tion with morphine. However, in evaluating these data, it must be considered that Noludar is a much longer acting hypnotic than pentobarbital and hence discrepancies in the time necessary to produce respiratory arrest might be expected. Nevertheless, 4 out of 7 animals survived for 4 hours or longer following Noludar while all 7 animals receiving equi-depressant doses of pentobarbital expired.

Summary. Noludar and pentobarbital alone and in combination with morphine were compared as to their respiratory minute volume depressant effects in rabbits. No statistically significant difference could be found between Noludar alone and in combination with 0.603 mg/kg of morphine. Pentobarbital, on the other hand, was significantly more depressant in combination with morphine at low pentobarbital doses but not at anesthetic levels.

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Received September 10, 1956. P.S.E.B.M., 1956, v93.

Carbohydrate Metabolism and Phosphate Turnover Rate in Scorbutic Guinea Pigs. (22818)

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Metabolism of carbohydrate is altered in ascorbic acid deficiency. Banerjee *et al.*(1-4) and Bacchus and Heiffer(5) observed decreased glucose tolerance in scorbutic guinea pigs. Murray *et al.*(6) and Stewart *et al.*(7) reported diminished glucose tolerance in scorbutic monkeys. Liver glycogen content was lower in such animals than in paired fed normal controls. Banerjee *et al.*(3,8,9) ascribed the decreased carbohydrate metabolism to diminished insulin content of the pancreas in scurvy. Recently Banerjee and Ghosh(10) reported diminished hepatic and muscle hexokinase activity in scorbutic guinea pigs possibly due to decreased insulin synthesis and supply in such animals. Insulin was shown to stimulate hexokinase(11) and reduce liver glucose-6-phosphatase activities(12). Considering the possible role of ascorbic acid in the biosynthesis of insulin and the functions of insulin at these levels of glucose metabolism, the glucose-6-phosphatase activity was determined in tissues of scorbutic and paired fed normal guinea pigs. Decrease in hexokinase activity and increase in glucose-6-phosphatase activity might consequently lead to diminished phosphorylated intermediates of the glycolytic system. The changes in phosphorylation and dephosphorylation processes might eventually alter inorganic phosphorus content of tissues in scurvy. Studies were, therefore, carried out to elucidate the pattern of metabolic changes in relation with carbohydrate metabolism in scorbutic guinea pigs. The hexokinases are known to be sulfhydryl enzymes, and it is now well accepted that ascorbic acid protects and maintains the sulfhydryl groups of the enzymes(13). As an introduction to further study on the role of ascorbic acid in carbohydrate metabolism, fructokinase activity of the brain tissue of scorbutic and paired fed normal guinea pigs was determined.

Methods. Female well-growing guinea pigs of weights varying between 230 and 260 g were distributed into 16 pairs. Feeding a scorbutic diet, with or without supplement of ascorbic acid, by paired feeding technic and care of animals were the same as described previously(14). Pairs of animals were sac-

TABLE I. Effect of Scurvy on Inorganic Phosphorus Values of Blood and Liver of 10 Guinea Pigs in Each Series.

	mg P/100 ml blood	mg P/100 g liver
Normal	4.7 ± .25*	34.1 ± 2.1
Scorbutic	6.2 ± .50	46.1 ± 1.2
t	2.5	4.8

* Stand. error.

rificed after an over-night fast on 25th day of experiment, and inorganic phosphorus contents of blood and liver, glucose-6-phosphate, fructose-6-phosphate and fructose-1, 6-diphosphate contents of liver, glucose-6-phosphatase activity of liver and kidney and fructokinase activity of brain were determined. Total weights of livers of some pairs of guinea pigs were recorded and the values calculated per 100 g body weight. For estimation of *inorganic phosphorus,* blood was collected in an oxalated tube and 1 ml aliquot was analysed by the method of Fiske and SubbaRow (15). For liver inorganic phosphorus and phosphorylated intermediates, the tissue was immediately frozen after killing the animal and was extracted with ice cold 10% trichloroacetic acid. A portion of the extract was neutralized, in cold, to phenolphthalein and inorganic phosphate was precipitated with magnesia mixture over night and then estimated. Another aliquot was analysed for glucose-6-phosphate, fructose-6-phosphate and fructose-1, 6-diphosphate according to the methods described by LePage and Umbreit(16). Results are given in Tables I and II. For estimation of *glucose-6-phosphatase* and *fructokinase* activity, tissues were removed from body immediately after killing the animal and cooled in cracked ice for 3-5 minutes. Glucose-6-phosphatase activity of liver and kidney was assayed by the method of Swanson(17). The tissues were homogenized with exactly 3 volumes of ice-cold 0.25 M sucrose in an ice-cold all glass homogeniser. 0.1 ml of this homogenate, 0.1 ml of 0.04 M glucose-6-phosphate (pH 6.5) and 0.1 ml of 0.1 M citrate buffer of pH 6.5 were mixed in 1 cm diameter duplicate tubes, incubated for 30 minutes at 37° and the reaction terminated by addition of 0.3 ml of 10% trichloracetic acid. In control tubes, water was added in place of the substrate. The samples were diluted to 3 ml, centrifuged and analysed for inorganic phosphorus (15). Dry weights of tissues were obtained from 1 ml homogenate, less the weight of sucrose present. Inorganic phosphorus liberated by the enzymatic hydrolysis in one hour per mg dry tissue is presented in Table III.

TABLE II. Effect of Scurvy on Phosphorylated Intermediate Contents of Liver of Guinea Pigs (mg/100 g Fresh Liver). 6 animals/series.

	Glucose-6-phosphate	Fructose-6-phosphate	Fructose-1,6-diphosphate
Normal	1166 ± 41	17.0 ± 1.1	20.2 ± .97
Scorbutic	995 ± 44	11.1 ± 1.5	15.7 ±1.0
t	2.7	3.2	3.1

TABLE III. Effect of Scurvy on Glucose-6-phosphatase Activity of Tissues of Guinea Pigs (γ P Liberated/mg Dry Tissue/ Hr). 8 animals/series.

	Liver	Kidney
Normal	27.4 ± 4.2	31.0 ± 5.5
Scorbutic	40.5 ± 4.5	36.8 ± 15.0
t	2.1	.7

For determination of *fructokinase activity,* brain tissue was homogenized in an ice-cold all glass homogeniser with 4 volumes of ice-cold 0.15 M potassium phosphate buffer of pH 7.5 containing 0.15 M potassium fluoride. Fructose phosphorylation studies were studied in an incubation medium proposed by Vestling *et al.*(18), using 1 ml portions. Incubation was carried out for 30 minutes at 37° and the reaction stopped by addition of 1 ml of 0.3 N barium hydroxide, followed by 1 ml of 5% zinc sulfate and water to 10 ml, mixed and filtered. Control experiments were conducted using complete medium with omission of adenosinetriphosphate. Fructose disappearance was measured by the method of Roe(19). 1 ml homogenate was dried at 105° to constant weight, and weight of salts deducted to get dry weight of tissue. Fructose disappeared/mg dry tissue/hour is presented in Table IV.

Results. A change in tissue phosphorus content in scurvy is apparent from Table I. Though there is a significant increase in inorganic phosphorus content of blood, only 5 pairs out of 10 studied show this change. A

TABLE IV. Effect of Scurvy on Fructokinase Activity of Brain of 10 Guinea Pigs per Series. (γ fructose disappeared/mg dry tissue/hr.)

Normal	Scorbutic	t
123.3 ± 12	100.5 ± 35	.6

significant increase in liver inorganic phosphorus content in scurvy was observed. This increased inorganic phosphorus level of blood and liver in scorbutic animals suggests that either the mechanism responsible for removal of free phosphate is inefficient, or that hydrolysing the organic phosphates, *i.e.*, phosphatase activity, is increased.

We reported that hepatic and muscle hexokinase activity is diminished in scorbutic guinea pigs(10). Table III shows that glucose-6-phosphatase activity of the hepatic tissue is increased in scurvy. These changes are those which would be expected if an insulin insufficiency occurred in such animals. As a consequence it is likely that inorganic phosphorus level of tissues will tend to increase and glucose-6-phosphate content of liver would diminish in scurvy. The data presented in Table II clearly indicate that this ester content of livers of scorbutic guinea pigs is significantly decreased. This table further shows that fructose-6-phosphate and fructose-1, 6-diphosphate levels of livers in scurvy are lowered.

Table III shows that the hepatic glucose-6-phosphatase activity is increased in scurvy, and remains unchanged in the kidney. Langdon and Weakley(12) reported that alloxan diabetes in rats is accompanied by a more than 2-fold increase in hepatic content of the enzyme. In scurvy, however, there is comparatively less change, which could be due to a smaller decrease in insulin synthesis in scurvy than in alloxan diabetes. These findings, therefore, suggest that the quantities of phosphorylated intermediates turned over in unit time are reduced below normal and further add to the evidence that the defect in carbohydrate metabolism in scurvy is quantitative rather than qualitative. It is, however, difficult to assess whether these quantitative changes in the phosphorylation cycle are of sufficient magnitude to account for the disturbed glucose tolerance in scorbutic animals.

No change in fructokinase activity in the brain of guinea pigs has been observed in scurvy. Table IV shows that, though there is a tendency to decrease, in some scorbutic animals, the individual variations are too great to make the change in average values significant. Fructose phosphorylation is also not hampered in insulin insufficiency due to alloxan diabetes(19-21). It has been observed that in scorbutic guinea pigs the weights of livers were considerably increased. The values calculated in g/100 g body weight of normal and scorbutic guinea pigs are 2.8 ± 0.2 and 4.8 ± 0.4 respectively. If the inorganic phosphorus and glucose-6-phosphatase activity of livers of scorbutic guinea pigs is expressed on the basis of total liver, these values will be further increased.

Summary. Inorganic phosphorus content of blood and liver and hepatic glucose-6-phosphatase activity are increased in scorbutic guinea pigs. Glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate contents of liver are decreased in scorbutic guinea pigs. Fructokinase activity of brain remains unchanged in scorbutic guinea pigs. Considerable increase in weight of livers of scorbutic guinea pigs has been observed.

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Received September 13, 1956. P.S.E.B.M., 1956, v93.

Enzymatic Estimation of Urinary Steroids.* (22819)

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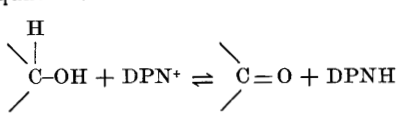
Existing procedures for the determination of urinary steroids depend upon either chemical reactions or bioassay. Despite great interest in this subject and its clinical importance, simple and specific micro-methods for the estimation of the amounts and types of urinary steroids have not been perfected(1). The novel procedure to be described here permits the simple microestimation of certain types of steroids in urine. This procedure depends upon selective enzymatic oxidations and reductions of steroids and the spectrophotometric measurement of the associated changes in the concentrations of diphosphopyridine nucleotide coenzymes. Enzymatic estimations are characterized by a high degree of specificity and sensitivity. It is thus possible to obtain quantitative information on the principal types of steroid ketones and alcohols present in a few milliliters of urine without resorting to tedious isolation and separation techniques. *Pseudomonas testosteroni* is a soil bacterium capable of growing on testosterone and related steroids as its only carbon source, and of oxidizing such steroids to carbon dioxide and water(2). The presence of steroids in the growth medium of this bacterium causes the induction of certain diphosphopyridine nucleotide-linked enzymes which reversibly interconvert particular hydroxy- and ketosteroids with a high degree of steric specificity(2-4). These enzymes have been named *hydroxysteroid dehydrogenases,* and their potential usefulness for the micro-estimation of steroids was pointed out in 1952. Subsequently, steroid dehydrogenases have been applied to the measurement and identification of single steroids(2-5). The purification and substrate specificities of 2 enzymes suitable for analytical purposes have been described(5-7). Briefly stated, these enzymes are: 1) a *3α-hydroxysteroid dehydrogenase* (designated *α* enzyme) which catalyzes the reversible oxidation of 3α-hydroxysteroids of the C-19 and C-21 groups; and 2) a *3β- and 17β-hydroxysteroid dehydrogenase* (designated *β* enzyme) which catalyzes the reversible oxidations of 3β- and 17β-hydroxysteroids to their respective ketones. Thus, the specificity of these enzymes fortunately coincides with the principal types of urinary steroids *i.e.* 17-ketosteroids as well as 3α-hydroxy- and 3β-hydroxysteroids of both the C-19 and C-21 series. The *analytical procedure* depends upon interconversions of steroid alcohols and ketones according to the general equation:



* Supported by grants from Am. Cancer Society, upon recommendation of National Research Council Com. on Growth.

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