TABLE III. Effect of Nephrectomy on Liver Vitamin A.

	No. of rats	Vit. A, μg/g, avg (range)	No. of rats	Wt of liver, g, avg (range)	μg vit. A/liver, avg (range)
Nephrectomized	9	60.7 (45.8- 75.1)	7	12.7 (11 -14)	746.8 (560 - 902)
Sham operated	7	70.7 (50.4-101)	5	11.7 (10 -13.2)	831.5 (630 -1013.6)
Nephrectomized but not given vit. A test dose	3	25.8 (14 - 36)	2	10.4 (10.2-10.5)	326 (365.5- 287)

drome may be attributed to a change in the function of some organ other than the kidneys. Previous work(3) in this laboratory showed that the liver may be responsible for the changes in vit. A metabolism observed in the nephrotic syndrome. The present study suggests that this may be independent of the kidney.

Table III shows that absence of the kidneys in these short term experiments had no significant effect on liver vit. A content or concentration.

Summary. Mature white male rats were subjected to bilateral nephrectomy and the serum and liver vit. A concentrations and liver vit. A content were studied with and without test doses of vit. A. No differences were observed between the serum vit. A values in nephrectomized and sham operated control rats. There was also no difference in the liver vit. A concentrations or content. The results are interpreted as indicating that the normal mature kidney plays no significant role in vit. A metabolism in such short term experiments in the rat.

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Hexose-6-Phosphatase Activity in the Seminal Vesicles and Prostate Gland of the Rat.* (20917)

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Spermatozoa, according to Mann(1) appear to utilize fructose rather than glucose as an energy source. The fructose of seminal fluid is produced in the glands of the male reproduction tract.

Chiquoine(2) has demonstrated histochemically that glucose-6-phosphatase is located in hepatic cells and in renal convoluted tubules. This enzyme, according to Cori(3,4) is necessary for the transformation of cellular stores of carbohydrate into blood glucose, and in its absence, excessive cellular storage of glycogen occurs. By analogy, a hexose-6-phosphatase capable of transforming a phosphorylated precursor into fructose might be expected in the seminal vesicles and prostate gland of the rat, since these are the glands which furnish the fructose of the seminal fluid. Using suitable modifications of Chiquoine's method, these accessory glands have been tested for their capacity to hydrolyze fructose-6-phosphate, glucose-6-phosphate and fructose-1-6-diphosphate.

Material and method. Mature male rats were killed by a blow on the head. The seminal vesicles and prostate gland were removed and immediately frozen in containers maintained at —20°C. Pieces of tissue were sectioned at 10 μ in a Linderstrøm-Lang

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cryostat by the technic described by Coons and Leduc(5). Fructose-6-phosphate, glucose-6-phosphate and fructose-1-6-diphosphate, converted to their potassium salts, were utilized as substrates. These salts were added to lead nitrate solutions and the pH adjusted to its final value as described by Chiquoine (2). To conserve substrate, small quantities were used in small weighing bottles as suggested by Engle and Souders(6). After incubations of 15 minutes to one hour at 30°C, the lead phosphate precipitated in the tissue was converted to the sulfide by treatment with yellow ammonium sulfide as employed in Gomori's acid phosphatase technic(7). The slides were dehydrated, cleared in xylene and mounted in clarite. For comparison with the results obtained with the methods outlined above, some sections were stained with Gomori's(7) alkaline phosphatase procedure employing glycerol phosphate.

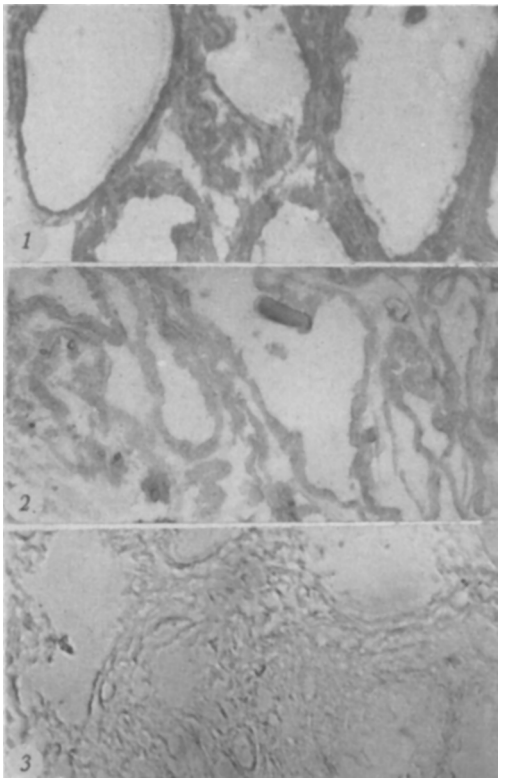

FIG. 1. Frozen section of rat seminal vesicle exhibiting phosphatase reaction when fructose-6-phosphate was used as substrate. ×150.

FIG. 2. Section similar to that shown in Fig. 1, in which the substrate was glucose-6-phosphate. The reaction is weaker than that obtained with the fructose ester. ×150.

FIG. 3. Control section, treated identically with those shown in Fig. 1 and 2 except that the incubating mixture lacked either glucose or fructose esters. The reaction is absent. ×150.

Results. The epithelia of the seminal vesicles and prostate gland contained deposits of lead phosphate distributed rather evenly throughout the width of the cells when either fructose- or glucose-6-phosphate were used as substrates. The connective tissue stroma beneath the epithelium was negative (Fig. 1 and 2). The optimum pH for the reaction lay between 6.3 and 7; the intensity fell off rapidly above and below these values and was absent at pH 5 and pH 10. The reaction was perceptibly more intense with fructose-6-phosphate as compared with the corresponding glucose ester.

Slides immersed in 5% formalin at pH 7.4 for 15 minutes were entirely lacking in reactivity when tested with either of the hexose-6-phosphates. The enzyme appeared to be extremely labile, weak reactions being given by tissues not frozen immediately after death of the animal or after prolonged storage at —20°C.

Negative reactions were obtained with fructose-1-6-diphosphate at neutral and acid pH values. Between pH 8-10, reactions in the basement membranes and subjacent stroma were obtained. Similar locations of reactivity were observed after the conventional alkaline phosphatase procedure. These reactions, unlike those after the hexose-6-phosphates, were unaffected by prior treatment of the sections with formalin.

Discussion. Unlike alkaline and acid phosphatases, which are resistant to heat, and to fixing agents such as alcohol and formalin, glucose-6-phosphatase is extremely labile and easily destroyed. Moreover, alkaline phosphatase hydrolyzes glucose-6-phosphate very slowly as compared with its activity when tested with glycerol phosphate. These characteristics permitted Chiquoine(2) to determine differentially the presence of glucose-6-phosphate in liver and kidney, and to show that this enzyme is located in cellular regions different from those in which acid or alkaline phosphatases are found. Similar procedures

employed on the male accessory gland, described above, have permitted detection of a labile enzyme localized differently from the more stable acid and alkaline phosphatases.

The specificity of the enzyme dealt with here remains an open question. The tissues of the male accessory glands are capable of hydrolyzing both glucose-6-phosphate and fructose-6-phosphate. Whether this fact implies the presence of separate enzymes for the 2 substrates, or of an enzyme which will nonspecifically split either hexose ester, is not known. In any event, it is of interest that high concentrations of fructose occur in seminal fluid and that an enzyme capable of splitting fructose phosphate occurs in the glands where the fructose originates.

Summary. The seminal vesicles and prostate gland of the rat are capable of hydrolyzing both fructose-6-phosphate and glucose-6-phosphate. This hydrolytic activity is located in different regions from those containing acid and alkaline phosphatase. The activity toward the hexose phosphates is easily destroyed by fixing agents. The significance of the hexose phosphatase activity is briefly discussed in relation to the fructose metabolism of the male reproductive system.

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Effect of Atabrine on X-Irradiation-Induced Leucopenia in Chick, Rat and Mouse.* (20918)

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It has been previously noted that the administration of atabrine at relatively high levels causes a marked generalized leucocytosis in rats and chicks(1,2). Since x-irradiation is known to produce a marked leucopenia in experimental animals it seemed of interest to determine the effect of atabrine on leucocyte level following irradiation. In connection with this study it has been observed that the failure of chicks to feather normally after exposure to x-irradiation is overcome by the administration of atabrine(3).

Methods. The x-irradiation treatments reported here were carried out using the following factors: 15 ma, 220 KV, inherent filtration only, HVL 0.25 mm Cu, 32 inches to the center of the animal, rate of 35.5 r/min. (in air), field size covering the entire animal. Day-old White Rock chicks were placed on the purified diet described by Keith *et al.*(4) supplemented with 2 mg of folic acid per kilo of diet. One half of the chicks received a supplement of 500 mg of atabrine per kilo of diet. After 10 days on these diets the birds were subjected to 725 r. In experiments to determine the effects of atabrine given after irradiation, all the chicks were fed the unsupplemented diet for 13 days prior to irradiation (675 r). Immediately after irradiation the birds were divided into 2 groups. The birds of one group were injected intraperitoneally with a 5% solution of glucose and

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