CHANGES IN THE ACTIVITY OF THE HEPATIC GLUCOSE-6-PHOSPHATASE AFTER CASTRATION AND UNDER THE EFFECT OF THYROID HORMONES. PRELIMINARY REPORT

D. S. Kalitzin

Glucose-6-phosphatase is an enzyme of key importance for intermediate metabolism processes. It ends the chain of reactions in the liver, which are responsible for the production of glucose, at the expense of glycogen, of other sugars and intermediate products of the carbohydrate metabolism, and likewise of non-carbohydrate sources (9). Glucose-6-phosphatase plays an essential role in the metabolic processes and is subject to multilateral hormonal influences (4, 3, 11, 12, 13, 24, 25).

In this study we aim at investigating the effect of castration and treatment with extract of the thyroid gland, on the activity of hepatic glucose-6-phosphatase in male rats.

Material and Method

The experiments were carried out on 38 common breed young, sexually mature white rats, distributed as follows; group I — controls — 14 rats, group II — castrated — 12 rats and group III — castrated and treated with thyroid gland exstract — 12 rats.

The rats of group II and III were castrated by surgical removal of the testes and results were studied 1 $^{1}/_{2}$ months after the operation. In the animals of group III, hyperthyroid state was experimentally induced by means of daily administration of thyroid extract by tube, per os, for a period of 10 days (at daily dose 15 mg per 100 g body weight). The animals received, ad libitum, bread and fresh milk. Prior to the experiment, they were kept without food for an equal period of time (15 hours), since it is well known (24) that during fasting an increase of the hepatic glucose-6-phosphatase activity occurs, increasing with the length of the fasting period. The rats were killed by blow on the occiput, always at the same time of the day (11,00 hrs), in order to avoid differences caused by the 24-hour fluctuations in the phosphourus fractions level in the liver, as demonstrated by *Barnum and Halberg* (6).

The glucose-6-phosphatase activity was estimated in total homogenates of liver, according to the method of *Swanson* (20) *and Cori—Cori* (8), as modified by the authors of this paper.

The liver was promptly removed, stripped from the connective tissues, washed out with cold distilled water, dried on filter paper, weighed and homogenized at 0° C for 1—2 minutes in glass homogenizer — type Potter—Elvehjem (18) — with water cooled to 0° at a 1:15 ratio. The homogenate thus obtained was rapidly filtrated through gauze in an ice-immerged test-tube for the removal of remaining connective tissue fibers (8).

The homogenate was examined immediately, for it has been established that glucose-6-phosphatase reduces its activity when kept at room temperature (9,20). Barium salt of glucose-6-phosphate (Chinoin—Budapest: $C_6H_{11}O_6PO_3.Ba.7H_2O$) not containing mixtures of inorganic or acid labile phosphate, was employed as substrate. For the purpose of the experiments the barium salt was converted into potassium salt.

The incubation mixture was prepared in centrifugal test tubes at total volume amounting to 1,3 ml by adding 0,5 ml freshly prepared homogenate to 0.8 ml buffer substrate solution, temperated in advance at 37° (containing 0,3 ml 0,025M solution of barium salt of glucose-6-phosphate and 0,5 ml 0,1M citrate buffer). The pH of the incubation mixture was 6,5. The latter value is considered as the optimal pH of hepatic glucose-6-phosphatase (20).

The samples were incubated in ultra-thermostat of Höpler at 37° for 60 minutes. The reaction was discontinued by the addition of 0,7 ml, 10% solution of trichloracetic acid precooled to 0°. The precipitated samples were centrifuged for further 15 minutes.

Control samples were prepared for the determination of the initial level of inorganic phosphate, by adding 0.5 ml homogenate to centrifugal test tubes, containing 1,3 ml buffer substrate solution, kept at 0° C immediately thereafter, the mixture was precipitated with 0.7 ml 10% solution of trichloracetic acid.

Determination of the inorganic phosphate was made in the following fashion (10,14): to 8,4 ml distilled water, 0,2 ml supernatant liquid, 0,5 ml 10 normal sulfuric acid, 0,5 ml 5% solution of ammonium molybdate and 0,4 ml 0,2% solution of ascorbic acid were added. Within 30 minutes, the optical density of the solution was measured in photoelectric colorimeter FEK—M (with red filter), in a cuvette with thickness of the layer 1 centimeter. The inorganic phosphate concentration was calculated by means of a previously prepared calibration curve.

The activity of the enzyme was determined by the difference in the quantity of inorganic phosphate after and before incubation, i. e. in the control sample.

The difference was measured as inorganic phosphate increase in micrograms per 100 mg fresh liver tissue.

Results

In the animals of group II (castrated) and group III (castrated, treated with thyroid extract), a clearcut reduction was established of the weight of the accessory sexual organs: seminal vesicles and prostate. This weight averaged 0,7887±0,0007 g (for group I — the control animals), 0,0559±±0,0010 g (for group II) and 0,0452±0,0009 g (for group III).

The changes in the weight of the animals were also traced up. The group II animals, prior to castration had an average weight amounting to 159,0±±5,3 g, whereas 1,5 months after the castration — 246,0±7,6 g.

The rats of group III, with initial weight 143,0±5,2 g (before castration), weighed 206,6±6,5 g prior to the thyroid extract treatment which was reduced to 196,0±6,2 g following 10-day-long treatment. Similar changes in the weights of animals under the effect of castration and treatment with thyroid hormones were reported by Kochakian and co-workers (16,17).

The data concerning the changes in weight and glucose-6-phosphatase activity are given in Table 1:

Table 1

The Influence of Castration and Treatment with Thyroid Gland Extract upon the Total Weight of Animal and Glucose-6-Phosphatase Activity in Hepatic Homogenates of Male White Rats

Group	Weight in grams			Glucose-6-phosphatase activity (increase of inorganic P in mcg/100 mg fresh tissue
	Castration at the beginning of treatment		At the killing of the animals	
I — controls (14)	—	—	158,0±3,5	414,1±2,6
II — castrated (12)	159±5,3	—	246,0±7,6	359,1±8,4
III — castrated treated with thyroid extract	143,0±5,2	206,6±6,2	196,0±6,2	385,4±6,5

The data show a 13,3% decrease ($t=6,2$; $p<0,0001$) in the hepatic glucose-6-phosphatase activity after castration.

Upon treatment male rats with thyroid extract (III), the activity of the enzyme shows a statistically significant increase ($t=2,5$; $p<0,05$) without, however, reaching the level in the control animals.

Discussion

The investigation performed demonstrates an increase of the hepatic glucose-6-phosphatase activity in castrated male rats, treated with thyroid extract over a period of ten days.

The studies of *Tata* (21,22), *Knox* (15) *and Bargoni* (5) have show stimulation of the microsomal glucose-6-phosphatase in the liver under the influence of hormones of the thyroid gland. The latter effect exerted on glucose-6-phosphatase, as well as on other enzymic systems, is explained by the protein biosynthesis activation under the action of thyroid hormones. Reference is also made to increased incorporation of labelled amino acids in the proteins of hepatic sections from thyrotoxic animals. The transfer of amino acids, bound to soluble RNA into the microsomal proteins is considered as the most likely site of action of thyroid hormones (19).

Our experiments demonstrated that after castration, the activity of the hepatic glucose-6-phosphatase is decreased. It may be assumed that to a great extent, this effect is related to the influence exerted upon the thyroid gland.

The question of the influence of castration and of sexual hormones upon the thyroid function has been subject to numerous studies. Nevertheless, literature data on this problem are controversial (1, 2).

Thus, according to Tixier and assoc. (23), castration leads to intensification of the functional activity of the thyroid, manifested by increase of synthesis and thyroid hormones' release. Loskutova (2) established the significance of the animals's age in terms of the effect exerted by castration upon the thyroid gland. Castration, performed on rats after sexual maturity has been reached, inhibits the influence upon the functional activity of the thyroid, whereas in castration prior to sexual maturity, a similar effect is not observed.

It is possible that the fall in the hepatic glucose-6-phosphatase activity, established in young, sexually mature rats (weighing at castration from 140 to 160 g), might be accounted for by inhibition of the thyroid function, subsequent to castration. This reduction is compensated for, to a great extent, by the introduction of thyroid extract in the castrated animals.

Inference

1. The activity of hepatic glucose-6-phosphatase in castrated young, sexually mature male rats is reduced as compared to controls.

2. After treatment of castrated male rats with thyroid extract, the activity of the enzyme shows an increase without however, reaching the control animals' level.

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ИЗМЕНЕНИЯ В АКТИВНОСТИ ПЕЧОНОЧНОЙ ГЛЮКОЗО-6-ФОСФАТАЗЫ ПОСЛЕ КАСТРАЦИИ И ПОД ВОЗДЕЙСТВИЕМ ГОРМОНОВ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

Д. С. Калицин

РЕЗЮМЕ

Исследованы 38 беспородных молодых, полово зрелых мужских белых крыс, из которых 14 контрольных (I-ая группа); 12 кастрированных (II-ая группа) и 12 кастрированных и третированных экстрактом из щитовидной железы (III-я группа).

Кастрированные животные исследовались через полтора месяца после операции. Животным из III-ей группы вводился ежедневно через зонд per os экстракт из щитовидной железы в течение 10 дней (суточная доза 15 мг/100 г веса).

Устанавливается, что после кастрации активность печоночной глюкозо-6-фосфатазы понижается, в сравнении с контрольными животными. После третирования кастрированных мужских крыс экстрактом из щитовидной железы, активность энзима повышается, не достигая однако уровня у контрольных животных.

Допускается, че эффект кастрации связан до большой степени с воздействием на щитовидную железу.