Physiological Regulation of Phosphopyruvate Formation from 3-phosphoglycerate in Rat Tissues.* (18346)

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Previous experiments in this laboratory(1) have shown that tissues of adult male rats convert 3-phosphoglyceric acid to phosphopyruvic acid at a higher rate than the corresponding tissues of female animals. This observation suggested a further investigation of the regulatory mechanisms of this coupled enzyme reaction (phosphoglyceromutase ⟶ enolase). The experiments here described demonstrate that castration and hypophysectomy as well as injection of diethyl stilbestrol, testosterone, cortisone, and adrenocorticotrophic pituitary extract (ACTH) markedly influence this phase of glycolysis.

Experimental. Castration was performed on both adult male and female white rats. Hypophysectomized adult male rats were obtained from the Hormone Assay Laboratories, Inc.† and were received one day after operation. Controls for this group came from the same source. The body weight of adult rats ranged between 300 and 350 g. It appeared to be of interest to determine the effect of age on the rate of phosphopyruvic acid formation; therefore, young male and female rats were used in the experimental and control groups in which the effect of diethyl stilbestrol, testosterone, cortisone, and ACTH was tested. The weight of these young animals was between 120 and 150 g. The enzyme activity of tissues of individual animals in an experimental group was found to agree within the limits of the standard error reported previously(1). Control and experimental groups consisted of 5 to 8 animals each. The difference between the average enzyme activities of control and experimental groups was considered significant when it exceeded at least twice the standard error of the controls. Diethyl stilbestrol, testosterone and cortisone were injected intraperitoneally in form of freshly prepared dilute ethanol (30%) solution in a volume of 0.1 ml, ACTH was suspended in saline, 1 ml containing 10 mg extract.

Results. The experimental data are summarized in Fig. 1, 2, and 3, each column representing the average enzyme activity of each

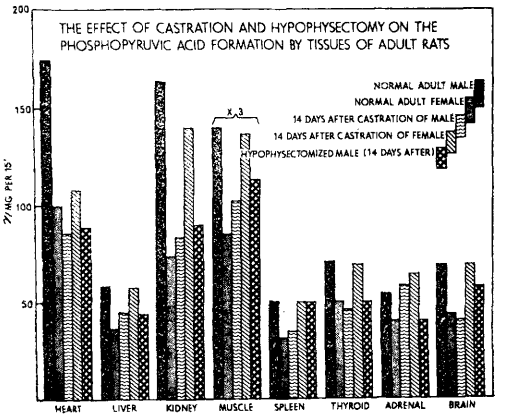

FIG. 1.

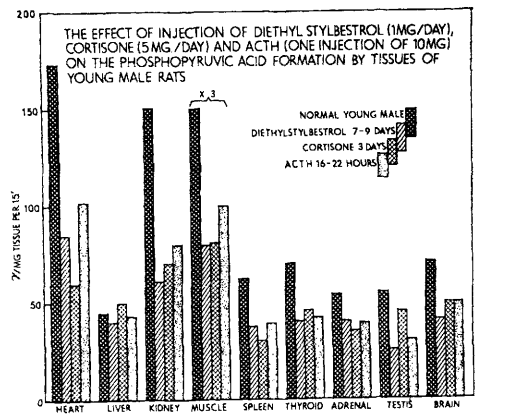

FIG. 2.

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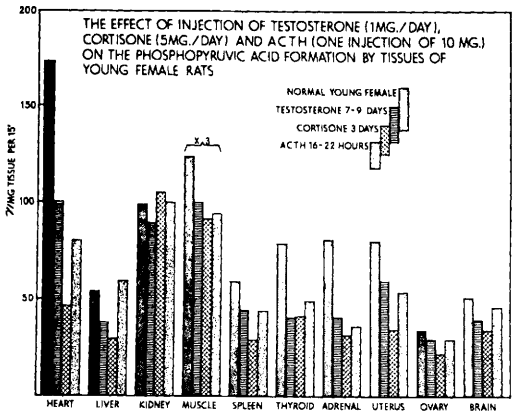

FIG. 3.

particular tissue. As early as 1 to 2 days after castration in both adult male and female rats, marked changes in enzyme activity could be observed. These changes persisted and were recorded 14 days after operation (Fig. 1).

In adult males castration caused a sharp decrease in the rate of phosphopyruvate formation, while removal of ovaries in females resulted in an increase. Heart, kidney, and muscle tissues exhibited the most pronounced changes, but this effect was also noticeable in other tissues. Hypophysectomy generally decreased the enzyme activity of tissues, but this effect was significant only 14 days after operation. Sham operations had no effect on the rate of phosphopyruvate formation.

In the course of enzyme assays of tissues of young rats it was noticed that the sex difference, observed on adult animals, was less pronounced in the young ones. Enzyme activities significantly higher in young males than in young females were observed only in kidney, muscle, and brain tissue. The enzyme activity of the adrenals of young females was somewhat higher than in young males. The rate of phosphopyruvate formation in the ovaries of young females was only about one-third of that in adult animals. Enzyme activities in young males was not significantly different from adult male animals.

The pharmacological action of diethyl stilbestrol on the rate of phosphopyruvate formation in young males, that of testosterone on young females, and the effect of cortisone and ACTH on enzyme activities of tissues of both sexes is summarized in Fig. 2 and 3. The action of these compounds was qualitatively similar; they caused a decrease in the rate of phosphopyruvate formation. Injection of diethyl stilbestrol (1 mg per day for 7 to 9 days) in young males resulted in a large decrease of enzyme activity in all organs, but less marked in the liver and adrenal. Testosterone injection of females (1 mg per day for 7 to 9 days) had a similar inhibitory effect, but this inhibition was small in the kidney. Cortisone (5 mg per day for 3 days) caused a marked diminution in phosphopyruvate formation, except in the liver of males, and kidney of females. The single injection of 10 mg ACTH resulted after 16 to 22 hours in a decrease of enzyme activity in all tissues, except in the liver of both sexes, and in the kidney and brain of females (Fig. 2 and 3).

Discussion. According to the observations reported in this paper, the phosphoglyceromutase-enolase reaction in tissue homogenates is a suitable test system for the study of hormones on enzyme reactions. At present it is difficult to visualize the exact mechanism of this hormonal regulation, since the injection of a hormone or the removal of an endocrine gland undoubtedly influences the whole regulatory system. Preliminary experiments *in vitro* with testosterone, dehydroandrosterone and desoxycorticosterone acetate showed that these compounds can activate or inhibit—depending on the amount of hormone—the phosphopyruvate formation in homogenates in amounts of 0.005 to 10.0 μg. Similar *in vitro* effects of sterole hormones were recorded by Dirschel and Hauptmann(2) on the glycolysis of mouse and rat liver slices and diaphragms. It is of interest that marked sex differences were found in tissue respiration of muscles of male and female specimens of *Periplaneta americana* by Barron and Tahmisian(3). Recently Copenhaver, Stafford and McShan reported(4) that the esterase activity of tissues of male rats was higher

2. Dirschel, W. and Hauptmann, K. H., *Biochem. Z.*, 1950, v320, 199 and 228.

3. Barron, E. S. G., and Tahmisian, T. N., *J. Cell and Comp. Physiol.*, 1948, v32, 57.

than of females, a finding comparable to the sex difference in the rate of phosphopyruvic acid formation(1) in rat tissues. It must be noted, however, that this sex difference in the rate of phosphopyruvic acid formation was not found in mice, indicating the influence of species on the regulation of enzyme reactions. Pertinent to the experiments reported is the effect of diethyl stilbestrol and castration on the metabolism of prostatic tissue, described by Barron and Huggins(5). The regulatory effect of hormones may partly be due to their effect on the synthesis of enzymes and partly to their interaction on the catalytic protein. This question is being further studied by comparison of *in vivo* and *in vitro* effects of pure hormones.

Summary. Castration of male white rats resulted in a decreased rate of phosphopyruvic acid formation from 3-phosphoglyceric acid, by dilute tissue homogenates. Ovariectomy caused an elevation of enzyme activity in the tissues of females. Enzyme activity of hypophysectomized rats decreased only 14 days after the operation. The effect of age on this enzyme reaction was also demonstrated. Tissues of young females had a higher enzyme activity than those of mature females, except the ovaries which were considerably lower than in the mature animals. The effect of age was not noticeable in young males. Injection of diethyl stilbestrol in young males or testosterone in young females caused a decrease in phosphopyruvic acid formation. Cortisone and ACTH decreased the rate of this enzyme reaction in both sexes.

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4. Copenhaver, J. H., Jr., Stafford, R. O. and McShan, W. H., *Arch. Biochem.*, 1950, v26, 260.

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Identification of Sterols on Filter Paper and Their Separation by Paper Partition Chromatography.* (18347)

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Partition paper chromatography, in which the partition is between the water of the hydrated cellulose of the filter paper and the organic mobile solvent, has been applied to a wide variety of chemical compounds(1,2). However, the separation and identification of steroids by this method is difficult, due to extremely low solubilities in H_2O and to a lack of good color reactions capable of detecting these compounds on filter paper. Recently, paper partition chromatography has been successfully applied to *keto steroids* by the formation of hydrazones with Gerard's Reagent T prior to chromatography(3) and by the use of non-aqueous solvent systems (4). This communication describes methods and conditions for the identification and separation of several non-keto steroids on a micro scale on filter paper.

Identification. Two satisfactory methods were developed for the identification of cholesterol, ergosterol, 7-dehydrocholesterol, vit. D_2 and vit. D_3—using Whatman's No. 1

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3. Zaffaroni, A., Burton, R. B., and Keutmann, E. H., *J. Biol. Chem.*, 1949, v177, 109.

4. Zaffaroni, A., Burton, R. B., and Keutmann, E. H., *Science,* 1950, v111, 6.