

Demonstration of Urinary Gonadotropins in Normal Men, Using the Chick as a Test Animal.

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Extensive assays have been made of pregnancy urine and similar studies have been reported of normal or menopausal female urine, but comparable studies of male urine in normal or pathological conditions have been limited. It is the purpose of this paper to report some analyses of gonadotropic potency of normal male urine as measured by the response of the gonads in chicks.

Methods for quantitative concentration of urinary gonadotropic substances have attracted considerable attention in the past several years and particular interest has been attached to the application of these methods to the urine of normal individuals which, because it contains very small amounts of the active material, must be concentrated into small volumes of non-toxic extract in order to be suitable for assay. Although most assays have been made with pregnancy urine, some of these methods can be applied successfully in extraction of gonadotropins from normal male urine.

An alcohol precipitation method was introduced by Zondek (1928) which according to some investigators appeared to be the easiest and most nearly quantitative, but required additional purification procedure when applied to normal urine. Katzman and Doisy (1934) proposed a benzoic acid method of obtaining gonadotropic extracts from pregnancy urine. This was found to be too toxic for assay, when used in large amounts. Hellbaum, Fevold, and Hisaw (1935) introduced a tannic acid pyridine method, which is claimed to be quantitative since the purified extracts in comparable dose are as active as the original urine. Levin (1941) reported the first precipitation of gonadotropins from normal male urine by a new tannic acid method. The size of the yields, compared to those obtained by the other methods, suggested that tannic acid is a suitable and effective precipitant for the gonadotropins of normal urine. This method was claimed to yield the least toxic preparations. Evans and Gobrahan (1942) described a modified alcoholic precipitation method for preparation of gonadotropic concentrates from normal male urine which is claimed to be superior to all other methods as to its non-toxicity and its quantitative concentration.

In connection with our work it is of interest to note that very little work has been done on assaying of urine gonadotropic extracts in birds. Several investigators have performed such experiments by using pregnancy urine extracts. Riddle (1931) showed failure of pregnancy urine extracts, prepared by the Zondek method, on testes of immature pigeon or

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ring-dove as a test object for qualitative and quantitative measurement of urine gonadotropins, regardless of the dose level employed. Schockaert (1934) injected extracts from pregnancy urine, prepared by the Zondek method, to chicks, found that these extracts were inactive in these forms in regard to the endocrine and the gametogenic functions. No increase in growth of the testes or the comb was observed.

Materials and Methods

Pooled samples of urine of the male staff of the department and college men were precipitated in lots of 18-21 liters. For extraction of the gonadotropins from the urine, Fevold's method was used at first, but later it was replaced by Levin's method which was found to be easier and faster.

The gonadotropic potency of the normal male urine was determined by assaying the extract in chicks and observing its effect on chick testicular weight. The method employed was that described by Byerly and Burrows (1938) according to which the chicks were kept in shipping boxes without food or water during the 96-hour assay period. The chicks were divided into different series, each of which received different concentrations of the extract. The concentrations ran from 100-800 cc. equivalents of urine per chick. The final extract was dissolved in water of such volume that 1 cc. of the extract was equivalent to 1000 cc. of urine. All injections were made subcutaneously and each chick received six injections of .25 cc. each at intervals of 12 hours. The control chicks were injected with water. At the fifth day, the chicks were killed by breaking the neck, testes were removed, weighed, and placed immediately into Helly's modification of Zenker's fixative. The tissues were embedded, sectioned in the customary manner, and then stained with Harris hematoxylin and eosin.

The estimation of the gonadotropic potency was made in terms of "chick units" since rarely more than ten birds were used in each series, 25 per cent increase of gonad weight above the control average was considered to constitute a chick unit. In this paper only standard errors for percentage gonad weights are presented, because according to Breneman (1945), the standard errors for percent body weight do not differ significantly from those for the gonad weight. In all the experiments, only single comb White Leghorn cockerels received 12 hours after hatching were used.

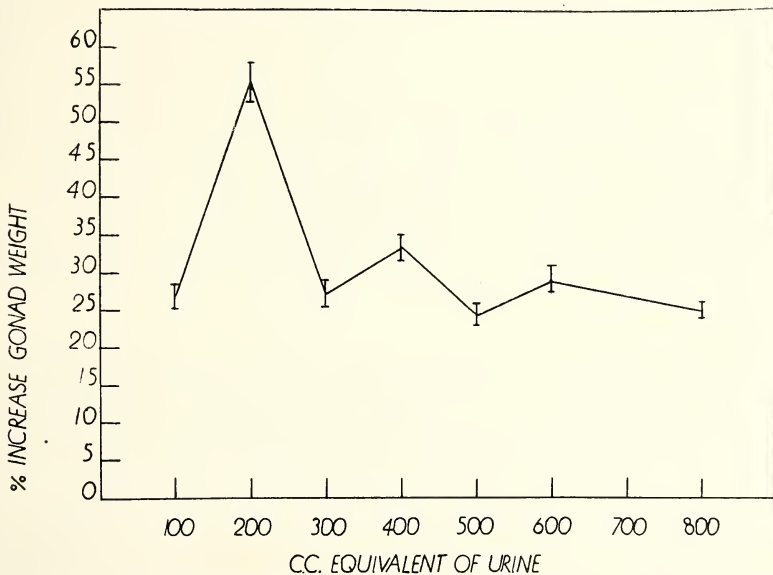
Results and Discussion

The consistent increase in gonad weights of the injected chicks over the controls, which was observed throughout the period of experimentation, indicates that there are some gonadotropic substances present in the normal male urine and that the chick gonads are very sensitive to such concentrations of gonadotropins.

Graphs I and II illustrate the results obtained with extracts prepared according to Fevold's and Levin's methods respectively for the

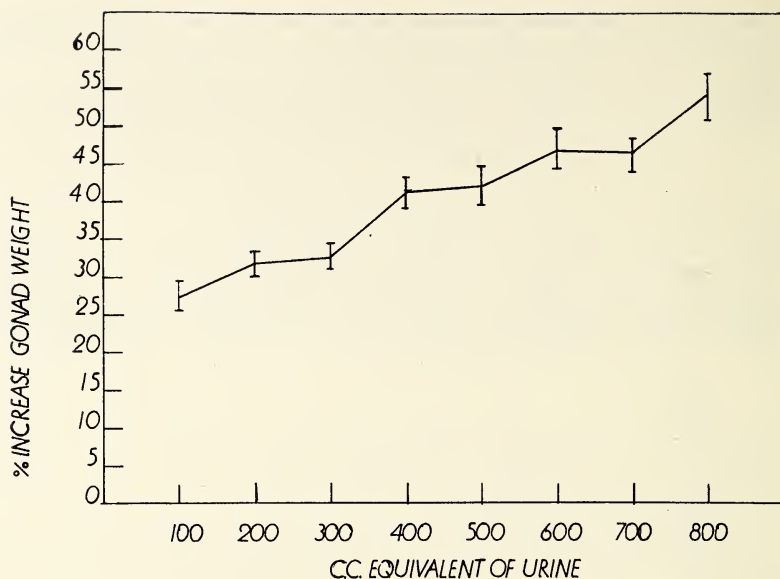
extraction of gonadotropins from pregnancy and normal male urines. An analysis of these graphs demonstrates a striking difference in the gonad weights, following injection of extracts prepared by the first method (Fevold's) when compared with those which resulted from the administration of extracts using the second method (Levin's).

The extracts prepared by the Fevold's method exceeded slightly the potency of those prepared by the Levin's method at low dosage level (note 200 cc. equivalents of urine per chick), but it was toxic when used at higher dosages than 600 cc., because most of the birds injected with such dosage began to die after the second injection. The curve representing the increase of the gonad weight of injected chicks over controls, after reaching a peak at 200 cc., abruptly fell to the level



Graph I. Gonadotropic potency of extracts prepared by the Fevold's method. Vertical lines represent standard errors. A total of 200 animals were used to determine the curve.

of the lowest dosage, and rose again in two peaks each smaller than the first one. The greatest increase of the gonad weights was obtained with extracts of 200 cc. (equal to 2.22 chick units) and the smallest with extracts of 500 cc. (equal to .93 chick units). The irregularity of this curve can probably be explained by the toxicity of the extracts which may be due to the incomplete purification of the final extract, or by the presence of some toxic or inert substances the toxicity of which could be noticed only at high dosage levels. The toxicity of the extract and the irregularity of the results obtained by this method necessitated the application of another method which could give better results.



Graph II. Gonadotropic potency of extracts prepared by the Levin's method. Vertical lines represent standard errors. A total of 500 animals were used to determine the curve.

Levin's method was found to be less toxic than Fevold's even when given in concentrations as high as 800 cc. It was also found to be simpler and required very little time for extraction. In the results obtained by extracts of this method, a correlation was shown between the increase in the gonad weight and the increase in concentration of the extract. There was a general tendency for progressive increase in a straight line with a little deviation due to the fact that results with concentrations of 300 and 700 cc. were a little below, and with concentrations of 400 cc. a little above this line. The increase in gonad weights with this extract ranged from 1.08 chick units for the lowest dosage level (100 cc.) to 2.17 chick units for the highest dosage levels (800 cc.).

Histological data failed to demonstrate any significant increase in the size of the tubules or relative increase of the interstitial tissue. There are some indications of formation of lumina and increase in the total diameter of the gonads of the injected chicks. In the control chicks, a general retrogradation of the gonads was observed which is probably due to the process of inanition used during the period of experimentation.

From the above data we may conclude that in the normal male urine the concentration of the gonadotropic substances is very low. Although it is sufficient to keep the gonads of the injected chicks from retrogradation, it is not sufficient to stimulate them.

Summary

Assays of gonadotropic substances of normal male urine were made using the 96-hour chick test. The gonadotropic potency was determined in terms of chick units, using 25 per cent increase of gonad weight over controls as a chick unit. Two different methods were used for extraction of the gonadotropins from the urine, Fevold's and Levin's, but the first extract was found to be toxic when given in high dosages.

The preparations obtained by the Levin method were less toxic than Fevold's and showed a correlation between the increase in the gonad weight and increase in the concentration of the extract. The potency of extracts prepared by the Fevold's method ranged from .93 chick units (for concentration of 500 cc.) to 2.22 chick units (for concentration of 200 cc.). For Levin's method extracts the potency ranged from 1.08 chick units (or concentrations of 100 cc.) to 2.17 chick units (for concentrations of 800 cc.).

Histological data failed to demonstrate any significant increase in size of tubules or relative increase of interstitial tissues. There was some indication of formation of lumina and increase in total diameter of the gonads of the injected chick.

These data indicate that there are some gonadotropic substances present in the normal male urine which will affect the avian testes.

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