These data show that a radiation of 40 seconds produces in *P. caudatum* a depression of the division rate and the zone of stimulative action is therefore located between 0 and 25 seconds. The curve for *P. bursaria* goes above that of *P. caudatum* and correspondingly the zone of stimulation is extended more to the right somewhere between 0 and 80 seconds. On the whole *P. caudatum* is about 2 times more susceptible to the stimulative and depressive action of the ultraviolet radiation as compared with *P. bursaria.*

The second series of experiments during the first 3 days confirms these conclusions.

Beginning with the fourth day the susceptibility of *P. bursaria* turned out to be practically the same as that of *P. caudatum.* The explanation is as follows: During the whole period of experimentation the cultures were kept in darkness and microscopical observation has shown that *P. bursaria* has lost almost completely the green color of the symbiotic algae. It seems therefore that the presence of pigmented algae is the cause of a greater resistance of *P. bursaria* against the influence of the ultraviolet radiation.

7576 C

Oestrus in Hypophysectomised Rats Parabiotically Connected with Castrates.*

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It is known that unoperated female rats, when united in parabiosis with castrates, first pass through a period of irregular oestral and anoestral activity and later go into a condition of constant oestrus. Hill[1] has reported that during this second period the ovaries always contain large numbers of mature follicles but no corpora lutea. On the contrary in the first period the ovaries are crowded with corpora lutea which, especially during prolonged anoestral phases, are increased in number far beyond anything observed under normal conditions. Cryptorchid males (Martins[2]) as

* This investigation was supported by grants from the Committee for Research in Problems of Sex of the National Research Council.

[1] Hill, R. T., *Endocrinol.,* 1933, **17,** 414.

[2] Martins, Th., *Compt. Rend. Soc. de Biol.,* 1930, **105,** 789.

well as X-ray sterilized males and females (Levine and Witschi[3]) produce the same effects as castrates (Fig. 6) if put in parabiosis with normal females. This is remarkable since the secondary sex characters are maintained in these cases. An histological examination of the hypophyses proves that the characteristic "castrate cells" are present not only in castrates but also in cryptorchids (Fig. 7) and in X-rayed males and females (Levine and Witschi[3]). Destruction of the germ cells by either treatment causes similar or identical histological and functional changes in the hypophysis.

Fels[4], Kallas[5], and Martins[6] suggested that the known hyper-function of the "castrate hypophysis" might be responsible for the striking reactions in the unoperated parabiont. These authors gave little consideration to the fact that the non-castrate too possesses a hypophysis which may participate in evoking ovarial reactions. The experiments here described will furnish proof that each hypophysis is responsible for one of the 2 main reactions, prolonged anoestrus and constant oestrus.

In a first experiment it was attempted to unite females hypophysectomised for over one month with male castrates. This was not very successful. The females, which were weakened by hypophyseal deprivation, died in all but one case shortly after the operation. In the one surviving case the female recovered to a healthy condition only after several weeks. She remained in anoestrus during the first month of parabiosis and then went into constant oestrus. Obviously, the anoestral phase was due to the poor general condition of the female, following the parabiosis operation. The oestral phase, which has lasted now for 2 months with only one short interruption, must be due to the influx of hypophyseal hormones from the castrate.

A second experiment starts with females that had been in parabiosis with castrate males from 2 to 6 months. All had established the constant oestrus condition. In 5 pairs of this type the hypophysis of the female was removed with no effect upon the oestral type of vaginal smears (Fig. 1, pair 192). It is true that some of the females fell once or twice into anoestrus for periods of 1 to 3 days, immediately following hypophysectomy; though apparently this was due merely to general disturbances caused by the operation.

[3] Levine, W. T., and Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1152.

[4] Fels, E., *Arch. f. Gynaek.*, 1929, **138**, 16.

[5] Kallas, H., *Pflüg. Arch. ges. Physiol.*, 1929, **223**, 232.

[6] Martins, Th., *Compt. Rend. Soc. de Biol.*, 1929, **103**, 1341.

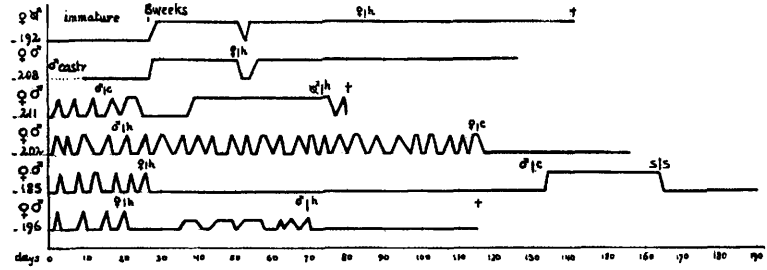

FIG. 1.

Selected oestrus curves of parabiotic pairs of rats. The base corresponds to the anoestral and dioestral condition, the top to full oestrus (stage 2-3). *Pair 192:* male was castrated at birth, united with female at 3 weeks. The female, when 8 weeks old, starts oestrus which is constant with only one interruption of 2 days. Eight weeks later the female is hypophysectomized (♀ h) but remains in constant oestrus. *Pair 208:* Adult male is castrated and united with adult female on same day. No smears taken for first 8 days. Constant oestrus established on 28th day. Female hypophysectomized on 51st day of experiment. *Pair 211:* Normal pair, female has shown regular cycles for some time. Male castrated on 15th day. Female establishes constant oestrus on 39th day. Castrate hypophysectomized on 73d day. Female resumes normal cycles on 75th day. *Pair 202:* Normal pair. Male hypophysectomized on 20th day. Female continues normal cycles until castration on 115th day. *Pair 185:* Normal pair. Female hypophysectomized on 26th day, falls immediately into anoestrus. Castration of male on 130th day brings female into constant oestrus within 4 days. After separation from the castrate on the 164th day the female returns immediately into anoestrus (165th day). *Pair 196:* Normal pair. Female hypophysectomized on 20th day; goes immediately into anoestrus but after the 35th day shows periods of incomplete oestrus. After hypophysectomy of the male complete anoestrus is established again.

Later, constant oestrus is maintained in all cases. (Fig. 1, pair 208).

The ovaries of these hypophysectomised females in constant oestrus are extremely enlarged and contain large numbers of growing follicles (Fig. 2), some of excessive size, others equal to normal mature follicles. New follicles obviously are added constantly while the older ones degenerate (Fig. 2, follicles of irregular shape). These ovaries are larger even than those of non-hypophysectomised females under similar conditions (Fig. 6, Levine and Witschi[3]). The uteri are widely distended and their lumen is filled with cell-debris and leucocytes (Fig. 3). In 4 cases the females were separated from the castrate males 5 or more weeks after hypophysectomy. Within 2 days they fell into anoestrus (compare Fig. 1, pair 185 S,S). Ovaries preserved at different intervals show a rapid atresia of all mature follicles, without ovulation. It is evident, therefore, that the follicular stimulation was due to a hormone received from the castrate.

That this hormone originates from the hypophysis of the castrate is definitely shown in a third set of experiments in which the castrate is hypophysectomised (2 cases), whereupon the unoperated

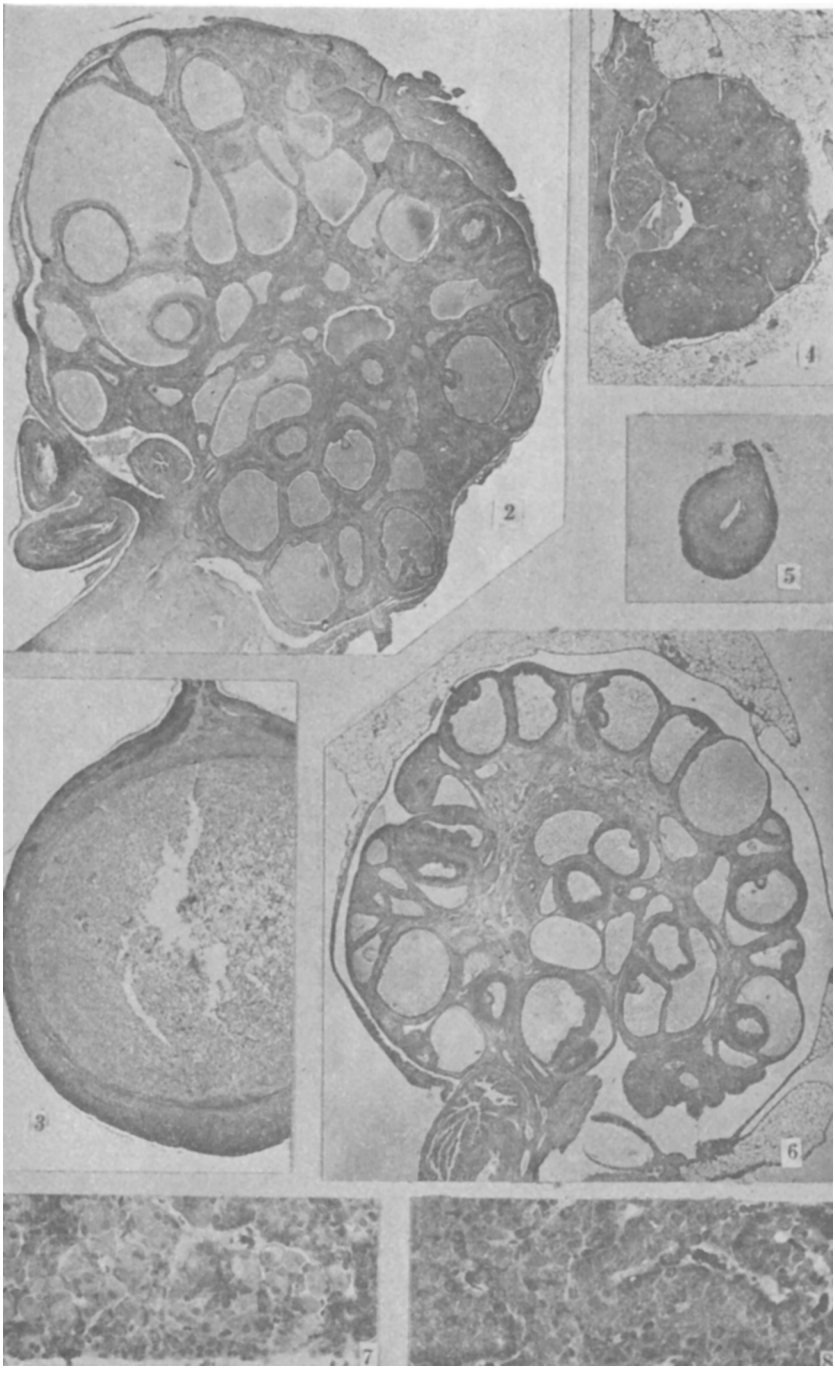
2
4
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FIG. 2.
Parabiotic pair 186. Ovary of a hypophysectomized female, united with castrate male, after constant oestrus of 5½ months. × 12.

FIG. 3.
Parabiotic pair 210. Similar conditions as in previous case. Cross-section of uterus. × 12.

FIG. 4.
Parabiotic pair 205. Ovary of a hypophysectomized female united with normal female. Preserved after 2½ months of anoestrus. × 12.

FIG. 5.
Same pair as Fig. 4. Uterus of hypophysectomized female. × 12.

FIG. 6.
Parabiotic pair 175. Ovary of female in parabiosis with X-ray sterilized female. Preserved after 3½ months of constant oestrus. × 12.

FIG. 7.
Parabiotic pair 178. Anterior lobe of hypophysis of the male twin, one-half year after experimental cryptorchidism had been established. Note the large "castrate cells" filled with colloid. × 200.

FIG. 8.
Same pair as Fig. 7. Anterior lobe of hypophysis of the female twin, which had been in constant oestrus for more than 4 months. Nearly normal histological picture. × 200.

female returns from the constant to cyclical oestrus (Fig. 1, pair 211). Normal cycles are maintained also by females in parabiosis with hypophysectomised females or males (Fig. 1, pair 202).

The reported experiments show clearly that the condition of constant oestrus is due to an influx of follicular growth stimulating hormone which is released in great quantities by the hypophysis of the castrate co-twin. There remains to be ascertained where the luteinizing hormone comes from, which causes the extensive formation of corpora lutea and the prolonged phases of anoestrus during the first period after castration. The case 211 (Fig. 1) may illustrate once more the sequence of typical reactions that follow upon castration of the male twin. The female still runs one normal cycle, then a prolonged cycle and then falls into a period of anoestrus. Constant oestrus is established only during the fourth week. In more than 20 similar cases Hill (1. c.) and the present authors have found that the period of irregularity preceding constant oestrus can last from 3 to 18 weeks. So, evidently, the hypophyseal system which produces the luteinizing hormone subsists slowly under the influence of the continuous stream of follicle stimulating hormone. Evidence that the hypophysis of the unoperated female is the source of the luteinizing hormone comes from a set of experiments in which we observe that constant oestrus follows castration of the male within 4 or 5 days, if the female had been hypophysectomised 5 to 15 weeks previously. This experiment (4 cases) runs in the following way.

We start with normal pairs (Fig. 1, pair 185). After normal cycles of the female had been observed for some time she is hypo-

physectomised. Immediately she falls into anoestrus, though in some cases she recovers partly, showing irregular and incomplete oestral changes in the vaginal smears. That this activity is due to stimulation by the normal male parabiont can easily be demonstrated. Subjecting him to hypophysectomy brings the female into permanent anoestrus at once (Fig. 1, case 196). Even if the vaginal smears show constantly the anoestral condition, as in case 185, the ovaries are slightly larger than in single hypophysectomised females or in females of double-hypophysectomised pairs. These ovaries contain large numbers of small egg follicles (white dots in Fig. 4) which later undergo fibrous degeneration. No luteinization is observed, and the output of oestrin must be negligible, as indicated by vaginal inactivity and reduced size of uteri (Fig. 5). If now, after an anoestral period of 5 to 15 weeks, the male parabiont is castrated, then the increased amount of hypophyseal hormones coming in from the castrate immediately causes a rapid growth of the Graffian follicles and constant oestrus is established within 4 or 5 days (Fig. 1, case 185).

This quick and clear cut reaction of hypophysectomised as compared with normal females makes it quite certain that in the latter the mainly anoestral period preceding constant oestrus is due to the production of luteinizing hormones by the female hypophysis. As long as this lasts, excessive corpus luteum formation will result because of the simultaneous stimulation of follicular growth by the castrate hypophysis. However the maintenance of a constant and high concentration of follicle stimulating hormone in the blood stream tends to suppress the production and the release of the luteinizer. Nevertheless the hypophysis of the female in constant oestrus retains the potency of producing the luteinizer. For such females, if separated from their castrate twins, resume cyclical changes and may become pregnant and have litters again. Histologically the hypophysis preserves the normal appearance even after months of constant oestrus (Fig. 8). The fact that the two gonadotropic hormones of the hypophysis thus act as an antagonistic pair probably plays an important rôle in the establishment of normal oestrus cycles.

Conclusions. Our experiments with parabiotic rats corroborate the contention of Zondek[7], and Hellbaum[8] gained from entirely different sources of evidence, that the castrate hypophysis emits an increased quantity of follicle stimulating hormone but no luteinizing

[7] Zondek, B., *Arch. f. Gynaek.*, 1930, **144**, 133.

[8] Hellbaum, A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 641.

hormone. Furthermore we conclude that the maintenance in the blood stream of a constant and relatively high level of follicle stimulating hormone suppresses the production of luteinizing hormone by the hypophysis.

7577 P

Nervous Control of Thyroid Activity. I. Effect of Pilocarpin and Adrenalin on Metamorphic Action of Thyreoactivator.

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In the experiments reported here we have used amphibian metamorphosis (in the larvae of *Ambystoma tigrinum*) as a physiological sign of the effects of the thyroid hormone, pilocarpin and adrenalin as stimulators of the parasympathetic and sympathetic nervous system respectively, and injections of thyreoactivator from the anterior lobe of the beef hypophysis[1-4] as an activator of the thyroid function.

When pilocarpin or adrenalin alone is injected intraperitoneally, no visible effect on metamorphosis is obtained. It will be shown here that the injection of either of these drugs together with thyreoactivator increases the sensitivity of the larvae to the metamorphic action of the thyreoactivator.

In one representative experiment (CCCLVI, 1933) a number of the larvae of the tiger salamander were divided into 4 groups: Group "a", controls injected with Ringer solution; Group "b, c and d" received triweekly intraperitoneal injections of thyreoactivator extracted from approximately 30 mg. dried anterior lobe, per animal and injection; group "c" received in addition triweekly injections of 2 mg. pilocarpin-hydrochloride (Merck) per animal and injection; group "d" received in addition triweekly injections of 0.05 mg. adrenalin-chloride, 1:1000 (Parke, Davis Company), per animal and injection.

* Aided by the Weaver Fellowship Fund and Julius Friedenwald Fund of the University of Maryland School of Medicine.

[1] Uhlenhuth, E., *Anat. Rec.*, 1926, **34**, 119.

[2] Uhlenhuth, E., and Schwartzbach, S., *Brit. J. Exp. Biol.*, 1927, **5**, 1.

[3] Uhlenhuth, E., and Schwartzbach, S., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 149.

[4] Uhlenhuth, E., and Schwartzbach, S., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 152.