Zoology. — *Spermiation in Rana and Salamandra.* Preliminary note [1]). By G. J. VAN OORDT, F. CREUTZBERG and N. SPRONK. (Zoological Laboratory, Dept. of Endocrinology, University of Utrecht.) (Communicated by Prof. CHR. P. RAVEN.)

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In 1929 HOUSSAY and LASCANO GONZALEZ showed that after transplantation of the anterior part of the mammalian pituitary in male specimens of *Bufo arenarum* sperms are released from the testes in a remarkably short time. This observation has been confirmed later by RUGH (1935) in *Rana* and by many others; from it GALLI-MAININI (1947) derived his pregnancy-test, for which many species of *Bufo*, as well as of *Rana*, may be used.

In a recent communication (VAN OORDT and KLOMP, 1946) we have introduced the term s p e r m i a t i o n for the liberation of sperms from the testis, a term which is analogous to ovulation, the process of discharging the ova from the ovary.

The subject of the present investigation is a study of spermiation in representatives of Anurans and Urodeles. As experimental animals hibernating *Rana esculenta* and *temporaria* and *Salamandra salamandra* were used.

We have found on the one hand that spermiation can be provoked easily by gonadotrophins [2]) in these species; on the other hand important differences were observed in the way in which this process takes place in Anurans and Urodeles respectively.

Rana spec. In intact *Rana*-specimens spermiation is accomplished easily after administration of gestyl, a pregnant mare serum gonadotrophin, or pregnyl, a pregnancy urine preparation. In a specimen of *Rana esculenta*, which has been injected with a dosis of 2—3 I.U. of pregnyl pro gram body-weight, sperms are already found in the cloacal urine after about 1 hour.

As we were also interested in the resumption of spermatogenesis in the testis tubules after spermiation, we have tried, by repeated injections, to get testis tubules totally free from sperm. However, in *Rana* it is extremely difficult to accomplish this phenomenon. After 9 doses of pregnyl had been administered in 22 days to a specimen of *Rana esculenta*, most of the testis-tubules were not yet empty (fig. 1).

In this species it was difficult to ascertain, whether a resumption of spermatogenesis took place after administration of such a large quantity

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[2]) These preparations were kindly supplied by the Direction of Organon N.V., Oss.

of pregnyl, as control specimens may also show many spermatogenetic cysts in the tubules of the winter-testis. In *Rana temporaria* however, in which the testis-tubules contain only very few spermatogonia and numerous compact sperm-bundles in winter, the formation of spermatogenetic cysts and therefore the resumption of spermatogenesis was very distinct in several cases after 3 or more gestyl-injections (fig. 2). Of course this spermatogenesis may be provoked by the pituitary, as the experimental frogs were not hypophysectomized, but it seems more likely that the resumption is (directly?) caused by the injected gonadotrophins.

From the above follows that in *Rana* spec. there is a distinct, direct (?) influence of pregnyl on the testis-tubules, which results in the liberation of sperms from these tubules.

Salamandra salamandra. It is a well-known fact that in Urodeles the testis structure is very different from that of the Anurans. In the newts (*Triturus* spec.) as well as in the Spotted Salamander (*Salamandra salamandra*) the winter-testis possesses numerous tubules, filled with bundles of sperm, loosely attached to Sertoli-cells, and with very few spermatogonia and tubules, totally filled with germ cells in the early spermatogenetic stage (especially spermatogonia) which show very few or no cell-divisions. These tubules form two areas, which may be called the sperm- and the spermatogenetic part, with a transition-zone between them (fig. 3). The testis of *Salamandra* belongs to the multiple type; in every lobe a sperm- and a spermatogenetic part are present, whereas in the narrow connecting tissue only male primary sex cells are to be found.

Spermiation is very easily caused in the sperm-part. Already after 2 doses of 4—5 I.U. of gestyl pro gram body-weight (the second dose given 48 h. after the first) practically all sperms are released from the testis-tubules and 5 h. after the second injection the testis is totally devoid of sperms (fig. 4).

Moreover, it is interesting that in experimental as well as in control salamanders, killed by means of chloroform, sperm and mucus were present in the cloaca, even before they died, but that in the cloaca of control salamanders which had been decapitated, no sperm or mucus secreted by the cloacal glands could be found. Between the experimental animals and the controls, however, there was a conspicuous quantitative difference, which became especially distinct on microscopical investigation of the testes: control specimens which had been killed by chloroform showed little spermiation, whereas in gonadotrophin-treated animals spermiation was mostly total and the efferent ducts of the testis were packed with sperm.

After spermiation the following processes take place in the "empty" testis-tubules (fig. 4):

1. a distinct increase in size of the Sertoli cells, which pass over into large inflated cells, filling almost the whole diameter of the tubules, and
2. the formation of large cells, very similar to male primary sex-cells.

From the above it follows that spermiation can be caused by gonadotrophins in *Rana* as well as in *Salamandra*. The way in which spermiation takes place is very different, however: gradually in frogs, very rapidly in the salamander. Though we have given the salamanders a relatively larger quantity of gonadotrophic hormones than the frogs, we think that the differences in the way in which spermiation is accomplished may be explained with the help of the biology of the investigated animals:

Male frogs of most species generally possess large seminal vesicles in which sperm is stored before and during the breeding season. Therefore it is not necessary that sperms are liberated from the testis tubules at once, and we may assume that under a rather prolonged but weak influence of the anterior lobe of the pituitary, spermiation takes place gradually in spring. In the salamander, however, the formation of spermatophores, consisting of a large quantity of sperm and of mucus, secreted by the cloacal wall, is a process which must take place rapidly; hence, in the reproductive period the sperms are suddenly released from the testis in one large mass, presumably under the influence of a large amount of hypophyseal gonadotrophin. Therefore total spermiation can only be provoked experimentally by many repeated injections of gonadotrophins in *Rana* and by already 2 consecutive injections of these hormones in *Salamandra*.

Summary.

Spermiation, i.e. the process of liberation of sperms from the testis, can be provoked easily in *Rana* and *Salamandra* by means of gestyl as well as pregnyl. Consequently both gonadotrophins have a distinct (direct?) influence on the testis-tubules.

In *Rana* it is almost impossible to get total spermiation: after more than 9 dosages of pregnyl administered in 22 days, most of the testis-tubules of a specimen of *Rana esculenta* still possessed sperms. Resumption of spermatogenesis after gonadotrophin-injections was especially distinct in *Rana temporaria*.

On the other hand spermiation is easily accomplished in *Salamandra salamandra*; all sperms are released from the sperm-part of the Salamander-testis after 2 dosages, the second being given 48 h. after the first.

The differences in the way in which spermiation is accomplished in these Amphibians are explained with the help of the biology of the investigated animals.

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DESCRIPTION OF PLATE.

Fig. 1. Spermiation of *Rana esculenta* after 9 doses of 2—3 I.U. of pregnyl pro gram body-weight, administered in 22 days. Large quantities of sperm are still present in the testis-tubules.

Fig. 2. *Rana temporaria.* Distinct resumption of spermatogenesis after 3 injections of 2—3 I.U. of gestyl pro gram body-weight.

Fig. 3. *Salamandra salamandra.* Section through testis of a control specimen. At the upper side of the figure the spermatogenetic, at the bottom-side the sperm-part.

Fig. 4. Spermiation of *Salamandra salamandra* after 2 injections of 4—5 I.U. of gestyl pro gram body-weight. In the tubules of the sperm-part only enlarged Sertoli-cells and a few male primary sex cells are present; no sperms.

Fig. 1.

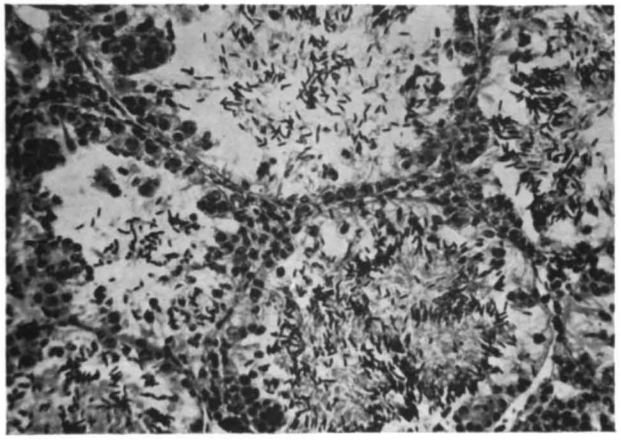

Fig. 2.

Fig. 3.

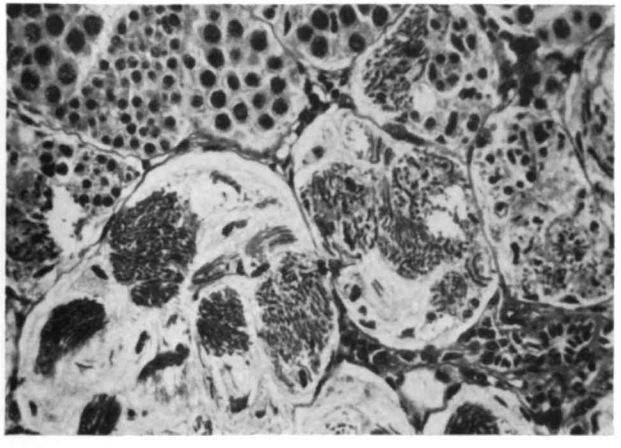

Fig. 4.