10347

Gonadotropic Action of Anterior Pituitary Extract after Tryptic Digestion.

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Work which we have had under way during the past 2 years is of interest in connection with the recently published observations of McShan and Meyer.[1] These authors concluded that trypsin largely destroys the luteinizing action of anterior pituitary gonadotropic extract. All our results are in accord with this statement and are based upon experiments requiring 492 normal immature rats and 246 hypophysectomized immature rats.

Three different commercial preparations of trypsin were used. Most of the experiments were performed with a preparation made by Merck (Darmstadt) which has since been found to hydrolyze casein at approximately the same rate as crystalline chymotrypsin made by the method of Kunitz and Northrop. Two lots of trypsin prepared by Grübler also were used; the manufacturer stated that one lot contained 50 Fuld-Gross units per gram, the other 1200 Fuld-Gross units per gram. Digestion usually was carried out in a 0.2% solution of Na_2CO_3 over a period of 15 hours at 38°C. At the end of digestion the pH of the solution was about 9.8. All control extracts were treated exactly the same way except that trypsin was not added. Extracts of acetone-desiccated sheep or horse pituitary,* which had been powdered, were ordinarily made by the method of van Dyke and Wallen-Lawrence[2] and represented, roughly, 3% of the weight of crude pituitary powder. Removal of nearly all the luteinizing action of such extracts could be accomplished by digestion of a 0.2-0.3% solution of the extract in 0.2% Na_2CO_3 containing 0.1% of Merck's trypsin. Similar results were obtained by employing the weak and strong trypsins of Grübler in concentrations of 0.4 and 0.03% respectively.

Figure 1 illustrates how the degree of ovarian hypertrophy induced by the injection of digested extract may vary according to

[1] McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1938, **126**, 361.

* We are indebted to Dr. A. S. Parkes whose cooperation made possible the purchase of the powdered horse pituitary.

[2] van Dyke, H. B., and Wallen-Lawrence, Z., *J. Pharm. Exp. Therap.*, 1933, **47**, 163.

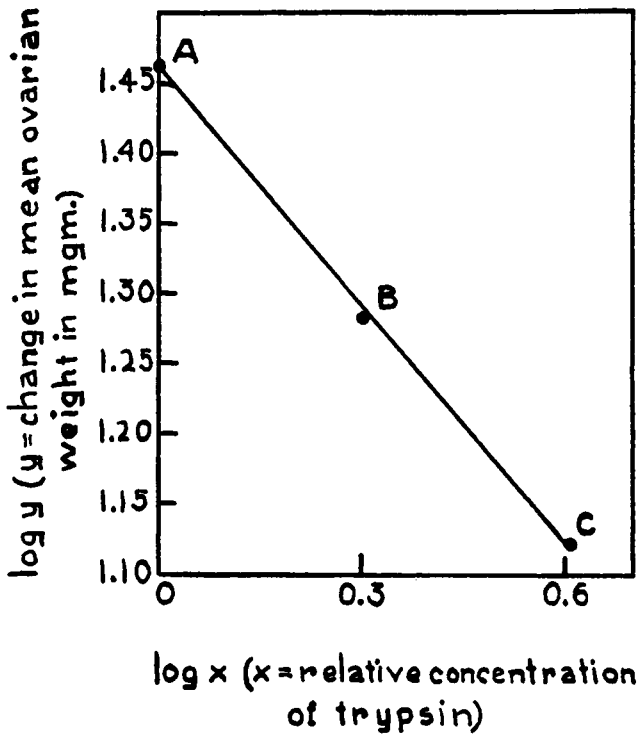

FIG. 1.

The effect of various concentrations of commercial trypsins on the potency of anterior pituitary extract as measured by the change in ovarian weight compared with non-injected controls. Each of the 3 dots represents 17 normal immature rats. All the animals compared were littermates. The same sheep anterior pituitary extract was always used. However, 3 samples of trypsin were employed.

The significance of the differences between the effects of the 3 concentrations of trypsins was calculated by the well known method of Fisher with the following results:

Groups compared	P
A and B	<0.02
A and C	<0.001
B and C	0.05

the concentration of trypsin used: the change in ovarian weight (hypertrophy) is greater if low concentrations of trypsin are used.† In this group of experiments a 0.2% solution of extract of sheep pituitary powder digested by trypsin in different concentrations was injected into normal immature rats. Although an aliquot of the alkaline solution of the extract incubated under the same conditions caused typical extensive luteinization without apparent loss of po-

† If a crystalline proteolytic pancreatic enzyme had been employed, presumably only the rate of hydrolysis would have been affected by varying the concentration of enzyme. The interpretation of the effect of varying the concentration of the crude preparations of trypsin used may be more complex.

tency, the digested extracts, which alone are indicated in the chart, brought about marked follicular growth associated with some luteinization, especially when low concentrations of trypsin were used. Experiments were therefore undertaken in hypophysectomized rats to determine whether digested extracts are completely free from luteinizing properties.

Both digested and control non-digested but incubated extracts of horse and sheep pituitary extract were injected into hypophysectomized immature female rats. We have not been able to convince ourselves that extracts can be completely freed from "luteinizing hormone" by means of tryptic digestion. Although a starting material such as horse-pituitary extract may be highly potent and produce chiefly a follicular stimulation, yet a large enough dose of this extract after digestion undoubtedly produces the formation of lutein tissue (Fig. 2). Smaller doses of extract may cause definite or marked follicular stimulation without detectable luteinization. In hypophysectomized immature male rats receiving injections for 3-4 days, digested extracts which were highly effective in female rats frequently caused doubling or trebling of the testicular weight (compared with that of non-injected hypophysectomized controls). However, the microscopic changes often were slight. Some tubules of a testis might be well maintained whereas neighboring tubules might appear as atrophic as if no treatment had been given. The number of mitoses in the germinal epithelium sometimes appeared to be increased. Usually tubular diameter was increased; the tubules themselves seemed to be separated by an unusual accumulation of non-cellular fluid. The interstitial cells were completely atrophic; the seminal vesicles were not enlarged.

Neither the crude nor the digested extracts of horse pituitary had any effect on the adrenals or thyroid of hypophysectomized rats receiving doses 20 times that capable of definitely stimulating the ovary.

It was considered possible that "antihormone" might not be produced by digested gonadotropic extract. Accordingly, littermate male and female normal rats, 21-24 days old and weighing about 50-60 g initially, were divided into 3 groups consisting of a non-injected control group, a group receiving non-digested horse pituitary extract, and a group receiving the same extract after digestion. There were 4-6 animals in each of the 6 groups. The injected rats received 0.05 mg of either extract daily at first; the dose was gradually increased until 0.4 mg was administered daily. The period of injection was 46 days. At the end of the experiment the mean weight of the ovaries of the injected females was less than that of

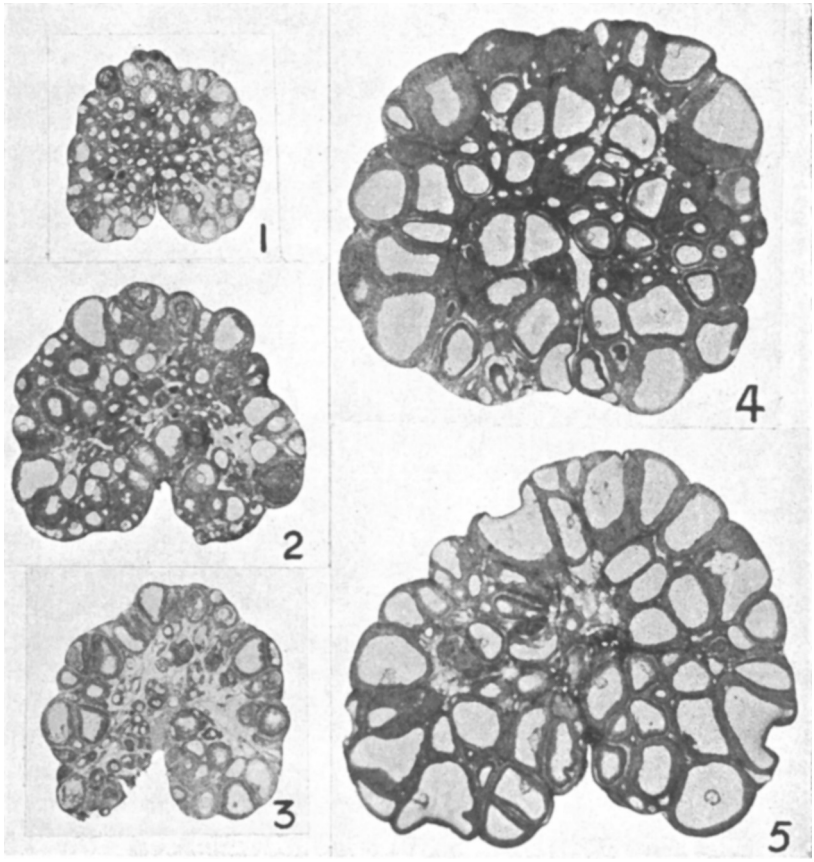

FIG. 2.

Photomicrographs of the ovaries of littermate rats (all × 17 before reduction). All except No. 2, which served as a normal control, were hypophysectomized at an age of 21 days. One-sixth of the dose of extract was given twice daily during the 24-26 days; the animals were killed on the 27th day. The dose of digested extract represented roughly 5 times the quantity of horse-pituitary extract without digestion. No lutein tissue can be found in 3. Lutein tissue is present in 5 but is much less extensive than in 4.

TABLE I.

No.	Treatment	Weight, in mg, of Ovaries	Empty uterus	Adrenals*
1	None	7.46	20.2	5.46
2	"	18.14	52.3	15.62
3	.05 mg digested H. P. extract	12.64	27.3	5.44
4	1.00 mg H. P. extract without digestion	94.2	101.7	7.62
5	1.00 mg digested H. P. extract	87.7	115.1	8.16

*Adrenal atrophy appears very rapidly after hypophysectomy. Therefore, in addition to examination of the sella, the adrenals were routinely weighed to make certain that hypophysectomy was complete.

the control group. However, it was significantly less only in the group receiving non-digested extract ($P=0$) and not in that receiving digested extract ($P=0.15$). On the other hand, the testes of both groups of injected males weighed significantly less than those of control animals (in both cases $P=<0.01$). Moreover, the testes of males receiving digested extract clearly weighed less than those of the group which was given non-digested extract ($P=0.02$). There is little reason to doubt that digested extract provokes the formation of "antihormone". Neither extract affected significantly the weight of the thyroid, adrenals, or seminal vesicles. Although serum was collected from these rats, circumstances beyond our control prevented tests for the presence of antigonadotropic substance.

A few remarks concerning the removal of gonadotropic extract from digested extract can be made. Alcohol (70 or 85% by volume) precipitates nearly all the hormone; the lower concentration appears as successful as the higher. (Two experiments indicate that substances insoluble in 35% alcohol contain little hormone.) In either case the pH of the digested solution of extract is adjusted to about 5.4 and the insoluble material removed before the addition of alcohol. This insoluble material was found to be inactive. The active preparations represent gravimetrically about 20% of the starting material and contain about 18% of inorganic material. Ammonium sulphate also can be used to precipitate the active principle. The latter does not dialyze through a cellophane membrane.

The procedure for the isolation of crystalline lactogenic hormone described by White, Catchpole, and Long[3] was repeated with a concentrated solution of digested horse-pituitary extract. The cautious addition of a 1% solution of NH_4OH was followed by the formation of an inactive, carbon-free crystalline precipitate identified as $MgNH_4PO_4 . 6H_2O$.

Tryptic digestion of a potent extract of the urine of a patient with chorionephithelioma, under the same conditions as those employed when pituitary extract was used, completely destroyed the activity of the urinary extract.

Summary. In agreement with McShan and Meyer it has been found that tryptic digestion abolishes most of the luteinizing action of extracts of sheep or horse pituitary. However, large doses of digested extract were followed by the formation of some lutein tissue in the ovaries of hypophysectomized immature rats.

The effects of digested extracts on the testes of hypophysectomized immature male rats are described.

[3] White, A., Catchpole, H. R., and Long, C. N. H., *Science*, 1937, **86**, 82.