LXXVI. THE PREPARATION OF GONADOTROPIC HORMONES FROM NORMAL URINE AND URINE OF PREGNANCY.

BY CASIMIR FUNK AND PETER ZEFIROW¹.

From the Casa Biochemica, Rueil-Malmaison, France.

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ZONDEK [1930] believes that apart from the growth hormone of Evans, three distinct anterior pituitary hormones are excreted in urine of pregnancy, a gonad-stimulating, a luteinising and a metabolism-stimulating principle. The last-named action may be partly due to the presence of the thyroid gland hormone. Recent work on the gland itself by Fevold *et al.* [1931] and others has corroborated Zondek's view on the separate existence of gonad-stimulating and luteinising principles. Urine, and particularly urine of pregnancy, shows the presence of many hormones, notably oestrin, placenta hormone, corpus luteum substances [Fevold *et al.*, 1932] *etc.*, often with similar chemical properties. This renders the chemical separation and purification particularly difficult, and great caution has to be exercised in attributing a certain activity in urine to the presence of anterior pituitary secretion. The gonad-stimulating substance behaves chemically like a polypeptide. The purity of such high molecular compounds is to be judged perhaps best by biological tests.

Testing the activity.

For the biological test we have used immature rats of both sexes just at the age when the sex can be clearly recognised. Animals which reach the weight of 25–35 g. after 5 daily injections are killed and the weight of seminal vesicles and uteri determined per g. of body weight. Controls of the same age and size gave values for the seminal vesicles of 0.15–0.2 mg. and for the uteri 1.5–2.0 mg. per g. of body weight. The opening of the vagina occurs on the sixth day of the experiment. For the actual assay we take only males, in which the weight of the organs can be determined more accurately, and we tentatively define the rat unit as the amount of hormone necessary to increase the weight of the seminal vesicles by 100 %, as compared with the controls. The dose is injected subcutaneously on 5 days. The female rats used in the same way give us indications regarding other hormonal admixtures. The accompanying Table shows a satisfactory response to increasing doses (the preparation used corresponded to 1–5 cc. of urine per day).

¹ With the technical assistance of Boris M. Viesnin.

Table I.

Dose cc.	Wt. of seminal vesicles in mg. per g. of body weight
0 (control)	0.21
1	0.25
2	0.60
3	1.23
4	1.64
5	2.00

Preparation of the active material and some of its properties.

The method of preparing the active material, used initially by Zondek, was the precipitation of the urine with alcohol and the extraction of the precipitate formed with water. Extracts prepared in this way are very crude and highly toxic to the test animals. Later Dickens [1930] described a method of salting out with ammonium sulphate. We prefer, however, the simple method to be described here, adopted after much experiment, which has rendered us good service for more than a year and a half. To the urine a solution of 30 g. per litre of quinine or benzoic acid, dissolved in the minimum volume of alcohol, is added in small portions with shaking. Both substances, and probably many others with similar properties, can be used successfully, the use of benzoic acid being now preferred. The next day the precipitate is separated and well washed with alcohol and ether. It is then extracted repeatedly with water and the resulting hormone solution purified by fractionation with alcohol of varying concentration, and finally by fractional evaporation and separation of impurities in vacuum at low temperature. The preparation of greatest purity was obtained by slow separation from 40 % alcohol, rubbing the vessel with a rod. The material under the microscope appears to be in a semi-crystalline state and showed the following activity. Five doses of 8 γ each injected on 5 days (a total of 40 γ) into rats caused a threefold increase in weight of seminal vesicles and twofold of the uteri, as compared with the controls. The yield was about 3 mg. per litre of urine of pregnancy. With purer preparations the opening of the vagina was delayed, compared with the effect of less purified material. The biological and chemical purity of the preparation is now under investigation, it retains most of its activity after preservation for a year in a dry form.

The gonad-stimulating principle is a colourless powder, partially soluble in 40 % alcohol and practically insoluble in 60 % alcohol. In contrast to insulin it is not precipitated by picric acid. The active material appears to be quite stable in urine; we have obtained an excellent preparation from pregnant urine kept for more than 2 years with traces of chloroform.

We have frequently used the benzoic acid method here described for the diagnosis of pregnancy with very good results, the main advantage being the lessened toxicity of the material to the test animals. By using this method we were able to demonstrate appreciable quantities of the gonad-stimulating principle in normal male and female urine. In these cases the material injected

corresponded to 150–200 cc. of urine. The results, however, were not constant and require reinvestigation.

The simultaneous administration of the gonad-stimulating substance and andronin (male sex hormone) to capons evoked less response than that of andronin alone, injections of the gonad-stimulating principle having no effect at all. Experiments with infantile rats were similar, combined administration showing less response than that of each substance separately.

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