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Experimental "Constant Oestrus" and the Notion of Anti-Gonadotropic Hormones.*

G. P. DU SHANE, W. T. LEVINE, C. A. PFEIFFER AND E. WITSCHI.

From the State University of Iowa.

In the recent literature frequent references[1, 2, 3] occur to refractoriness of the ovary toward prolonged or repeated stimulation with urinary and pituitary hormones. In many instances the unresponsiveness of an overstimulated ovary is due, partly, to extreme luteinization, follicular exhaustion or other degenerative changes. Moreover, the hypophysis of the injected or implanted animal undergoes transformations, mostly of a depressive nature. These 2 factors account as a rule for non-return to the normal condition immediately after experimental procedures, Hertz and Hisaw.[3]

Collip and his coworkers[4, 5] have shown that the blood of rats which have become unresponsive to urinary hebin is able to inactivate this hormone. Such a serological reaction may be interpreted either as due to the appearance of antibodies to the foreign (human) proteins, or to the increase in "antihormones" normally controlling hormonic levels in the blood. Collip[4] prefers the second alternative and Bachman[6] has attempted to rule out the first one, though obviously without success.

In view of the fact that the refractory condition has been obtained by the introduction of foreign proteins (mouse, rat, rabbit as recipients; man, horse, cattle, sheep as donors) immunity reactions should be expected *a priori*. That animals became "insensitive" even by injection of subthreshold doses of hormones, and remained so for months afterwards (Hertz and Hisaw[3]) further suggests an immunological interpretation. Therefore it becomes necessary to test whether the refractory state may be established also by gonadotropic

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1 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487.

2 Hill, R. T., Parkes, A. S., and White, W. E., *J. Physiol.*, 1934, **81**, 335.

3 Hertz, R., and Hisaw, F. L., *Am. J. Physiol.*, 1934, **108**, 1.

4 Collip, J. B., *Mount Sinai Hosp. Reports*, 1934, 1.

5 Selye, H., Bachman, C., Thomson, D. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1113. Bachman, C., Collip, J. B., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 544.

6 Bachman, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 851.

substances derived from identical species. Experiments of long duration with parabiotic rats, offer probably the best attainable conditions to solve this question. From colorimetric data published by Hill[7] we calculated that almost 0.1 cc. of blood per minute or 150 cc. per day are exchanged between the members of a pair. Since unoperated females thus connected run oestrus cycles independently and may become pregnant even after long duration of parabiosis we must conclude that the hormone levels in normal animals are at all times relatively low and also that hormones disappear quite rapidly from the circulation. These two facts are drastically brought out by pairs in which one member is hypophysectomized. If the hypophysis is removed at an early date (50 to 60 days) the deprived member soon lags in body size. After puberty the normal member starts regular oestrus cycles, has normal ovaries, thyroids and adrenals, while the hypophysectomized twin has ovaries less than 20 mg. in weight, undeveloped accessory genital organs, and the adrenals

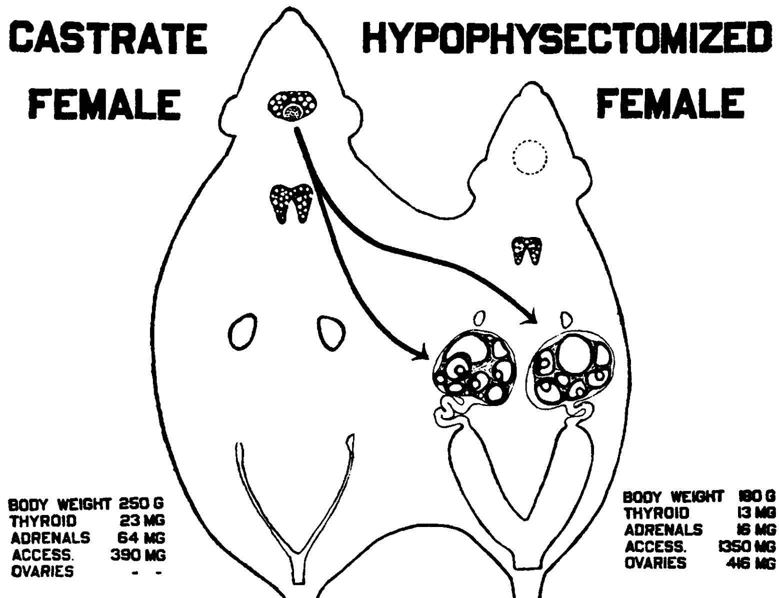

FIG. 1.

Diagrammatic representation of hormonal reactions in a pair of parabiotic female rats. *1st operation at 30 days:* litter mate females united in parabiosis. *2nd operation at 50 days:* hypophysectomy in right member (retardation of growth, reduction of thyroids, adrenals and genital system). *3rd operation after puberty:* castration of left member (reduction of genital ducts and excessive production of follicle-stimulating hormone in the castrate; exaggerated constant oestrus in the hypophysectomized twin.)

[7] Hill, R. T., *J. Exp. Zool.*, 1932, **63**, 203.

as well as the thyroids are of the reduced type characteristic for single hypophysectomized animals. In short, 150 cc. daily of normal rat blood do not carry enough gonadotropic, thyreotropic, adrenotropic, growth or oestral hormones to relieve any of the deficiencies caused by hypophysectomy. This picture changes at once if the normal twin is castrated (Fig. 1). The ovaries of the hypophysectomized animal begin to develop, follicles with antra become rapidly prominent and within 4 to 6 days the animal establishes a condition of constant oestrus. Of the many cases of this type among our parabiosis series we mention pair 192 (male castrate + female hypophysectomized), constant oestrus observed 3½ months; pair 199 (female castrate + female hypophysectomized), constant oestrus observed 5 months; pair 221 (female castrate + female hypophysectomized), constant oestrus observed 4 months. Particularly interesting is pair 208. Originally a combination of a male castrate with a hypophysectomized female the latter had been in constant oestrus for 3 months when she was castrated. The ovaries removed were of the extraordinary size characteristic for these combinations (416 mg., without capsule or oviduct). Uteri, vagina and vestibular glands were correspondingly enlarged. Following castration the female, naturally, fell in anoestrus. After 2½ months the ovaries of a newborn rat were implanted into her kidneys with the result that 20 days later oestrus reappeared, soon became constant and thus persisted for 5 months, until the animal was killed. Two of the present authors have shown[8] that the constant oestrus condition is due entirely to the influx of follicle stimulating hormone from the hypophysis of the castrate twin. This hormone obviously is the only one transmitted, for corpora lutea are never formed, thyroid and adrenals do not recover nor is body growth resumed.

It has been shown that constant oestrus is not only established by hypophysectomized females but also by normal ones if they are parabiotically united with castrated or with otherwise sterilized mates. However, the female in possession of its hypophysis goes into constant oestrus only after a certain period of irregularity, during which the capacity of the normal female hypophysis to release luteinizing hormones gradually becomes suppressed. Table I contains some cases in which constant oestrus has been observed for periods of several months. In these and in over a hundred more pairs of similar constitution, studied during the past years, constant oestrus never came to an end spontaneously. Sooner or later the

[8] Witschi, E., and Levine, W. T., PROC. SOC. EXP. BIOL. AND MED., 1934, 32, 101.

mucosa of the uteri and even oviducts and ovaries may become infiltrated with leucocytes. Therefore, leucocytes appear frequently besides cornified cells in the smears in later months of constant oestrus. While the smear resembles then stage IV, sections through the vaginal epithelium still give the picture characteristic of stage III. Shrinkage of the uteri and disappearance of cornified cells from smears follow presently after hypophysectomy in the co-twin, separation of the pair or castration of the female exhibiting the constant oestrus condition. Females that have been in constant oestrus for several months resume cyclical oestrus after separation from their co-twin. At the beginning these cycles are extremely prolonged and irregular. Fertility tests have not been carried out in a systematic way, though our records show that the females of the pairs 145 and 153 (Table I) became pregnant 4 and 3 months after separation from their sterile co-twins.

TABLE I.
Oestral Reactions in Unoperated Females Which Are United in Parabiosis with Castrated or Sterilized Cotwins.

No.	1st twin	2nd twin	irregular period* months	constant oestrus months
P111	♂ castrate	♀ unoperated	3	7½
P115	♂ ”	♀ ”	1½	14½
P205	♀ ”	♀ ”	3	10½
P145	♀ X-rayed	♀ ”	1	5
P151	♀ ”	♀ ”	1½	4½
P153	♀ X-rayed (later castrated)	♀ ”	1	4½
P175	♀ X-rayed	♀ ”	1	4
P149	♂ ”	♀ ”	½	6½†
P157	♂ ”	♀ ”	1	10‡
P161	♂ cryptorchid	♀ ”	2	8
P178	♂ ”	♀ ”	1	4

*Concerning the factors responsible for this period see Witschi and Levine.
†With an interruption in the third month, probably due to partial regeneration in the testes of the cotwin.
‡With an interruption in the third month; after a renewed X-ray treatment of the testes constant oestrus was resumed.

The amount of follicle-stimulating hormone received by these parabiotic "constant oestrus females" is not exactly known. Injection experiments indicate that it is far below 150 daily rat units. On the other hand, it is above the single unit. In consideration of the effect on the ovaries of hypophysectomized females one may venture an estimate of about 5 to 20 daily units. At any rate it is clear that the ovaries are constantly being overstimulated. The high level in follicle-stimulating hormone is maintained because the oestrin is not transmitted in physiological quantities to the sterile member. This is evident from the castrate condition of vagina and uterus in the sterile co-twin (Fig. 1). Even daily injections of as many as 100 rat units of oestrin in one member of a pair do not bring about oestrus in the ovariectomized co-twin. Obviously oestrin disappears so rapidly from circulation that it can not reach a sufficient concentration, in the sterile member, to check the increased hypophyseal activity.

Recently one of us (Pfeiffer[9]) has developed another method of constant follicular stimulation in female rats. Newborn rats are implanted with testes of male litter mates. In cases of successful takes their hypophyses assume the male character of secreting constantly follicle-stimulating hormone and not releasing any luteinizing hormone even in the presence of considerable amounts of oestrin in the circulating blood. Constant oestrus, therefore, is established in these rats as soon as they reach the age of puberty. In contrast to the described situation in the parabiosis experiment, the ovaries in this case are not overstimulated and oestrus is of a low grade, producing cornification in the vagina but only a very moderate enlargement of the uteri. Apparently the activity of the hypophysis is kept on a moderate level by the oestrin from the ovaries. A state of equilibrium between hypophysis and ovaries is thus established, which resembles very much the normal oestrus condition in the rabbit. This constant oestrus condition has been observed for periods of 4 or 5 months in many of these implanted females. Neither luteinization nor ovulation ever occur spontaneously and all indications of cyclical processes are wanting. After a 4 to 5 month period of continuous oestrus 10 of these females were injected, each with 4 mg. of a purified extract of the luteinizing hormone from powdered sheep pituitary (equivalent of 120 mg. dry powder). All of them ovulated within 10 hours, each ovary releasing 4-9 perfectly normal eggs (Witschi and Pfeiffer[10]). One must conclude therefore that the ovaries had constantly maintained about 5 eggs at the proper stage to be released and to proceed presently through the final maturation stages, upon the stimulus provided by a given amount of luteinizing hormone.

In a further series of experiments with these rats, it was found, however, that the ovulation reaction can not be repeated with the sheep luteinizing hormone. Even more than one month after a single injection or after repeated treatments, the rats are unrespon-

[9] Pfeiffer, C. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 603.

[10] Witschi, E., and Pfeiffer, C. A., 1935, *Anat. Rec.*, in press.

sive, *i. e.,* remain in constant oestrus. On the contrary, if the treatment is repeated with extracts from human pregnancy urine (4-10 cc.) ovulation follows the injection within 12 hours (3 cases).

These experiments prove irrefutably that continuous stimulation of the rat ovary with moderate or with large amounts of follicle-stimulating hormone results in a state of constant oestrus which may persist for more than a year, provided that this hormone has been derived from the same species. It is concluded that the loss of sensitivity of ovaries of rats and rabbits after injection of gonadotropic hormones of heterogeneous origin is of the nature of an immunity reaction. The fact that the gonadotropic substances so far isolated are proteins will of necessity set certain limits to the possibilities of therapeutic application of extracts from animal tissues. No evidence of the presence of specific anti-gonadotropic substances in the sense of Collip was found. The fact that the ovaries of hypophysectomized females in parabiosis with castrates enlarge to about 4 times the weight of those of females in possession of the hypophysis indicates that the hypophysis normally not only stimulates but also limits the follicular growth in the ovary. Preliminary experiments suggest that the thyroids and possibly also the adrenals enter as factors in this complex mechanism of control.

Of great interest in this connection is also the recent report by Wade and Doisy[11] on the prolonged administration of oestrogenic hormones (theelin and theelol) to male and female rats. These investigators find that the injury to the ovary produced by short period treatments becomes largely repaired after prolonged treatment. Female rats injected daily with varied (partly with excessive) amounts of female sex hormone resume nearly normal cyclical oestrus and become pregnant like normal controls. Even spayed females do not stay in constant oestrus but run "irregular cycles of 5 to 10 days' duration interspaced with long periods of oestrus and dioestrus up to over 30 days." From their observations the 2 authors draw the following conclusion: "Since the quantity of hormone administered was kept constant it would appear that there is a rhythmic response in the cells sensitive to the hormone." This inference seems unwarranted in view of the fact that not only the vaginal, but obviously also the ovarial cycles are maintained in spite of the injections. On the other hand, our own experiments show that vaginal oestrus becomes continuous if the ovaries are in the constant oestrus condition—no matter whether moderately or

[11] Wade, N. J., and Doisy, E. A., *Endocrinol.,* 1935, **19**, 77.

strongly stimulated. On the basis of the available data it would seem not unlikely that the oestrin-injected animals develop some means to dispose more rapidly of the injected dosages of hormone, probably by excretion, so that they have little chance to evoke physiological reactions in the genital organs.

Summary. Parabiotic twins of rats exchange daily about 150 cc. of blood. If one member of a female pair is hypophysectomized it exhibits deficiencies which prove that blood received from the normal mate carries not enough hormones to maintain body weight, thyroids, adrenals and ovaries at normal levels. Castration or sterilization of males and females increases the output of follicle-stimulating hormones to the extent that normal or hypophysectomized twin females go into a condition of constant oestrus. The ovaries are visibly overstimulated and contain large follicles but no corpora lutea. Uterus and vagina exhibit also the characteristics of a maximal or even an exaggerated oestrus condition, indicating the release of large amounts of oestrin by the ovaries. Not enough oestrin accumulates in the castrate member to prevent complete atrophy of the secondary sex characters. This indicates rapid disappearance even of high amounts of oestrin from the circulation. Constant oestrus with a moderate degree of ovarian and vaginal stimulation and almost dioestral size of the uterus is observed in females with experimentally masculinized hypophyses. Both types of constant oestrus can persist indefinitely, at any rate over a full year, without loss of sensitivity by either ovaries or genital accessory organs. It is concluded that no specific anti-hormones are formed, though the hypophysis, probably through the intermediary of the thyroid (and adrenals?) checks the excessive growth of overstimulated ovaries. The loss of reactivity in ovaries after prolonged stimulation with gonadotropic hormones of heterogeneous origin is considered as an immunity reaction to foreign proteins.