Phosphorylases in the Prepubertal Human Testis: A Histochemical Study

In the adult human testis phosphorylase a) (active form) is present in larger quantities than the inactive form b)[1]. This aspect is related to the fact that phosphorylase a) releases the energy from the glycogen for DNA synthesis during the early stages of spermatogenesis. The present report deals with the behaviour of phosphorylases in the prepubertal human testis.

Material and methods. Biopsy speciemens were obtained from 2 cases aged 8 and 12 years. Fragments were immediately frozen on dry ice and absolute ethyl alcohol. 12 μm sections were prepared with the cryostat, mounted on glass slides and incubated according to the technique described in a previous paper[1,2]. The sections were washed in 40% ethyl alcohol, fixed in absolute ethyl alcohol and stained according to Schiff-dimedone[3]. Control slices were incubated in a medium without substrate.

Results. The sections incubated in a medium for the histochemical demonstration of phosphorylase a) showed no reaction. Addition of AMP to the incubation medium for the study of phosphorylase b) revealed a large number of granules distributed quite regularly throughout the tubules, with higher positivity in the peripheral area of the preparation (Figures 1 and 2).

Discussion. There is no phosphorylase a) in the prepubertal human testis, whereas phosphorylase b) is present in small quantities in all the tubules. This finding which differs from the behaviour in normal human adult testis, appears to confirm the correlation between glycogen, phosphorylase and nuclear synthesis of DNA. It is probable that in the prepubertal human testis, in which there is still no sign of gonadotrophin stimulation, the glycogen present in the undifferentiated Sertoli cells is not used since b) phosphorylase is not activated.

Riassunto. Nel testicolo umano di tipo prepuberale la fosforilasi attiva a) é assente, mentre é presente in discreta quantità la fosforilasi inattiva b). E' probabile pertanto che, a differenza del testicolo umano normale, la fosforilasi b) non sia attivata poiché non c'é necessità di energia fornita dalla scissione del glicogeno per la sintesi del DNA.

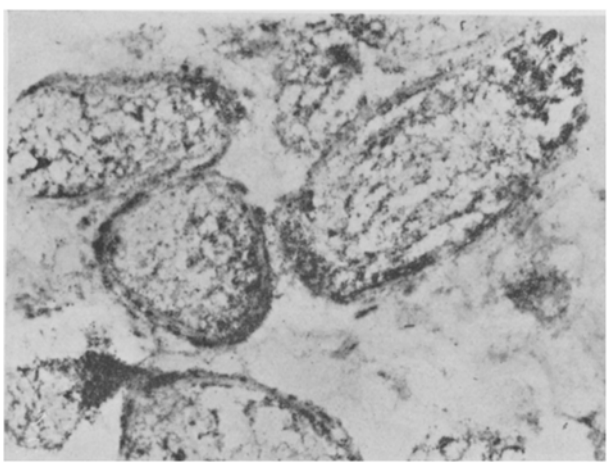

Fig. 1. The reaction product of phosphorylase b) is distributed quite regularly throughout the tubules. ×125.

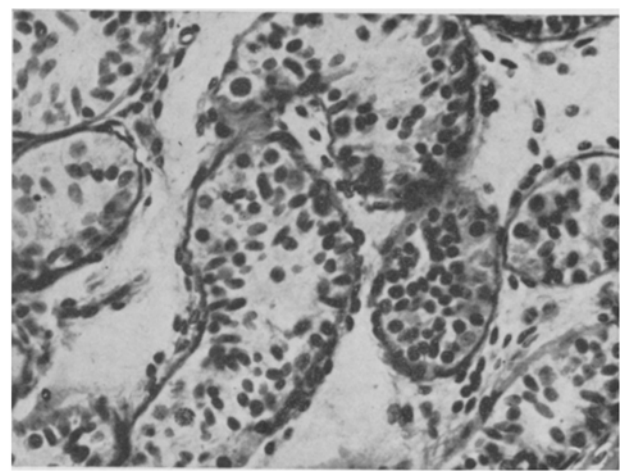

Fig. 2. Histological pattern of the same specimen (Hopa staining) ×125.

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Increase in Rabbit Hypothalamic Histidine Decarboxylase Activity after Oophorectomy and Thyroidectomy

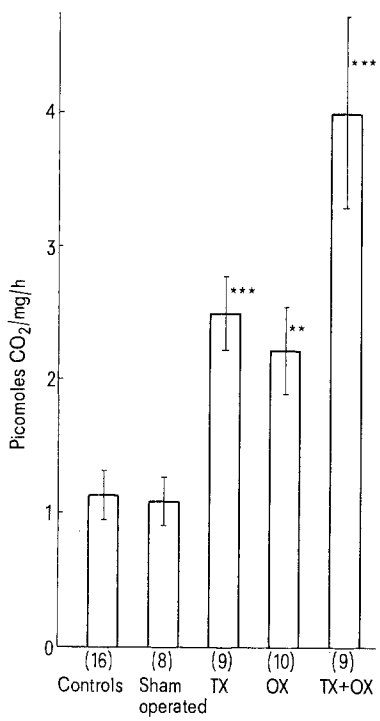
Brain histamine and histidine decarboxylase are preferentially associated with the hypothalamus[1-3]. Their cellular localization is as yet unknown. Because of the intimate functional relationship between the hypothalamus and the pituitary, it was considered worthwhile to examine whether major alterations in the endocrine functions of the adenohypophysis, induced by thyroidectomy and oophorectomy, would cause changes in hypothalamic histidine decarboxylase activity.

Female adult albino rabbits (2–3 kg body weight) were used. Hypothalamic histidine decarboxylase activity was assayed 20 days after removal of the thyroid gland, bi-

lateral removal of the ovaries, or after a combined resection of both the thyroid and the ovaries. Sham operations involved opening of the abdomen. All operations were performed under fluothane-N_2O-O_2 anesthesia. The rabbits were killed by i.v. injection of air. The hypothalamus preparation dissected out extended from the anterior margin of the optic chiasm to the interpedunculary fossa, and the horizontal cut was placed at the level of the anterior commisure. The average weight of the preparation was 250 mg. It was homogenized in 0.1 M phosphate buffer, pH 7.0, to a final concentration of 100 mg tissue (wet weight) per ml.

The homogenate was centrifuged at $10{,}000 \times g$ for 10 min at 0°C. The supernatant was used as enzyme source. Aliquots of 0.5 ml were incubated with carboxyl-labelled ^{14}C-L-histidine ($4 \times 10^{-4} M$; 1.3 mc/mmol, New England Nuclear) in the presence of pyridoxal-5-phosphate ($10^{-5} M$) and glutathione ($4 \times 10^{-4} M$). The total volume never exceeded 0.55 ml. The samples were incubated under nitrogen at 37°C for 1 h under continuous agitation. The histidine decarboxylase activity was determined by estimating the $^{14}CO_2$ released. All assays were made in duplicate and the enzyme activities were expressed as picomoles CO_2 produced per mg and hour. The results were corrected for non-enzymatic decarboxylation by incubating identical samples with 1-^{14}C-D-histidine ($4 \times 10^{-4} M$; 1.3 mc/mmol, New England Nuclear) instead of 1-^{14}C-L-histidine, or by using boiled tissue extracts incubated with 1-^{14}C-L-histidine. Usually, both types of blanks were run with each series of determinations. (For details on the procedure, see ref.[4]).

The results are summarized in the Figure. Thyroidectomy, oophorectomy, as well as the combination of the two, caused a statistically significant increase in hypothalamic histidine decarboxylase activity, whereas no alteration was seen in the sham operated controls. The increase upon thyroidectomy and oophorectomy was of similar magnitude, about 100%. Interestingly, the effect of these resections appeared to be additive, since the combined operation induced an increase in histidine decarboxylase activity that was approximately equal to the sum of the enzyme activations seen after thyroidectomy alone and oophorectomy alone (Figure). No attempt was made to identify the histidine decarboxylating enzyme and it cannot be excluded that the non-specific DOPA decarboxylase, which also occurs in the hypothalamus[5], may have contributed to the enzyme activities measured.



Histidine decarboxylase activity in rabbit hypothalamus (means± S.E.M.). TX, thyroidectomy; OX, oophorectomy. Figures within parentheses give number of determinations. Differences to controls (or sham operated), Student's t-test. ***, $p < 0.001$; **, $0.01 < p < 0.001$.

It is well established that extirpation of the endocrine target organs, the thyroid and the ovaries, will cause marked changes in the hormone secretion of the adenohypophysis, and that these changes are to a large extent mediated via the hypothalamus (see e.g. ref.[6]). During the recent years it has also been shown that hypothalamic catecholamines and 5-hydroxytryptamine probably participate in controlling pituitary functions[7,8]. Against this background the present finding raises the interesting question whether the histidine decarboxylase activity reflects the functional state of histamine-containing structures in the hypothalamus, and whether the changes seen in enzyme activity following thyroidectomy and oophorectomy reflect a functional interrelation between hypothalamic histamine-containing structures and pituitary endocrine cell systems. It is interesting that an increase in the turn-over rate of brain histamine, possibly mediated through endocrine mechanisms, was described recently by TAYLOR and SNYDER[9] in cold stressed rats. Work is in progress to examine the possibility of a neuroendocrine involvement of hypothalamic histamine and histidine decarboxylase[10].

Zusammenfassung. Die Histidin-Decarboxylase-(HD)-Aktivität im Hypothalamus des Kaninchens wurde nach Oophorectomie und Thyroidectomie gemessen. Die Resultate zeigten, dass die HD-Aktivität sich 20 Tage nach der Oophorectomie und der Thyroidectomie verdoppelt. 20 Tage nach kombinierter Oophorectomie und Thyroidectomie stieg die HD-Aktivität ungefär 4mal, verglichen mit der Aktivität des Kontrollmatrials. Sham-operierte Kontrollen zeigten keine Veränderungen der HD-Aktivität im Hypothalamus.

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[10] This work was supported by grants from Harald and Greta Jeansson's Foundation, AB Ferrosan, and Ford Foundation (grant No. 68–383).