increased adrenocortical secretion. Since Romanoff *et al.*(16) have observed that the excretion of adrenocortical steroids in man is greater during the morning and the rest of the day than during sleep, and that this excretion of steroids undergoes a constant diurnal variation, it might be postulated that the decrease in the level of plasma iron during the day may be associated with the increase in adrenocortical activity and similarly that the increase in the plasma iron during sleep may be associated with the decrease in the secretion of cortical hormone. However, it has been observed that the levels of eosinophils and lymphocytes in the peripheral blood increase rather than decrease as the day progresses(17,18). Furthermore, two patients with Addison's disease whom we have studied showed a normal diurnal variation in the level of plasma iron. There is even less evidence to suggest that the decrease in plasma iron during the day is due to sympathetic tone and that its rise during the night is due to an increase in parasympathetic tone(8). Thus, the physiological mechanisms responsible for the diurnal variation in plasma iron of man remain obscure.

Summary. It has been shown that the plasma iron of man undergoes a regular diurnal variation. These results have been compared with those of other investigators.

We are indebted to Miss Helen Ashenbrucker for technical assistance as well as to the subjects who took part in this investigation.

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Received July 21, 1950. P.S.E.B.M., 1950, v75.

Conversion of 3-Phosphoglycerate to Phosphoenolpyruvate by Tissue Homogenates.* (18103)

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A series of recent observations(1-3) made it interesting to pursue the problem of regulatory mechanisms of enzyme reactions participating in the glycolysis of Harden-Young ester. The present paper is a part of this study and describes a method for the determination of phosphopyruvate formation from 3-phosphoglycerate, catalyzed by dilute tissue homogenates. Evidence is also presented suggesting that this enzyme reaction might be under endocrine influence.

Experimental. Two enzymes, phosphoglyceromutase and enolase(4-6), are necessary to bring about the formation of phosphoenolpyruvate from 3-phosphoglycerate. Both enzymes are present in tissue homogenates. The principle of the assay of these two consecutive enzyme reactions is the determination of

* This investigation was supported by grants from the National Multiple Sclerosis Society, the Life Insurance Medical Research Fund, and the U. S. Public Health Service.

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the formation of iodine labile phosphorus, which according to Lohmann and Meyerhof (4) and Meyerhof and Oesper(7) is a reliable measure of phosphoenolpyruvate. As pointed out by Lardy and Ziegler(8), alkali labile phosphates (triose phosphates) interfere with phosphopyruvate analysis. Since the possibility of triose phosphate formation from 3-phosphoglycerate in fresh tissue homogenates cannot be excluded, phosphopyruvate phosphorus was determined as the increment in inorganic phosphate, due to iodine treatment, above the inorganic phosphate present in the alkalinized samples. This procedure also includes the correction for inorganic phosphate, formed during the incubation period. The pH optimum for phosphopyruvate formation was found to be pH 6.8, which value agreed well with that reported by Warburg and Christian(6) for crystalline enolase in the presence of inorganic phosphate and Mg^{++} ion. The test was carried out in centrifuge tubes of 15 ml volume. The tubes containing the reaction mixture were incubated in a shaking device at 37°C for 10 minutes prior to the addition of the enzyme (0.1 ml tissue homogenate, equivalent to 1-5 mg tissue). Each centrifuge tube contained 0.5 ml 0.05 M potassium 3-phosphoglycerate† (brought to pH 6.8) + 0.5 ml trishydroxymethyl-aminomethane buffer (pH 6.8)(9) + 0.2 ml 0.05 M $MgSO_4$ + 0.1-0.2 ml tissue homogenate in 0.9% KCl + 0.9% KCl to make a final volume of 1.5 ml. At the end of the incubation period the reaction was stopped by the addition of 1 ml of 1 N trichloracetic acid, the precipitated proteins centrifuged down at 2,000 r.p.m. in an International Centrifuge for 15 minutes. For the determination of phosphopyruvate phosphorus a 0.2 ml sample of the trichloracetic acid supernate was pipetted into each of 2 colorimeter cuvettes (Coleman spectrophotometer Model 6-A). Thereafter 1 ml N KOH was added to each cuvette and 0.2 ml 0.1 N iodine solution (KI + I_2) pipetted to *one* of the 2 tubes. The tubes were shaken and allowed to stand at room temperature for 15 minutes. To each cuvette 0.5 ml 5.0 *N* H_2SO_4 was then added and in the iodine treated tube the discharged iodine decolorized by 0.2 ml 0.1 N Na-thiosulphate which was added, followed by vigorous shaking of the tube. This precaution was necessary to avoid occasional turbidity probably due to the precipitation of colloidal sulphur. In the tube which contained no iodine, equal volumes of distilled water were substituted for the iodine and thiosulphate. Finally the contents of each tube were made up to 3 ml by the addition of 0.9 ml distilled water. A reagent blank was always made up simultaneously, in which the 0.2 ml trichloracetic acid supernate was replaced by 0.2 ml H_2O. The determination of inorganic phosphate was carried out by the method of Gomori(10) because, under the condition of this method, the molybdate color reaction was not altered either by the acidity or by the iodine-thiosulphate treatment, as determined by comparing phosphorus values of iodine treated and untreated samples of a standard phosphate solution. The colorimetric phosphate analysis was carried out as follows: 2.5 ml molybdate-H_2SO_4 reagent and 1 ml of Elon-bisulphite solution(10) were pipetted to the contents of each colorimeter cuvette. After shaking the optical density was read in 45 minutes at 660 mμ on the Coleman spectrophotometer. The phosphopyruvate phosphorus was calculated from the reading difference between the alkali and alkali + iodine treated cuvettes from a phosphorus calibration curve. The iodine labile phosphorus read in micrograms, multiplied by 12.5, gives the total amount of phosphopyruvate phosphorus present in the 2.5 ml trichloracetic acid treated incubation mixture. This value can be converted to phosphopyruvic acid by multiplication by 5.9.

Results and discussion. The rate of phosphopyruvate formation was followed for 60

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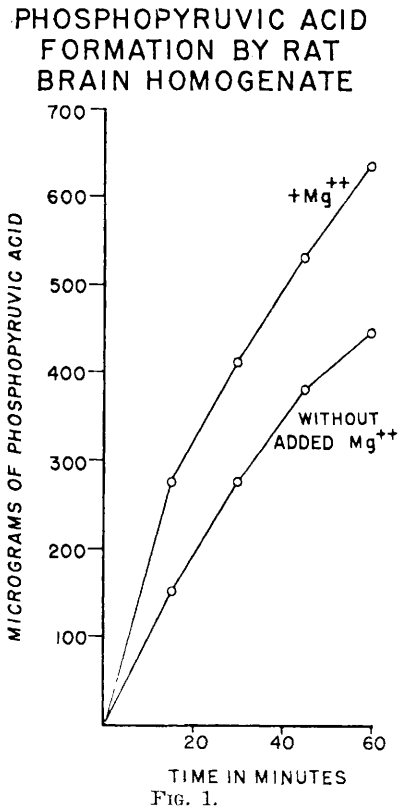

FIG. 1.

Enzyme = 5 mg male rat brain cortex homogenate. $MgSO_4$ solution was replaced by 0.2 ml H_2O. The reaction was stopped at 15' intervals by the addition of trichloracetic acid.

minutes using various tissue homogenates and extracts as the source of enzymes. A typical rate curve is presented in Fig. 1 where the activating effect of added Mg^{++} ions can also be observed. A linear relationship was found between the amount of enzyme (expressed in protein content of the tissue extract) and the amount of phosphopyruvic acid formed during a 15 minute incubation period (Fig. 2). Complete enzyme inhibition occurred when NaF was present in 0.01 M final concentration. The relative catalytic activity of various tissues could be compared by determining the phosphopyruvic acid formed by 1 mg tissue homogenate during 15 minutes incubation. Organs of 5 male and 5 female white rats, weighing between 250-340 g, were assayed by the described method. Results are summarized in Table I, where the mean values of determinations carried out on separate organs of 5-5 rats, the standard error (S.E.), and also the "t" values(11) existing between the means of comparable male and female organs are shown. It is apparent that from animal to animal, reproducible values can be obtained on the same organs. The phosphopyruvate formation by tissue homogenates of female organs was significantly lower in all tissues except the adrenal as compared to male animals. Interestingly, the activity of ovaries significantly exceeds that of the testis (+50% "t"=3.66).

It is well known that the formation of phosphopyruvate and the transphosphorylation to the adenylic system(12-14) is an important step of glycolysis since it is directly

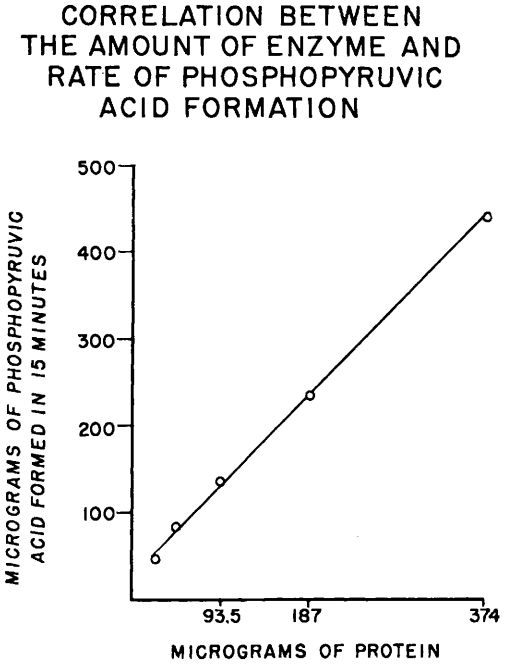

FIG. 2.

The enzyme was prepared by homogenizing 0.5 g rat brain cortex (male) in 10 ml 0.9% KCl and centrifuging at 14,000 × g for 20 min. at 0°C. Each test system (see text) contained 0.2 ml of the enzyme (supernate) which was serially diluted (1:1) with 0.9% KCl. The undiluted brain extract contained 374 μg of protein per 0.2 ml.

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TABLE I. Phosphopyruvic Acid Formation by Tissue Homogenates of Male and Female Rats. (Results expressed in terms of phosphopyruvic acid formed by 1 mg tissue during 15' incubation).

	Phosphopyruvic acid					
	Male		Female			
Organ	Mean	S.E.	Mean	S.E.	% difference	"t" values
Leg muscle	418.0	± 19.80	258.8	± 14.60	—38	6.5
Heart muscle	174.8	± 24.70	100.1	± 16.30	—43	8.55
Kidney	162.8	± 17.6	72.7	± 7.50	—55.5	4.72
Liver	58.1	± 2.24	36.36	± 3.38	—37.5	5.36
Brain	68.7	± 5.55	44.17	± 2.68	—35.7	3.96
Spleen	50.8	± 7.00	31.72	± 2.78	—37.6	6.35
Thyroid	72.4	± 4.45	49.57	± 2.00	—31.4	4.7
Adrenal	54.3	± 9.53	38.92	± 6.20	(—28.4)	1.35
Testis	45.3	± 6.75		—		
Ovary		—	91.0	± 10.5		
Uterus		—	78.0	± 5.7		

S.E. = Standard error.

5% homogenates (0.1 ml) were used for enzyme assays of liver, brain, spleen, thyroid, adrenal, testis, and ovary; 1% homogenates (0.1 ml) for leg and heart muscle, kidney, and uterus.

concerned with storage of readily available energy in the form of energy-rich phosphate bonds. The marked differences found between male and female organs suggests that this energetically important phase of glycolysis might be regulated by hormones. Further data on the effect of endocrine organs on this enzyme reaction will be published in a subsequent paper.

Summary. It was shown that dilute homogenates of rat organs catalyze *in vitro* the conversion of 3-phosphoglycerate to phosphoenolpyruvate as measured by the formation of iodine labile phosphorus. A marked sex difference was observed between the rates of phosphopyruvate formation by male and female organs.

The valuable assistance of Mr. Donald R. McCurley, who carried out part of the enzyme assays, is gratefully appreciated.

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Received July 21, 1950. P.S.E.B.M., 1950, v75.

Properties of the Methanol Soluble Factor Required by the Mink.* (18104)

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Previous work has shown that mink given a purified diet require a heat stable methanol soluble liver factor, and preliminary results indicated that a methanol insoluble (residue) factor was also required(1). The methanol soluble factor was found in liver, whole milk and whey, but not in tomatoes, alfalfa or yeast. Similar results have been obtained with the fox(2). Further studies on these

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were supported by Project 614 of the Wisconsin Agricultural Experiment Station and grants from the Borden Co., New York, New York and Fur Farm Food and Supplies, St. Paul, Minn.

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