Histochemical Demonstration of 1,4-Amylophosphorylase in the Human Testis Under Normal and Pathological Conditions

Glycogen seems to play an important role in the testis as a source of energy for spermatogenesis[1-7]. However, the synthesis and breakdown of glycogen in this organ are still unknown.

TAKEUCHI[8,9] applied his techniques for the histochemical demonstration of amylophosphorylase in the testis correlated with the metabolism of glycogen, but was unable to reveal its presence either in the seminiferous tubules of man or experimental animals.

Since it is well-known that there is a high glycogen content in the testis[1] and that the amount varies according to the spermatogenetic cycle, and pathological conditions[10], we were tempted to carry out investigations on the behaviour of amylophosphorylases (1–4 AP) in the human testis.

Material and methods. Bioptic specimens of human testis were removed by the method already described[11] from 6 normal adults and from 6 subjects with azoospermia. The fragments were frozen immediately at – 20 °C. Cryostat sections of 16 μ were incubated in a medium of glucose 1-phosphate and muscular adenilic acid according to the technique of TAKEUCHI et al.[8,9], fixed in absolute alcohol and then treated with a diluted iodine solution. By this method, polysaccharides which are resynthesized by the enzyme, are stained blue; whereas the endogenous glycogen is removed from the sections by the incubation liquid. Control slices were incubated without glucose 1-phosphate and adenilic acid. Any endogenous glycogen residue is easily recognized by reddish brown staining with iodine solution.

Results. Normal subjects. Positive results were obtained both in the tubular epithelium and in the interstitial tissue. In the tubular epithelium the enzyme varied in quantity from one tubule to another and even from one section to another in the same tubule (Figure 1). Elongated cellular elements of the fibroblastic type, concentrically placed along the tubule itself (Figure 2) together

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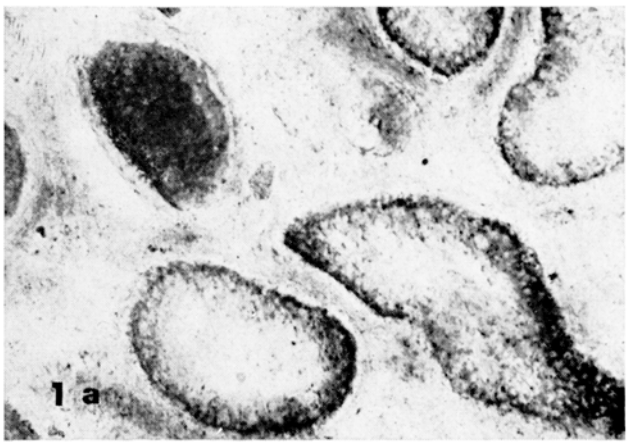


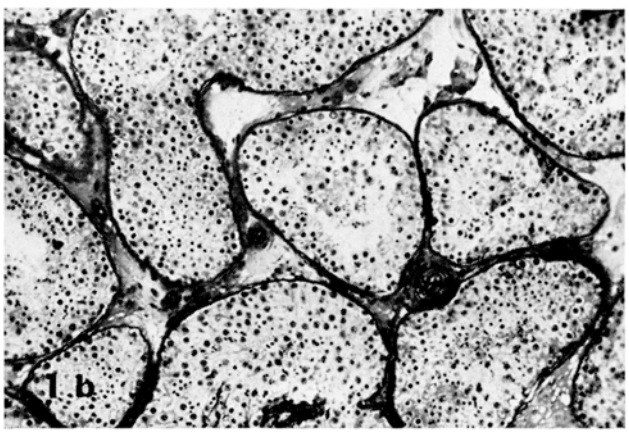
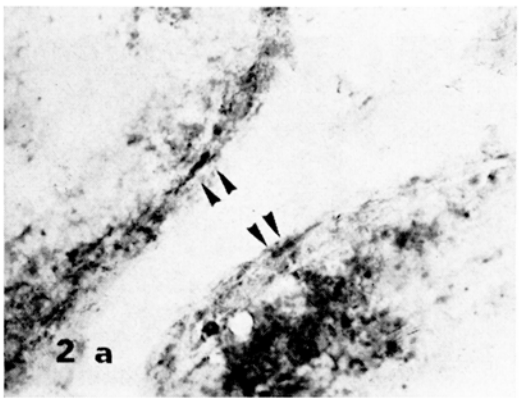


Fig. 1. Normal human testis. (a) Strong positivity of the 1–4 AP can be observed in the epithelium of seminiferous tubules. (b) Histological pattern of the same specimen (PAS). × 140.



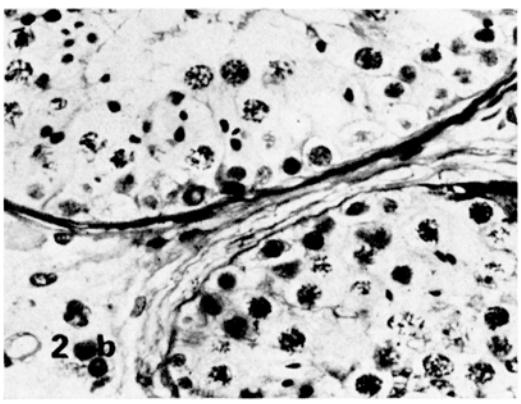


Fig. 2. Normal human testis. (a) Some positive elements can be observed in the area corresponding to the tunica fibrosa. (b) Histological pattern of the same specimen (PAS). × 560.

with some interstitial cells were also found to be positive. The small arteries were highly positive.

Patients with azoospermia. In 3 of these patients, histological examinations revealed the existence of a germinal cell arrest at the spermatid stage, whereas the 3 other patients were found to be suffering from germinal aplasia.

In the patients affected by germinal cell arrest, the reaction for 1–4 AP was markedly more positive than in normal subjects, affecting the whole tubular epithelium (Figure 3). It was difficult to determine whether the enzyme was localized only in the Sertoli cells or whether it was present also in the germinal cells. The peritubular elements of a fibroblastic type appeared to be less positive when compared with those of normal subjects.

In the cases of germinal aplasia Sertoli cells resulted negative; however a large amount of the enzyme was found in the peritubular area corresponding to the elongated elements localized on the outside of the tunica fibrosa (Figure 4).

Discussion. 1–4 AP can be demonstrated histochemically in the normal human testis. The enzyme is to be found in the tubular epithelium, some elongated peritubular elements, the arteriolar wall and some Leydig cells.

The presence of this enzyme is correlated to the breakdown and/or the synthesis of testicular glycogen and therefore also to the spermatogenetic mechanism. It is a matter of dispute whether this enzyme is part of the glycosynthetic system, whereas it appears certain that it plays a part in the glycolysis[12–16].

The increased amount of 1–4 AP observed in cases of germinal cell arrest could be correlated to the decreased utilization of glycogen in these patients. In fact glycogen is an important energetic material in the spermatogenetic cycle.

The absence of the enzyme in the tubules of patients suffering from germinal aplasia is in accordance with the absence of glycogen in the same cases. It is probable that the absence of the germinal cells coincides with the fact that Sertoli cells are congenitally incapable of synthesizing and therefore of breaking down glycogen.

Electron microscopic studies have revealed the presence of contractile cells in the peritubular connective tissue of the human, rat and guinea-pig[17–20] testis. It was therefore presumed probable that the 1–4 AP positivity in this

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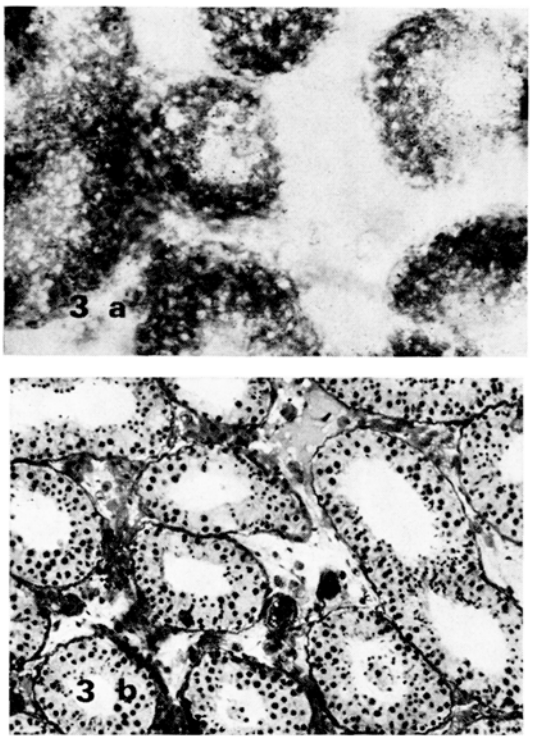

Fig. 3. Testis of a patient affected by germinal cell arrest (spermatid stage). (a) Positivity of the 1–4 AP in the tubular epithelium appears to be even stronger in normal subjects. (b) Histological pattern of the same specimen (PAS). × 140.

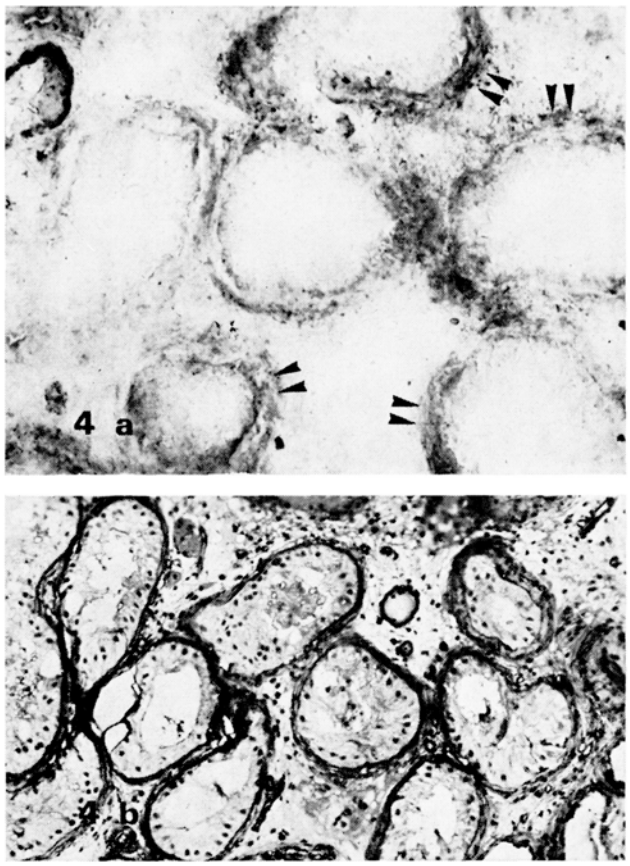

Fig. 4. Testis of a patient affected by germinal aplasia. (a) The epithelium is almost negative whereas moderate positivity of the tunica fibrosa can be observed (arrows). Note the strong positivity of the small arteriola (top left). (b) Histological pattern of the same specimen (PAS). × 140.

area was correlated to the presence of these contractile cells which have many features of the muscular elements.

The larger quantity of 1–4 AP found in the peritubular cells of germinal aplasia, as compared with normal subjects and other pathological cases, could be explained by the fact that in germinal aplasia there is no utilization of muscular glycogen which is necessary when spermatogenesis is active[21].

Riassunto. La 1–4 amilofosforilasi è presente nel testicolo umano normale ove è localizzata nell'epitelio tubulare e in alcuni elementi peritubulari. Nella aplasia germinale la reazione è intensamente positiva solo in sede peritubulare, nell'arresto maturativo solo nel tubulo. L'enzima è probabilmente in rapporto con l'energia necessaria per la spermatogenesi e con la funzione contrattile delle cellule peritubulari.

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Effect of 3-Hydroxy-3-methylglutaric Acid on Blood Lipids in Normal and Cholesterol-Fed Rats

In view of the increasing association of elevated levels of blood cholesterol:phospholipid (C/P) ratio and triglycerides content with an increased risk of manifestation of atherosclerotic heart disease, numerous hypocholesterolemic agents have been intensively investigated for their possible hypolipemic action and prevention of atherosclerotic vascular diseases[1]. Since in this laboratory 3-hydroxy-3-methylglutaric acid (HMG) has been shown to possess hypocholesterolemic property[2,3], a systematic study on the hypolipemic role of HMG in male albino rats was undertaken.

Methods. The following methods were used for biochemical determinations: total, esterified and free cholesterol by the method of Bloor et al.[4]; phospholipids as described by Zilversmit[5]; total esterified fatty acids and triglycerides by the method of Reinhold et al.[6].

For Table I, 2 groups of young male albino rats, weighing about 110 g and each containing 5 animals were caged separately. Group I was kept as control and fed on basal diet. In addition to this diet, animals in group II were given i.p. injections of 20 mg HMG/kg/day in water for 3 or 6 days. For studies made in Table II, 5 animals weighing about 100 g (cholesterol-fed group) were kept on basal diet containing 2% cholesterol, 1% cholic acid and 5% hydrogenated vegetable oils. In addition to the above fat-rich cholesterol diet, other 5 animals (cholesterol plus HMG-fed group) were given 20 mg HMG/kg/day i.p. in water. The animals were fed the diet ad libitum for 25 days. For Table III, 10 male rats weighing about 100 g were maintained on fat-rich cholesterol diet for a period of 25 days and then divided into 2 groups of 5 each. The hyperlipemic control group was given basal diet only whereas the HMG-fed group received in addition to this diet i.p. injections of 20 mg HMG/kg/day in water for

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Table I. Effect of HMG on serum lipids of normal rats (average ± S.E.)

	Basal diet		Basal diet + HMG	
	3 days	6 days	3 days	6 days
Total cholesterol, mg %	70 ± 2	100 ± 2	60 ± 4[a]	83 ± 2[b]
Ester cholesterol, mg %	50 ± 2	74 ± 2	40 ± 4[a]	60 ± 2[b]
Free cholesterol, mg %	20 ± 3	26 ± 1	20 ± 3	23 ± 1
Total esterified fatty acids, meq/l	6 ± 0.36	5.2 ± 0.28	5.1 ± 0.44[a]	4.11 ± 0.14[b]
Phospholipids, mg %	186 ± 14	153 ± 5	187 ± 19	140 ± 8
Triglycerides, meq/l	1.45 ± 0.17	1.1 ± 0.16	0.53 ± 0.44[b]	0.4 ± 0.14[c]
C/P ratio	0.389 ± 0.001	0.660 ± 0.0004	0.339 ± 0.004	0.596 ± 0.011

[a] Difference as compared to parallel basal diet control statistically significant $P < 0.05$; [b] $P < 0.001$; [c] $P < 0.01$.