8192 C

Relation of Potency of Anterior Pituitary-Like Hormone to Hydrogen ion Concentration.

C. A. ELDEN AND MARVEL-DARE FELLOWS. (Introduced by J. R. Murlin.)

From the Department of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry.

In a previous publication[1] concerning the separation of the anterior pituitary-like hormone from the urine of pregnant women by adsorption and elution, it was noted that the separation was dependent upon H-ion concentration. Therefore, it was decided to adjust the urine of pregnant women to various H-ion concentrations and determine the potency. The present communication deals with this relationship.

The method of procedure was to collect a mixed sample of the urine of pregnant women from the obstetrical clinic. The H-ion concentration was determined electrometrically using the hydrogen and calomel electrodes, and the urine was standardized in the following manner. Rabbits were injected in the marginal ear vein at 4:30 in the afternoon, with amounts varying in tenths of a cubic centimeter, and a laparotomy done the next morning to examine the ovaries. The smallest amount in tenths of a cubic centimeter causing the graafian follicles to rupture, in 3 out of 5 test animals, was then considered as the standard of potency of the fresh sample. Aliquots of the original mixed sample were adjusted to varying H-ion concentrations in the alkaline and acid range with a few drops of 18 normal sodium hydroxide or concentrated hydrochloric acid, and the exact pH determined as before.

The results are shown graphically in the accompanying curve where the pH is plotted against the cubic centimeters necessary to bring about a rupture of the graafian follicles. There is a range between a pH of 6.1 and 7.3 where the potency is greatest, with the curve rising in the form of a parabola from a pH of 7.3 to 12.0; and from a pH of 6.0 to 3.0. No ruptured follicles were found at any time with as much as 5 cc. at a pH of 12.0.

In order to determine whether the hormone potency was partially destroyed or inhibited, one collection was divided into 3 parts. The first having a pH of 6.48 was standardized. The second part was made acid with concentrated hydrochloric acid to a pH of 3.85, left one day and brought back to a pH of 6.12 with 18 normal

1 Elden, C. A., *J. Biol. Chem.*, 1933, **101**, 1.

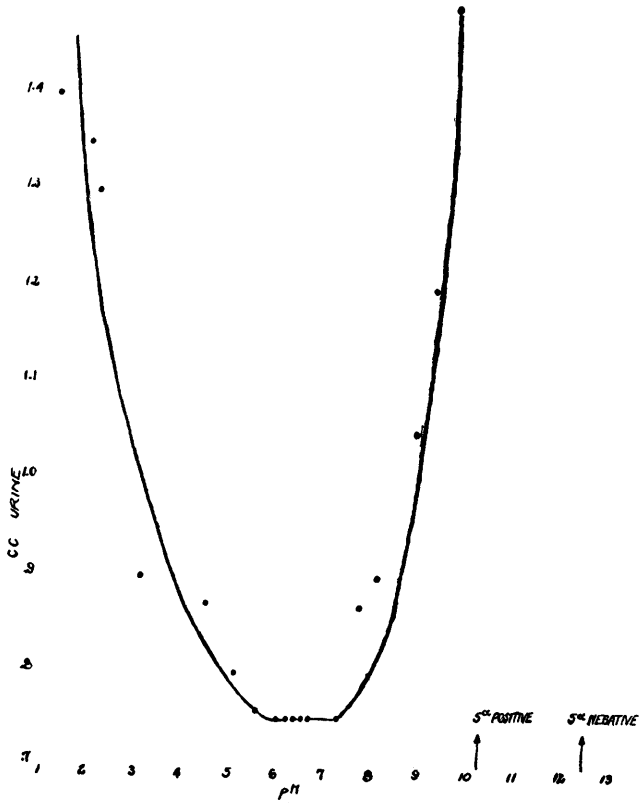

FIG. 1.

sodium hydroxide, where the potency was found to be unchanged. The third portion was made alkaline to a pH of 8.8, left 2 days, and adjusted to a pH of 5.0 where it was found to have decreased 50% in potency. Other samples which became more alkaline on standing for as long as 3 months in the icebox, and were then readjusted to the pH of maximum potency, were less affected than those artificially made alkaline with concentrated sodium hydroxide, and readjusted as above.

Zondek, *et al.*,[2, 3] have reported the best precipitation at a pH of 4.0. The findings in this investigation show the hormone to be most potent at a pH of 6.1 to 7.3. It seems highly probable that adsorption and elution may take place best at this pH; however it is possible that adsorption alone may take place equally well or even better at a different H-ion concentration, which would tend to keep

2 Zondek, B., and Aschheim, S., *Klin. Woch.*, 1928, **7**, 831.

3 Zondek, B., *Zent. Gynak.*, 1929, **53**, 834.

any foreign proteins in solution. In this case the hormone should be more readily removed from contaminating mixtures, and obtained in a more pure form.

The curve showing the effect of H-ion concentration on the potency of the urine of pregnant women suggests that the hormone may be protein-like in nature, and have an isoelectric point not unlike hemoglobin. If this is the true state, its physical and chemical properties, such as solubility in water, precipitation by ammonium sulphate, and the fact that it is most potent at a pH which may well be its isoelectric point, confirm the facts. On the other hand the hormone may be adsorbed on a protein, and the various urinary proteins may have varying isoelectric points, where different amounts of the hormone are carried down.

It has been noted by some observers[1, 2, 3] that the biuret test has been negative, perhaps due to the dilute concentration of hormone present. However, since most observations have been done on rather crude preparations, with the protein as a possible contaminant, the validity of these statements awaits further elucidation, with the preparation of a chemically pure hormone.

Conclusions. From the foregoing results, conclusions are drawn that the urine of pregnant women is most potent in anterior pituitary-like action at a pH of 6.1 to 7.3. Potency is defined as the smallest amount of urine causing the graafian follicles to rupture in 3 out of 5 test animals. The hormone may be protein-like in nature, or carried down with the foreign protein at the isoelectric point of 6.1.

Potency is little affected by acid, but varies with the alkali content, sometimes being greatly reduced, as when sodium hydroxide is added, and again showing less change after 3 months in the icebox at an alkaline pH which has developed on standing.

These observations in no way affect the Friedman modification of the Aschheim-Zondek test,[4] since larger amounts varying from 5 to 10 cc. are always used in the latter.

[4] Friedman, M. H., *Am. J. Physiol.*, 1929, **90**, 619.