anterior pituitary injection shows all of the mature sperm liberated into the lumen of the seminiferous tubule (Fig. 2). This effect can be achieved only by anterior pituitary treatment, the controls having received other frog organs and having been subjected to temperature changes from 4°C. to 28°C. The reaction is comparable to the follicle changes induced in females by anterior pituitary treatment[5] except that in the female the egg is released into the body cavity.

In sections of the kidney adjacent to the stimulated testis, spermatozoa may be seen in the uriniferous tubules, Bowman's capsule, and ureter (Figs. 3 and 4). The exact path of these spermatozoa, through the kidney, is being worked out for *Rana pipiens, Rana catesbiana, Bufo fowleri,* and *Hyla crucifer.*

Studies are at present being made to determine the source of the male gonad hormone, and the seasonal differences in maturation exhibited by *Rana pipiens* and *Rana catesbiana.* In the one there is a single breeding period, with a single expulsion of spermatozoa. In the other there seems to be an extended breeding period, at a different time of the year, with staggered maturation of spermatozoa.

9254

Water Intake and the Blood Sugar Level.

M. CAROLINE HRUBETZ. (Introduced by H. B. Williams.)

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In our studies which have involved blood sugar determinations on rats, considerable effort has been expended to secure uniformity both in the animal material and in the routine care of the colony. But in spite of all this care to secure uniformity we have found at times that the normal blood sugar level showed considerable variation.[1] Recently, upon our return from the summer vacation, the routine normal control series of sugar determinations was made. At the same time, determinations were made upon a group of rats used in the study of prolonged high carbohydrate feeding.[2] These animals represented the 4th, 5th and 6th generations which had been kept continuously on the high carbohydrate diet. It was noticed that the sugar levels in both groups were not only high but far above

[5] Rugh, R., *J. Exp. Zool.*, 1935, **71**, 163.

[1] Hrubetz, M. C., *J. Biol. Chem.*, 1934, **107**, 731.

[2] Hrubetz, M. C., *J. Lab. Clin. Med.*, 1936, **21**, 1142.

any mean value thus far obtained. Two and a half months previously the water supply had been changed from the open jar to the tubulated-bottle system. It had been observed that some of the water bottles did not deliver properly. The question arose, therefore, as to whether a possible water deficiency due to difficult access to the water by the tubulated-bottle method was associated with the high sugar level. The purpose of the observations to be reported was to determine the influence, if any, of the water consumption by the open jar and the tubulated bottle upon the blood sugar level.

After the initial observations were made, the entire colony was supplied with open water jars and the blood sugar levels were determined one week later and at subsequent intervals for 8 weeks. Approximately 50 observations were made on each point on the curve for the normal controls. There were 30 animals in the high carbohydrate group. The determinations were made by the Somogyi Micro Method[3] with the use of reagent No. 1. The chart shows a prompt drop in the normal animals to a level very near the mean for the colony. The individual values fluctuated rather widely the second week and then remained constant throughout the remainder of the 8 weeks period. The high carbohydrate rats maintained a high blood sugar for 3 weeks; not until the 4th week did they return to a normal level. After the return, however, the level remained constant for the remainder of the period.

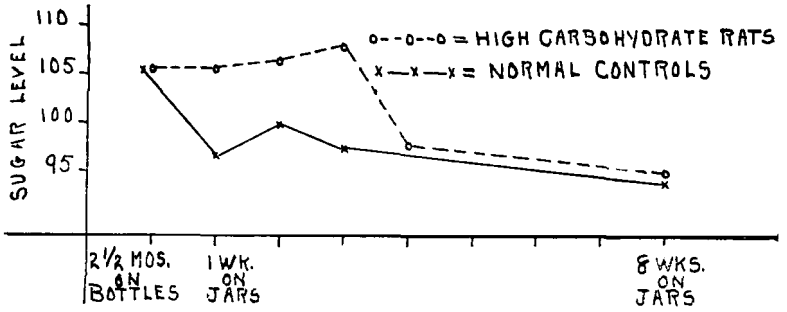

FIG. 1.

Naturally, we were somewhat surprised to find this increase associated with water intake and at present are quite unable to suggest its mechanism. It may possibly be associated with the general concentration of the blood though no measurements were made to determine this. Another point worthy of note and for which we have at present no information was the delayed return to the normal by

[3] Peters and Van Slyke, *Quantitative Clinical Chemistry*, Williams and Wilkins Co., Baltimore, 1935, Vol. II, p. 466.

the animals on the high carbohydrate diet. Apparently, diet is a factor influencing the ability of the organism in maintaining a more or less constant concentration of the blood as well as of some of its specific constituents. In spite of our inability at present to suggest the mechanism involved in the reactions reported, the practical bearing on standardization of experimental material is of sufficient importance to justify prompt publication.

9255 P

Determination of Sulfanilamide in Blood and Urine.

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Recently a method was described for the estimation of sulfanilamide (para-aminobenzenesulfonamide) in blood and urine.[1] In the rabbit and human subject this drug is partly excreted in the form of an acetylated derivative.[2] In developing a method for determining this conjugated derivative in blood, we have been able to improve the original procedure.

The present modification possesses the advantages of being more sensitive, of giving a more stable color, and of requiring fewer manipulations. Sulfanilamide can be determined very accurately by this method, and in addition one can obtain a fairly good estimate of the conjugated form present in blood. The present method consists of preparing a blood filtrate with toluenesulfonic acid, utilizing the acidity of the precipitant to perform diazotization and coupling with the amine. In determining the conjugated sulfanilamide in blood, the acidity of the blood filtrate is sufficient for hydrolysis on heating.

One volume of oxalated blood is measured into a flask, diluted and laked with 7 volumes of 0.05% saponin solution.* After laking is complete (1 or 2 minutes) 2 volumes of para-toluenesulfonic acid

[1] Marshall, E. K., Jr., Emerson, Kendall, Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

[2] Marshall, E. K., Jr., Cutting, W. C., and Emerson, Kendall, Jr., *Science*, 1937, **85**, 202.

* Laking can be accomplished by diluting with water instead of saponin solution at least 15 minutes before adding the protein precipitant.