INCREASE OF (Ca^{2+} – Mg^{2+})-ADENOSINE TRIPHOSPHATASE ACTIVITY IN RAT TESTIS UNDERGOING REGRESSION OF THE GERMINAL EPITHELIUM

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The pattern of testicular energy metabolism changes when spermatogenesis is impaired. Testes undergoing regression of the germinal epithelium show a higher QO_2 (Tepperman, Tepperman & Dick, 1949; Featherstone, Nelson, Welden, Marberger, Boccabella & Boccabella, 1955; Steinberger & Wagner, 1961) and a lower glucose utilization (Tepperman & Tepperman, 1950; Means & Hall, 1968; Free, Massie & VanDemark, 1969) than normal testes. These phenomena are also observed in immature rat testes (Tepperman *et al.*, 1949; Tepperman & Tepperman, 1950) and are believed to involve the intratubular tissue rather than the interstitium (Leiderman & Mancini, 1968).

The catabolism of ATP also varies between adult and immature testes. Although both tissues hydrolyse ATP at a very high rate (Hollinger, 1971; Setchell, Smith & Munn, 1972), mainly by means of a (Ca^{2+} – Mg^{2+})-ATPase, there are marked changes in ATPase activity throughout maturation. It has been shown to increase during the proliferation of the germinal epithelium, decrease during spermiogenesis, and attain stable lower values at the beginning of adulthood (Delhumeau-Ongay, Trejo-Bayona & Lara-Vivas, 1973). In the present study, investigations were carried out to see whether (Ca^{2+} – Mg^{2+})-ATPase would change its activity in testes undergoing regression of the germinal epithelium.

This regression was induced in normal adult rats of the Wistar strain by various means: (a) using a parapharyngeal approach, hypophysectomy was performed on adult rats weighing 180 ± 20 g; serial sections of the brains of these animals at autopsy showed no traces of pituitary tissue; (b) adult rats weighing 180 ± 20 g were made unilaterally cryptorchid by placing the left testis in the abdomen and suturing the inguinal canal; (c) adult intact rats, weighing 225 ± 25 g were injected daily for periods of up to 17 days with either 50 i.u. HCG or 250 μg fluoroacetamide in 0.25 ml saline, controls receiving the same volume of saline alone; rats were killed 24 hr after the last injection.

For histological observations, the tissue was fixed in either formol or Bouin's solution and stained with haematoxylin and eosin.

Within each treatment, and for each experimental point shown in Text-fig. 1 and Table 1, the testes of three or four rats were pooled and homogenized, and the rate of ATP hydrolysis was measured as previously described (Delhumeau-Ongay *et al.*, 1973). Reported values are the means of twelve phosphate determinations with their corresponding standard deviations. Values of statistical analysis were determined by Student's *t* test. The enzymatic activity responsible for ATP hydrolysis was characterized as a $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$ following the same criteria applied by Delhumeau-Ongay *et al.* (1973).

TEXT-FIG. 1. $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$ activity in rat testis undergoing regression of the germinal epithelium. The incubation mixtures contained: 1 mM- Mg^{2+} , 58 mM- Na^+ , 5 mM- K^+ , 10 mM- CN^- , 0.1 mM-EDTA, 157 mM- Cl^- , 2 mM-ATP, 92 mM-tris (hydroxymethylamino methane) buffer (pH 7.5) and 0.1 ml of a 10% total aqueous homogenate of rat testis (0.6 to 1.2 mg protein) per ml. Δ , Control; $\bullet$, hypophysectomy; $\circ$, cryptorchidism.

The effect of hypophysectomy on the testicular $(\text{Ca}^{2+}\text{-Mg}^{2+})\text{-ATPase}$ activity is shown in Text-fig. 1. The activity started to rise 1 week after hypophysectomy, reached a plateau at the end of the 2nd week, and remained stable until the 4th week of the experiment ($P < 0.001$). Regression of the germinal epithelium started to become apparent histologically on the 4th day after surgery. Maturing spermatids and spermatozoa were still present, but their numbers were reduced. Their reduction became more evident after 1 week and by the 14th day they were absent.

Rats were made unilaterally cryptorchid on Day 0 (Text-fig. 1). The enzymatic activity of the ectopic testes was compared with that of the contralateral scrotal ones (control testes in Text-fig. 1) at different times after surgery. Little variation was observed in the enzymatic activity of the eutopic testes throughout the 6 weeks of the experiment. In the ectopic ones, a significant ($P < 0.001$) continuous increase was observed from the 2nd to the 5th week, at

which time activity began to stabilize. When data were expressed in terms of the protein content of testicular homogenates, the enzymatic activity showed almost identical patterns to those shown in Text-fig. 1. A similar pattern was also obtained when homogenates from scrotal testes were incubated at 33° C.

High doses of HCG are known to block the maturation of the germinal cells (Morabito & Strazzulla, 1963). After 1 week of a chronic course of treatment with daily intraperitoneal injections of 50 i.u. HCG, $(Ca^{2+}-Mg^{2+})$ -ATPase activity was determined. The experiment was repeated four times. Control groups showed values ranging from 31.54 ± 0.76 to 38.04 ± 2.98 μ mol inorganic phosphate (Pi)/min/g wet tissue, whereas treated animals significantly increased their enzymatic activities ($P < 0.001$) to values ranging from 54.62 ± 4.34 to 71.0 ± 7.06 μ mol Pi/min/g wet tissue.

Table 1. Effect of fluoroacetamide administration on $(Ca^{2+}-Mg^{2+})$ -ATPase activity in rat testis

Days of treatment	Enzymatic activity* (μ mol inorganic phosphate produced/min/g wet tissue)
0	64.33 ± 3.78
4	64.28 ± 3.01
6	57.21 ± 4.03
8	60.10 ± 2.15
13	73.56 ± 4.89
17	98.53 ± 3.89

Fluoroacetamide was given in consecutive daily subcutaneous injections of 250 μ g in 0.25 ml saline.

* The incubation mixture contained: 5 mM- Mg^{2+} , 156 mM- Cl^- , 2 mM-ATP, 146 mM-tris (hydroxymethylamino methane) buffer (pH 7.5) and 0.1 ml of a 10% total aqueous homogenate of rat testis (0.6 to 1.2 mg protein) per ml.

Fluoroacetamide is known to inhibit spermatogenesis specifically at the spermatid level (Steinberger & Sud, 1970). Data on the effect of the chronic administration of fluoroacetamide on testicular $(Ca^{2+}-Mg^{2+})$ -ATPase are shown in Table 1. Numerical values were higher than those of the previously mentioned experiments because a 5 mM- instead of 1 mM-magnesium concentration was used (see Delhumeau-Ongay *et al.*, 1973). During the 2nd week of treatment, the enzymatic activity showed a significant increase ($P < 0.001$). Histological observations of the testes at this time indicated that spermatids and many spermatocytes had disappeared. The interstitium showed no detectable variations.

A significant increase ($P < 0.001$) in $(Ca^{2+}-Mg^{2+})$ -ATPase activity was found to occur in rat testis when regression of the germinal epithelium was induced by any one of the means used. Each one of them is known to affect the interstitial tissue in a different manner. Thus, hypophysectomy induces both regression of the germinal epithelium (Clermont & Morgentaler, 1955) and atrophy of the interstitium (Hooker, 1970). Cryptorchidism has a major effect upon the development of the germinal cells (Hooker, 1970). High doses of

HCG appear principally to block the maturation of the germinal epithelium (Morabito & Strazzulla, 1963) and fluoroacetamide was reported to have a specific effect on spermiogenesis (Steinberger & Sud, 1970). Our own histological observations basically agreed with these previous findings. The fact that $(\text{Ca}^{2+}-\text{Mg}^{2+})$ -ATPase activity always increased when the germinal epithelium was undergoing regression, in the presence or absence of a functional interstitial tissue, points to the intratubular cells as the main site of the enzymatic activity.

The different methods used to induce regression of the germinal epithelium raised the enzymatic activity to different levels. Chronologically, this increase became generally significant when the bulk of spermatids had disappeared and became even more marked with the partial disappearance of the spermatocytes.

During the regression of the germinal epithelium, the germ cells remaining in the seminiferous tubules pursue their evolution at their normal rate until they encounter unfavourable conditions (hormonal, thermal, or other) which prevent their further differentiation. They then degenerate and are eliminated from the system (Clermont & Harvey, 1965). The increase in the enzymatic activity could therefore be related to one or more of the following phases: (a) the normal proliferation and differentiation of the more primitive type of germ cells, since it has been suggested that this process has a very high energy requirement (Free, 1970; Hollinger, 1971); (b) the disposal of degenerating cells, which are phagocytosed by the Sertoli cells (Clermont & Morgentaler, 1955; Clegg, 1963), since phagocytosis is also an energy-requiring process (Selvaraj & Sbarra, 1966); (c) the disappearance of spermatids, since if these cells were low in ATPase activity, they would produce an apparent increase in the enzymatic activity.

None of the above interpretations can be definitively accepted at the present time. It is worth noting, however, that throughout both maturation and regression of the germinal epithelium, rat testis shows comparable modifications in its pattern of energy metabolism in terms of QO_2 (Tepperman *et al.*, 1949; Featherstone *et al.*, 1955; Steinberger & Wagner, 1961; Leiderman & Mancini, 1968), glucose utilization (Tepperman & Tepperman, 1950; Means & Hall, 1968; Free *et al.*, 1969), UDPase activity (Xuma & Turkington, 1972) and $(\text{Ca}^{2+}-\text{Mg}^{2+})$ -ATPase activity (Delhumeau-Ongay *et al.*, 1973).

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