Table II. Effect of medrogestone on accessory sex organs in immature male rats treated with HCG

	Ventral prostate (mg)	Seminal vesicles (mg)
Control[a]	53 ± 4	18 ± 2
Medrogestone[b]	39 ± 2[d]	19 ± 1
HCG[c]	97 ± 5[d]	44 ± 2[d]
HCG plus Medrogestone	69 ± 3[e]	26 ± 2[e]

[a] 10 albino rats/group (Charles River), final body weight, 75 ± 1 g. [b] 100 mg/kg/day given s.c. for 4 days. [c] 2 IU (Ayerst A.P.L.) given s.c. for 4 days. [d] $p < 0.01$, compared to control group. [e] $p < 0.01$, compared to HCG-group.

Table III. Effect of medrogestone on testicular testosterone synthesis in hypophysectomized rats treated with HCG

	Testosterone (ng/g)
Control[a]	3 ± 2
Medrogestone[b]	5 ± 2
HCG[c]	202 ± 54
HCG plus Medrogestone	36 ± 6[d]

[a] 8 albino rats/group (Charles River), final body weight, 177 ± 2 g, maintained on 1% dextrose + 0.9% NaCl in the drinking water. [b] 20 mg/kg/day given s.c. for 3 days. [c] 50 IU (Ayerst A.P.L.) given s.c. on 3rd day, 24 h before sacrifice. [d] $p < 0.01$, compared to HCG-group.

In vivo, in rats (final body weight, 162 ± 2 g) receiving for 7 days a daily dose of 100 mg/kg s.c., medrogestone decreased the weights of the ventral prostate (control, 56 ± 5 mg; treated, 30 ± 3 mg; $p < 0.01$) and seminal vesicles (control, 88 ± 10 mg; treated, 36 ± 2 mg; $p < 0.01$). Similarly, in immature male rats, medrogestone significantly reduced the HCG-induced increase of the ventral prostate and seminal vesicles (Table II). Subsequently, the effect of medrogestone on the testicular synthesis of testosterone was studied in hypophysectomized rats (final body weight, 174 ± 3 g) given on the second day a subcutaneous dose of 50 IU of HCG. Testosterone was isolated by thin-layer chromatography and measured fluorometrically[7]. A dose-dependent reduction in testosterone content was observed: 3% at 5 mg/kg, 49% at 20 mg/kg and 82% at 100 mg/kg of medrogestone given daily for two days ($p < 0.01$ when compared to rats treated with HCG). After 3 days of treatment with 20 mg/kg of medrogestone, the HCG-induced increase in testicular testosterone content was decreased by 84% (Table III).

The dependence of a hyperplastic prostate on functioning testicular tissue has been established earlier[8,9] and it has been reported that some progestogens capable of lowering plasma testosterone levels produced clinical improvement in benign prostatic hyperplasia[10,11]. Our experimental findings are correlative to the recent clinical finding that medrogestone reduced the severity of benign prostatic hyperplasia in man[12].

Résumé. In vitro, la medrogestone bloque la synthèse des hormones dans les gonades par inhibition de la 3β-hydroxystéroide déshydrogénase-$\Delta^{4,5}$-isomérase. In vivo, la medrogestone réduit le niveau de testostérone dans le testicules de rat.

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[7] H. GERDES and W. STAIB, Steroids *6*, 793 (1965).
[8] R. A. MOORE, Surgery *16*, 152 (1944).
[9] C. HUGGINS and R. A. STEVENS, J. Urol. *43*, 705 (1940).
[10] J. GELLER, B. FRUCHTMAN, C. MEYER and H. NEWMAN, J. clin. Endocr. Metab. *27*, 556 (1967).
[11] J. GELLER, J. Am. Geriat. Soc. *17*, 861 (1969).
[12] R. E. RANGNO, P. J. MCLEOD, J. RUEDY and R. I. OGILVIE, Clin. Pharmac. Ther. *12*, 658 (1971).
[13] We wish to thank Mrs. S. GOSSELIN, Miss N. JAGARINEC and Mrs. F. FITZSIMONS for their technical assistance and Dr. G. SCHILLING for analyses by gas-liquid chromatography.

Phosphorylase Activity in the Normal Human Testis: A Histochemical Study

The first histochemical demonstration of phosphorylase was carried out by TACHEUKI and KURIAKI[1]. The technique has since been modified[2–6] thus facilitating histochemical differentiation of active (a) and inactive (b) phosphorylases. In a previous study[7], using the original technique of TACHEUKI and KURIAKI, we demonstrated the presence of phosphorylase in the normal human testis. The aim of the present research was to study the type of phosphorylase present in normal human testis.

Material and methods. Biopsy specimens from testes of normal subjects between 18–35 years of age were employed. Specimens were immediately frozen at –70 °C on dry ice and absolute ethyl alcohol. Sections 12 μm thick were cut in the cryostat and mounted on glass slides. The incubation medium for phosphorylase (a) contained glucose-1-phosphate, acetate buffer pH 5.7, EDTA and NaF according to MEIJER[6]. For the study of (b) phosphorylase 20 mg of AMP was added to the incubation medium. Sections were incubated at 37 °C for 2 h, washed in 40% ethyl alcohol and fixed in absolute ethyl alcohol. The Schiff-dimedone reaction was used to stain the slices according to BULMER[8]. Control specimens were incubated in a medium without substrate.

Results. Slices incubated in the medium without AMP showed a strongly positive reaction with fine irregular granules in the seminiferous tubules. The distribution of

[1] T. TACHEUKI and H. KURIAKI, J. Histochem. Cytochem. *3*, 153 (1955).
[2] S. GUHA and R. WEGMANN, Annls Histochim. *4*, 103 (1959).
[3] S. GUHA and R. WEGMANN, Bull. Soc. Chim. biol. *42*, 115 (1960).
[4] H. G. GODLEWSKI, Bull. Acad. pol. Sci. Cl. IV. *8*, 441 (1960).
[5] H. G. GOLDEWSKI, J. Histochem. Cytochem. *11*, 108 (1963).
[6] A. E. F. H. MEIJER, Histochemie *12*, 244 (1968).
[7] A. FABBRINI, M. RE and G. SPERA, Experientia *24*, 789 (1968).
[8] D. BULMER, Stain Technol. *34*, 95 (1959).

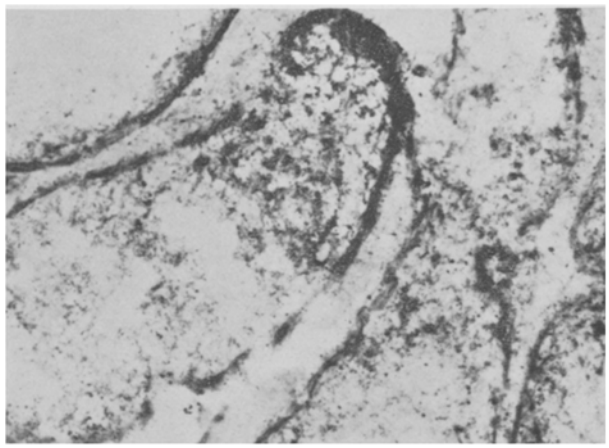

Fig. 1. Phosphorylase (a) is randomly distributed in the single tubules, and within the tubule itself, the reaction product is quite irregular. ×125.

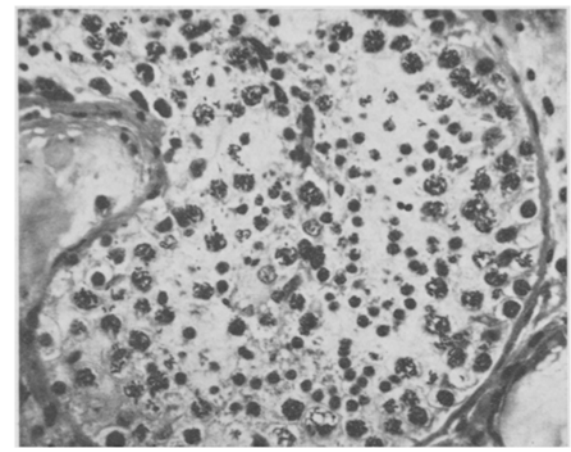

Fig. 3. Histological pattern of the same specimen (Hopa-Tonutti staining). ×350.

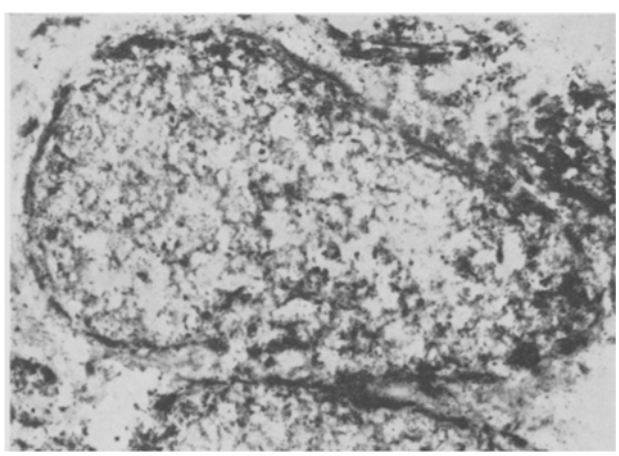

Fig. 2. The intensity of enzyme reaction did not change using a medium to which AMP had been added, but the polysaccharide granules were distributed more regularly within the same tubule. ×125.

the reaction product during the incubation period varied considerably in the individual tubules, even within a single tubule the reaction was not uniformly distributed throughout the various sections (Figure 1). It is not possible with this technique to determine the cellular localization of the enzyme since the germinal cells do not differentiate. The intensity of the reaction does not appear to be increased with the incubation medium containing AMP, however, in this case there is no difference in the staining from one tubule to the next, and within the tubules themselves the distribution is uniform (Figures 2 and 3).

Discussion. It has been shown that the histochemical demonstration of (a) and (b) phosphorylase in the normal human testis depends on the type of incubation medium used. Using this technique, the variation in the distribution of the 2 forms can be demonstrated in the seminiferous tubules. As already reported in a previous paper[7], the presence of this enzyme appears to be correlated to the metabolism of glycogen in the seminiferous tubule. Phosphorylase plays an important role in the first stage of glycogen catabolism. Moreover, it is well known that the most predominant phosphorylase in all tissues is type (b)[2,5,9]. It is possible, therefore, that in contrast to other tissues, the predominant form in the normal human testis is the (a) form which is derived from the (b) form by an enzymatic mechanism. This activation probably occurs when the glycogen is needed to supply energy necessary for nuclear synthesis and is probably triggered off by some hormonal action. According to the hypothesis of Fabbrini et al.[10], the variations in the tropinic stimulation are correlated to the amount of glycogen in the tubules, and this correlation could occur by means of the phosphorylase activation. The variation in the content of (a) and (b) phosphorylases may be related to the various stages in the cycle of the seminiferous epithelium, thus accounting for the differences seen between one tubule and another or between areas of a same section of the tubule[11]. Investigations are at present being carried out to determine whether phosphorylase (a) is predominant during the maturation of spermatogonia into spermatocytes I when new DNA is synthetized, and during the final stages of second meiosis.

Riassunto. Nel testicolo umano normale la fosforilasi (a) (attiva) é predominante nei confronti della forma (b) (inattiva), contrariamente a tutti gli altri tessuti. Tale comportamento é legato probabilmente al fatto che la fosforilasi (a), enzima del metabolismo del glicogeno, con la scissione di quest'ultimo fornisce l'energia necessaria alla sintesi degli acidi nucleici.

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[9] C. Ohanian, Histochemie. *24*, 236 (1970).
[10] A. Fabbrini, M. Re and C. Conti, J. Endocr. *43*, 499 (1969).
[11] A. Fabbrini, C. de Martino, F. Giacomelli and M. Re, *La Patologia della Gonade Maschile* (Ed. Fondazione Prof. D. Ganassini, Milano, Italy 1965).