathyroid results in tetany, the spasms observed in hypophysectomized toads might be attributed to the parathyrotrophic hormone being wanting, the existence of which however is not yet proved (cf. CAMERON, 1947, p. 100 and 349).

3. Effect of hypophysectomy on Bidder's organ (cf. table).

a. Liquefaction of oocytes. In hypophysectomized toads as well as in toads deprived of both pituitary and testes, a part of the oocytes of Bidder's organ shows very distinct changes. These relate to the protoplasm, taking the eosin stain poorly, and to their nuclei becoming eosinophile. The difference in staining between plasma and nucleus vanishes gradually. The nuclear membrane disappears and a distinct liquefaction of the oocyte takes place, resulting in the appearance of a cavity (fig. 2). During these changes the granulosa may or may not increase in size. Liquefaction also occurs in Bidder's oocytes which are generally found in the testes in the testis tubules. It also occurs in a very low grade in normal organs of Bidder (fig. 1), but in hypophysectomized toads it is already visible in a much higher degree 4 days after the operation.

Strong liquefaction appears only in hypophysectomized toads. Whether

liquefaction is due to the absence of the pituitary could be proved by injecting hypophysectomized toads with pituitary extracts or by implantation of pituitary glands. Then only it would be evident that in the toad the pituitary exercises an inhibiting influence on the formation of

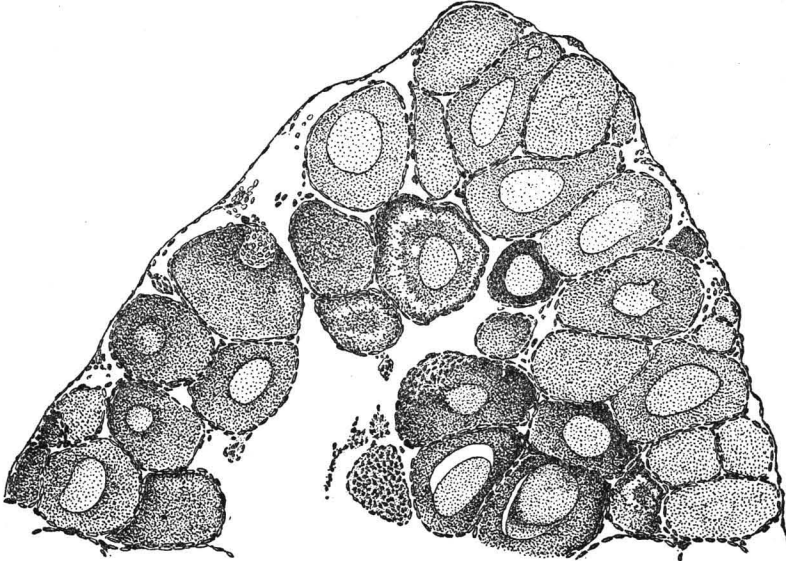


Fig. 1. Section of Bidder's organ of a normal male toad (*Bufo bufo*). $\times 72$.

preovulation corpora lutea. For liquefaction is a degenerative phenomenon and these corpora lutea originate from degenerating oocytes. It is likely that in hypophysectomized toads this degeneration is so quick or so strong that either the oocytes are unable to develop into corpora lutea, or the formation of corpora lutea starts only when the degeneration has proceeded further than normally. Therefore the liquefaction of the oocytes is considered to be a degenerative phenomenon that has proceeded very far.

	Number of oocytes	% of corp. lutea	% of liquefied oocytes	% of young oocytes	% of cortical tissue content
controls	174 \pm 21	4.5 \pm 1.6	0.5 \pm 0.4	6.2 \pm 1.3	82.4 \pm 2.2
hypophys-ectomized	121 \pm 13	6.6 \pm 1.9	20.5 \pm 3.1	3.6 \pm 0.9	70.9 \pm 3.3
hyp. + castrated	95 \pm 18	7.0 \pm 1.1	15.8 \pm 2.6	3.9 \pm 0.8	73.9 \pm 2.0
castrated	98 \pm 19	5.7 \pm 1.2	1.3 \pm 0.4	11.9 \pm 6.2	76.4 \pm 4.1

Table, showing details of Bidder's organs of normal and experimental toads.

b. Decrease of number of oocytes and content of cortical tissue.

From the table it follows that the number of oocytes decreases considerably in hypophysectomized and in hypophysectomized and castrated toads,

this being the result of liquefaction, As the toads left for castration were not as large as those used in the first two groups, the small number of

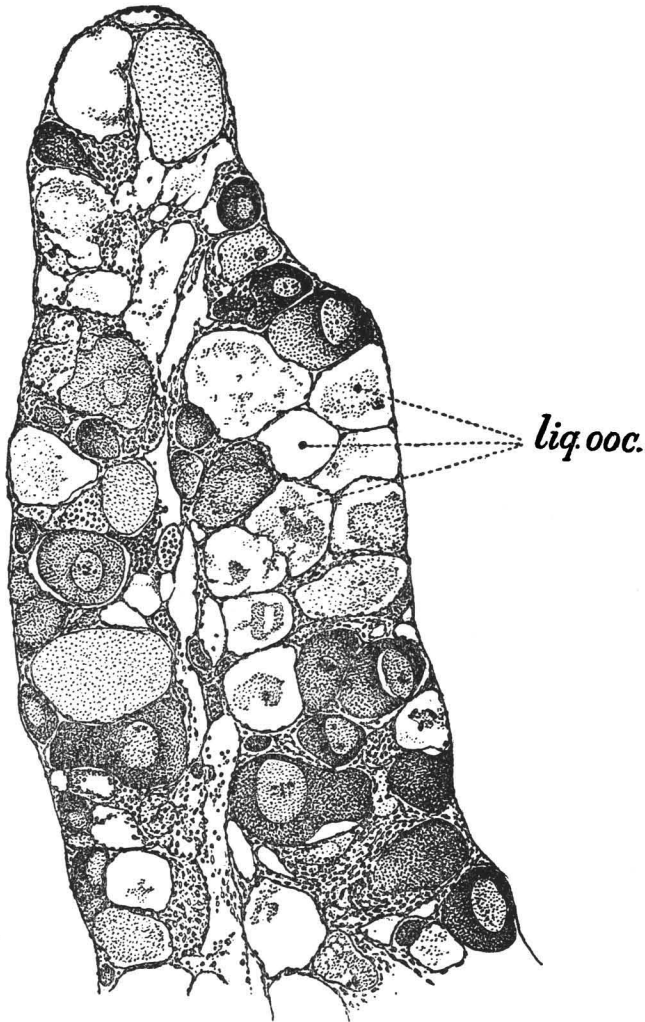


Fig. 2. Section of Bidder's organ of a hypophysectomized male toad (*Bufo bufo*) with many liquefied oocytes (*liq. ooc.*). $\times 72$.

oocytes in castrated toads can be ascribed to the small size of their organs of Bidder.

c. Number of preovulation corpora lutea.

Preovulation corpora lutea are found in Bidder's organs in normal as well as in experimental toads. In all groups (cf. table) their number was about equal. Especially in hypophysectomized toads the presence of the normal number of corpora lutea is interesting. It is therefore likely that in the toad the pituitary does not influence the development of corpora

lutea. This opinion is contrary to that of BRETSCHNEIDER and DUYVENÉ DE WIT (1941), investigating the Bitterling, in which however hypophysectomy was only successful in one case.

From the above it is concluded that hypophysectomy results in a decrease in size of Bidder's organ, being the consequence of the liquefaction of many of its oocytes. It therefore seems that the pituitary maintains the size of Bidder's organ.

Summary.

In male hypophysectomized toads, *Bufo bufo* L., the following effects occur.

1. Skin changes. *a.* Its colour changes from dark green into greenish yellow, this being the result of melanophore contraction. *b.* Occurrence of a distinct hyperkeratosis, resulting in the superficial layer of the epidermis being cast off periodically.

2. The experimental toads suffer from a distinct astheny, accompanied by a typical tetany. Hypophysectomized toads move with extended stiff legs, their body being lifted high above the ground. This phenomenon can possibly be ascribed to the lack of parathyrotrophic hormone.

3. Bidder's organ diminishes in size, being mainly caused by liquefaction, i.e. an abnormal strong degeneration of the oocytes, a decrease in number of oocytes and of the quantity of cortical tissue in the ovary. As the number of preovulation corpora lutea is not effected by hypophysectomy, it is suggested that the formation of these structures is not dependent on the pituitary.

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