

Hormonology. — *Testosterone and luteinization.* By F. J. A. PAESI. (From the Pharmacological Laboratory, University of Leiden, Holland.) (Communicated by Prof. J. VAN DER HOEVE.)

(Communicated at the meeting of April 26, 1947.)

The relations between male hormone and the ripening of the ovary.

In our laboratory (PAESI e.a. 1943, GAARENSTROOM e.a. 1944) and elsewhere too, it was found that testosterone may exercise a favourable influence on the development of the follicle cavity in rats. In immature mice it even led to ovulation (DE JONGH e.a., 1944); in the rat so-called "blood points" have repeatedly been observed by ourselves as well as by others (BAUER 1943, SELYE 1939). We could show, moreover, that treatment with chorionic gonadotrophin leads in the ovary of hypophysectomized rats to the production of an androgenic substance, and that its interstitium, but no other part, becomes hypertrophic (PAESI e.a. 1943). Such a substance was supposed to be present in normal animals too, and to influence the ripening of the follicle by a direct action on the ovary and not by acting through the intermediary of the hypophysis (GAARENSTROOM and DE JONGH 1946).

Somewhat older experiences with regard to a favourable effect of male hormone on the *luteinization* process (HOHLWEG 1937, rat; SALMON 1938a and b, rat; NATHANSON e.a. 1938, rat; SELYE 1939, mouse) appear now in a new light: GAARENSTROOM and DE JONGH are of opinion that the favourable influence exercised on the luteinization depends on the more advanced degree of follicle ripening, and that this influence too is therefore referable to a direct influence of the androgenic substance on the ovary, and not to one exercised by way of the hypophysis.

Are the corpora lutea influenced by the androgenic substance?

In connection with the considerations set forth in the preceding paragraph, it interested us to know whether already existing corpora lutea are influenced by male hormone. That oestrogens act in this way, has repeatedly been described.

LAQUEUR and KOETS obtained in 1945 by means of testosterone in *normal* adult rats corpora lutea that showed great similarity to corpora lutea graviditatis. It would be interesting to know whether this effect was obtained with the co-operation of the hypophysis, as in the case of the analogous activity of the oestrogens is highly probable, or without its intermediary, i.e. by a direct influence exercised on the corpus luteum.

In this respect experiments carried out at an earlier date by GAARENSTROOM (1941) are of importance. He injected a number of young rats

with a full gonadotrophic mixture¹⁾, and found that the ovaries became almost entirely filled with corpora lutea. After the hypophysis and the right ovary had been removed, the animals were during a period of ten days subjected to a treatment with ambinon in combination with various other substances. At the end of the experiment the weight and histological structure of the remaining ovary were compared with the weight and structure of the right one. The decrease in weight of the ovary, that at the end of the experiment still consisted almost entirely of corpora lutea, appeared to be far more effectively retarded by the combination of ambinon and testosterone than by ambinon alone. This result was ascribed to the formation of a larger number of new corpora lutea, for a measurement of the three *largest* corpora lutea showed that the latter had been no better protected against atrophy by ambinon plus testosterone than by ambinon alone. The possibility that testosterone might exercise a preservative effect on the corpora lutea, or even cause an improvement of their condition, was on this account, for the moment, set aside.

Our own experiments.

It seemed desirable to us to place GAARENSTROOM's conclusion on a more solid base. Although we too are of opinion that the largest corpora lutea should be the first to show a measurable difference in the degree of atrophy when the animals have been treated in different ways, we think that the possibility of a selective influence on the *smaller* ones should also be taken into account. Moreover, GAARENSTROOM confined himself to an examination of only *one section out of each group of ten*, which particularly with regard to the smaller corpora lutea may lead to erroneous conclusions. Finally, it seemed advisable to us to *count* the corpora lutea. Our experimental method resembled in the main that of GAARENSTROOM:

A number of adult female rats, weighing from 137 g. to 179 g., were treated during a week with ambinon (twice daily 2.5 R.U.) plus pregnyl (twice daily 2.5 I.U.). In this way the ovaries were intensively luteinized in all animals. On the eighth day of the experiment the hypophysis and the right ovary were removed, and preparations of them were made in the usual way. The animals were then divided in three groups: *a*, *b* and *c*, that were subjected from the 9th to the 18th day to the following treatments:

- a) ambinon (twice daily 2.5 R.U.) plus testosterone propionate (twice daily 0.5 mg.),
- b) ambinon (twice daily 2.5 R.U.) plus oil,
- c) 0,9 per cent NaCl plus oil.

On the 19th day the animals were killed. Preparations were made of the left ovary and the sella turcica. In the latter no rests of the hypophysis were found. The corpora lutea were counted, and in each ovary the four largest and the four smallest ones were selected for measurement: of each of them the two largest, vertically intersecting diameters were measured by the aid of an eyepiece-micrometer. By taking the third power of their mean a measure for the *content* of the corpora lutea was obtained. The four figures obtained for the smaller ones and those obtained for the larger ones were averaged.

¹⁾ Ambinon (hypophysis extract, Organon) plus pregnyl (chorionic gonadotrophin, Organon).

The experiment was repeated with a second group of rats, weighing from 137 to 166 g.

In a third series of experiments the first treatment was ended one day before the hypophysectomy, and the second started two days after the latter. The removal of the right ovary too took place two days after the hypophysectomy. We deviated here from our former course because we wished to find out whether certain phenomena that had been observed in the preceding series should be ascribed to the influence of some gonadotrophic hormone that remained in the circulation. These rests might belong to the amount produced by the animal's own hypophysis or to injected pregnyl and ambinon.

Results.

The results are given in the table. Data pertaining to nine rats that died during the course of the experiments are omitted.

The table confirms, to begin with, two observations made by GAAREN-STROOM:

1. In all three series the ovary weight (column C) showed the smallest decrease under the combined influence of ambinon and testosterone, and the largest decrease in the controls.

2. Neither of the treatments influenced in any series the mean size of the largest corpora lutea (column G) in a favourable way.

A number of new facts are also revealed. These will now be discussed.

TABLE.

In the columns C—G the values obtained for the left ovaries are expressed as a percentage of the corresponding values obtained for the right ones. The values themselves are averages; the number of animals from which the latter were taken, are given in column B.

	A	B	C	D	E	F	G
Series	Treatment after hypophys-ectomy	Number of animals	Ovary weight	Number of corp. lutea	Mean weight of corp. lut. calcul. from. C : D	Mean volume of the 4 smallest corp. lutea	Mean volume of the 4 largest corpora lutea
I	ambinon + testosterone	3	84	122	71	85	62
	ambinon + oil	2	67	115	62	55	67
	0.9 per cent NaCl + oil	3	50	113	52	27	64
II	ambinon + testosterone	2	85	127	67	20	68
	ambinon + oil	2	68	98	68	37	73
	0.9 per cent NaCl + oil	3	62	105	59	21	97
III *	ambinon + testosterone	5	119	137	81	124	83
	ambinon + oil	5	90	106	82	103	70
	0.9 per cent NaCl + oil	5	76	91	83	133	86

* The first and the second treatment separated from each other by an interval of a few days.

A. The counts of the corpora lutea (column *D*) have proved without any doubt that the increase under the combined influence of ambion and testosterone is larger than under the influence of ambion alone. Testosterone therefore must have exercised a favourable effect on the increase. Whether ambion alone gives an increase, remains uncertain.

In the third series, in which the right ovary was removed three days after the end of the first treatment, no new corpora lutea were found in the controls. In the second series their presence was dubious, in the first one conspicuous. In the latter the appearance of new corpora lutea will have to be ascribed to the presence of gonadotrophic substances produced in the previous period by the animal's own hypophysis or else introduced in its circulation during that period by means of the injections.

B. By dividing the relative ovary weights (column *C*) by the number of corpora lutea (column *D*) values are obtained that may serve as a measure for the average weight of the corpora lutea (column *E*). When these values are found to be equal in groups that have been treated differently, the difference in the ovary weight must be due to an increase in the number of corpora lutea, and then any influence that might be interpreted as a retardation of the atrophy ("preservation") of the existing corpora lutea, seems excluded. This doubtless applies to the *third series*. In view of the fact that the ovaries, before as well as after the second treatment, were almost entirely luteinized, we are of opinion that neither ambion alone nor ambion plus testosterone are able to preserve the existing corpora lutea when, as in the third series the hormonal influences from the preceding period must have completely disappeared. It is for the rest hardly conceivable that they would be able to achieve this when a small quantity of hypophysis hormone or some pregnyl (first and second series of experiments) would have remained in the circulation; signs that seem to point in this direction, should be regarded with a good deal of distrust. However, before considering them more closely, we want to say a few words with regard to the corpora lutea belonging to the smallest size class:

C. In the third series of experiments the smallest corpora lutea did not atrophy; it looks on the contrary more as if they increased in size. This is in contradiction to the behaviour of the largest ones, and also in sharp contrast to that of the smallest corpora lutea in the two other series. This cannot be accidental, and must have its cause in the three days that elapsed between the last injection with the gonadotrophic mixture and the removal of the right ovary; it is to be remembered that in two of these three days the hypophysis had also been absent. In these few days the atrophy must have taken place, for in the period of the second treatment there was no further sign of it. The exact nature of the second treatment is here of no importance. The differences in the degree of atrophy of the smallest corpora lutea observed in the first two series, in connection with the special nature of the second treatment, which will presently be discussed, must therefore

already have been established in the very first stage of the latter. This points to a particularly high vulnerability of the very small corpora lutea.

This is to us a motive for a partial revision of the views that we have elsewhere (PAESI, 1946) brought forward. At that time we had observed that the corpora lutea obtained in young mice by a treatment with aminon plus pregnyl were *larger and showed less tendency to degenerate* than those obtained by a treatment with aminon alone. As under the influence of aminon plus pregnyl more oestrogen is formed than with aminon alone, we supposed at that time that this oestrogen would have exercised, in the generally accepted way, a positively trophic influence on the corpora lutea. This may be right, but the results of the experiments described above show that another possibility should also be considered: if the *smallest* corpora lutea atrophy more rapidly than the larger ones, they will probably degenerate also *in a larger number*.

The particularly high vulnerability of the smaller corpora lutea points to a flaw in the luteinisation process of the latter. As the histological examination reveals that the theca of the smallest corpora lutea does not take part in the luteinization or is at least not over its whole extent concerned in this process, the vascularisation of these corpora lutea falls behind that of the larger ones, and in this difference, we believe, the vulnerability of the smaller ones may have its ground. The inadequacy of the blood supply may be responsible for the tendency to atrophy. It can, of course, not be regarded as accidental that corpora lutea in which these anomalies occur, remain small.

In the experiment with young mice to which reference was made above, the increase in the power of resistance of the corpora lutea under the influence of pregnyl would not have been due entirely to oestrogen production, but also to a more complete participation of the theca in the luteinization.

D. After this consideration of the behaviour of the smallest corpora lutea, we will now return to our main object, and try to find out what the *first two* series of experiments may teach with regard to an influence that might have been exercised on the corpora lutea themselves. We will begin with the influence exercised by aminon alone.

The table shows that the differences in the ovary weight cannot be entirely due to differences in the number of corpora lutea. The possibility that the corpora lutea are better preserved, should therefore be taken into consideration. On account of the data derived from the behaviour of the smallest corpora lutea, this possibility cannot be excluded, for under the influence of aminon the degree of atrophy was in both series in the smallest corpora lutea smaller than in the corresponding controls. However, with regard to the considerable differences between the values in column *F* that refer to groups of animals treated in the same way, the interpretation must be careful.

A second possibility is that the *new* corpora lutea in the aminon group and in the controls are of different size, for such a difference would also cause a difference in the average weight of the corpora lutea. However, to exercise such an influence, the *number* of new corpora lutea should not

be too *small*. As a complete disappearance of corpora lutea in the course of the second treatment need not be taken into account, we may estimate the production of new ones by diminishing the figures given in column *D* by 100. The number of new corpora lutea now appears to be but *small*, and it is therefore improbable that differences in size between these and the already existing corpora lutea might be responsible for the large difference in the average weight of *all* corpora lutea given in column *E*.

As a third possibility the follicle ripening should be considered. It is true that the ovaries at the beginning as well as at the end of the second treatment give the impression of being entirely luteinized, but a certain degree of follicle development not ending in luteinization may nevertheless have occurred, and although this may have been insignificant with regard to the total size of the ovary, it may have been of some importance in comparison with the new corpora lutea. When the average weight of the corpora lutea is calculated in the way described above, it will undergo a fictitious increase when follicles, perhaps large in number, although small in size, begin to ripen. This may have happened in our experiments, for that ambinon may be able to bring about some ripening of new follicles, cannot be denied, *and the number of corpora lutea did not increase under the influence of ambinon.*

E. We will now examine the possibility of an influence exercised on the corpora lutea themselves by comparing the influence of the combination ambinon-testosterone with that of ambinon alone. The answer is in this case less dubious than in the previous one.

The variability in the behaviour of the smallest corpora lutea is so large, that the atrophy of the smallest corpora lutea cannot be used as a base for discussion. We will have to build our conclusions therefore on the figures given in the columns *C*, *D* and *E*.

A comparison of the figures of column *E* (the average weight of the corpora lutea obtained by dividing the figures of column *C* by those of column *D*) suggests that the corpora lutea of the animals that had received both ambinon and testosterone are in the first series larger than those of the animals treated with ambinon alone, but that such a difference is not found in the second series. In view of the entirely identical treatment that the animals in these two series had received, this discrepancy is difficult to explain. It remains entirely incomprehensible when we assume that the corpora lutea are in the first series better preserved by the addition of testosterone, and it is not clear either when we ascribe it to a selective formation of large corpora lutea, against which moreover the same objection may be raised as has been done under *D*.

It is, however, not improbable that on account of minor differences in the treatment that escaped control, a number of newly ripened follicles may in the second series have succeeded in becoming luteinized, whereas a similar number in the first series just failed to reach this stage. Support for this supposition is found in column *D*: the number of new corpora lutea

formed under the influence of testosterone is in the first series small and in the second comparatively large. When it is right, the differences between the values of the first series in column *E* must rest on differences in follicle ripening, and then the real average weight of the corpora lutea may, as the values of the second series suggest, have been identical.

Testosterone therefore would not have helped to preserve the corpora lutea, but its action would have been confined to a stimulation of the production of new ones. An improvement in the condition of the corpora lutea by means of testosterone (LAQUEUR and KOETS) may be obtained in animals whose hypophysis has not been removed, but in this case it will have to be effected through the intermediary of the hypophysis.

Summary.

1. The weight of previously luteinized ovaries of hypophysectomized rats is better preserved by a treatment with pituitary gonadotrophin than without the latter, and still better when in addition testosterone is administered.

2. Pituitary gonadotrophin alone does not give a well-marked increase in the number of corpora lutea, but in co-operation with testosterone the increase is undeniable.

3. The corpora lutea may be better preserved when pituitary gonadotrophin is given; moreover the latter probably causes a modest follicle development.

4. Testosterone apparently does not preserve the corpora lutea. The retardation of the atrophy of the ovary may be due entirely to the formation either of new follicles or of new corpora lutea.

5. Arguments were brought forward, pointing to an especially short duration of life of the smallest corpora lutea.

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