CHANGES OF (Ca^{2+} – Mg^{2+})-ADENOSINE-TRIPHOSPHATASE ACTIVITY IN RAT TESTIS THROUGHOUT MATURATION

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Marked changes in the pattern of energy metabolism occur in rat testis throughout maturation (Free, 1970). The mature testis, as compared with the immature one, has a lower QO_2 (Tepperman, Tepperman & Dick, 1949; Leiderman & Mancini, 1968) and a different rate of aerobic and anaerobic glycolysis (Tepperman & Tepperman, 1950; Leiderman & Mancini, 1968). Adult testis utilizes glucose to a greater extent (Tepperman *et al.*, 1949) and ATP production is primarily dependent upon glucose metabolism in the mature but not in the prepubertal testis (Means & Hall, 1968). On the other hand, both adult (Tice & Barnett, 1963; Setchell, Smith & Munn, 1972) and immature (Hollinger, 1971) rat testes have been shown actively to hydrolyse ATP. However, the relationship of the rate of ATP catabolism to maturation is not clear. In the present communication, the pattern of the enzymatic hydrolysis of ATP by rat testis as a function of age is reported.

Fifteen groups by age, each consisting of three to eight albino rats of the Wistar strain, were used in the study. The rats were killed at the same hour of the morning by cervical dislocation followed by decapitation and exsanguination. Testes were immediately removed and rinsed in ice-cold twice distilled water. After removal of the tunica albuginea, testes were pooled to prepare a 10% w/v aqueous homogenate with a Potter–Elvehjem homogenizer provided with a loose Teflon pestle. The homogenate was then filtered through glass wool.

The method essentially described by Bonting, Simon & Hawkins (1961) was used to measure ATP hydrolysis. Bonting's medium A was used for the incubation, which was carried out at 37° C (Text-fig. 1). A 10-min pre-incubation without ATP was routinely performed in order to hydrolyse endogenous substrates. The reaction was started by the addition of ATP. All the incubations were run in quadruplicate and 0.5-ml aliquots were withdrawn at 30 sec, 1 and 2 min after the addition of ATP. The reaction was stopped by 2.5 ml ice-cold 6% perchloric acid. To separate zero-time aliquots, the substrate was added after perchloric acid. All the aliquots were allowed to stand for 30 min at 0° C and were centrifuged. Inorganic phosphate (Pi) was determined in the supernatant by the method of Bonting *et al.* (1961). Reported values are the

means of twelve phosphate determinations with their corresponding standard deviations. The protein content of the homogenates was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

The pattern of the enzymatic hydrolysis of ATP as a function of age is shown in Text-fig. 1. Twenty-four hours after birth, the enzymatic activity was already present. From the first week of life, it continuously increased until maximal values were attained at 21 to 24 days of age. These 3 weeks correspond to the appearance and differentiation of spermatogonia and spermatocytes, according to Clermont & Perey (1957), and to the observations of Sosa, Altamirano, Hernández & Rosado (1972) on the strain of rats used in the present work. After the 24th day of life, at ages corresponding to the appearance and further differentiation of spermatids, there was a statistically significant ($P < 0.001$) negative slope in the enzymatic activity. Stable lower values were

TEXT-FIG. 1. Enzymatic hydrolysis of ATP by rat testis as a function of age. The incubation mixtures contained: 1 mM-Mg²⁺, 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 total homogenate (0.6 to 1.2 mg protein) per ml.

reached at the 45th day of age when spermatozoa started to appear. These levels were maintained thereafter throughout adulthood.

Since Korman (1967) has shown that the testis/body temperature difference changes with age, it seemed important to investigate the effect of the incubation temperature on the enzymatic activity at various ages. Testis homogenates from groups of rats aged 20, 30, 35 and 60 days were incubated at 0, 20, 30, 33, 35, 37, 40 and 50° C (experimental conditions as described in Text-fig. 1). Within each age group, no significant differences were observed in the enzymatic activities over the temperature range from 33 to 40° C. Thus, the incubation temperature of 37° C, non-physiological for testes from certain age groups, did not affect the results shown in Text-fig. 1.

In order to determine whether the enzymatic hydrolysis of ATP was specific or not and whether the observed chronological changes of the enzymatic activity

were quantitative or qualitative in nature, some preliminary cation-dependence and substrate-specificity studies were undertaken. Testes from both adult and immature rats were used in these studies.

(a) Cation dependence. Using 2 mM-ATP as a substrate, Na^+ , K^+ , EDTA and cyanide could be eliminated from the original Bonting's medium without any detectable change in the activity. An absolute requirement of either Mg^{2+} or Ca^{2+} was observed, 5 mM being the optimal concentration for both divalent cations. When increasing amounts of Ca^{2+} were added to the incubation mixture containing 5 mM- Mg^{2+} , the activity was inhibited (40% at equimolar concentrations, 50% at a 2:1 Ca^{2+}/Mg^{2+} ratio). This effect was similar to the one reported in placental ATPase (Shami & Radde, 1972).

(b) Substrate specificity. Text-figure 2(a) shows that, in the presence of magnesium ions, adult rat testis homogenates were able to hydrolyse ATP, GTP, CTP and UTP at similar rates. Hydrolysis of ADP took place at a lower

TEXT-FIG. 2. Enzymatic hydrolysis of various substrates by immature and adult rat testis. The incubation mixtures contained: either 5 mM- Mg^{2+} (a) or 5 mM- Ca^{2+} (b), 156 mM- Cl^- , 2 mM-substrate, 146 mM-tris (hydroxymethylamino methane) buffer (pH 7.5) and 0.1 ml total homogenate per ml.

rate. No hydrolytic activity was observed towards AMP, 3', 5' cyclic AMP or β -glycerophosphate, but a very high inorganic pyrophosphatase activity was encountered. This enzyme became saturated at 0.5 mM-substrate concentration and showed an absolute requirement for Mg^{2+} in an equimolar pyrophosphate/magnesium ratio. It was completely inhibited by 2 mM-NaF and had no activity whatsoever when calcium was used instead of magnesium. These properties shown by rat testis pyrophosphatase were markedly different from those described for inorganic pyrophosphatase from bull semen (Heppel & Hilmo, 1951).

To determine whether ATP was hydrolysed by a phosphatase or by an organic pyrophosphatase, it was important to avoid inorganic pyrophosphatase activity. Determinations were therefore performed in the presence of 5 mM- Ca^{2+} instead of Mg^{2+} , since the pattern of nucleotide hydrolysis by adult

testis homogenates was similar with either cation (Text-fig. 2a and b). The inclusion of inorganic pyrophosphatase activity could have also been avoided by using fluoride since this did not appreciably inhibit ATP hydrolysis.

Text-figure 2(b) shows that the pattern of nucleotide hydrolysis was qualitatively similar in the adult and the immature testis, suggesting that the observed activity is mainly one of a $(\text{Ca}^{2+}-\text{Mg}^{2+})$ -dependent ATPase. It was, however, twice as great in the 6-day-old rat testis and over three times as great in the 20-day-old rat as in the adult testis.

Nucleoside diphosphatase showed only a slight increase in activity in the 5-day-old rat and 5'-nucleotidase was only apparent in the 20-day-old rat.

A high activity of ATPase that could be stimulated by either Ca^{2+} or Mg^{2+} but not by Na^{+} or K^{+} was found to occur in rat testis at all ages from birth to maturity. The activity, however, showed marked quantitative changes as a function of the age of the animal. During the first 3 weeks of life, a continuous increase in activity was encountered. This observation is consistent with a high energy requirement for the very active proliferation of the germinal epithelium, as previously suggested (Free, 1970; Hollinger, 1971). Throughout the ages corresponding to the appearance and differentiation of spermatids, ATPase activity continuously decreased until the 7th week of life. A cellular dilution effect could be evoked to interpret this finding. It may also suggest that during spermiogenesis, the testis progressively changes its energy metabolism until a different and stable pattern is attained in early maturity. In fact, previous studies have shown marked differences between the metabolic energy pattern of adult and immature testis in terms of QO_2 (Tepperman *et al.*, 1949; Leiderman & Mancini, 1968), rate of glycolysis (Tepperman & Tepperman, 1950; Leiderman & Mancini, 1968), glucose utilization (Tepperman *et al.*, 1949), ATP production (Means & Hall, 1968) and now ATP hydrolysis.

It has been suggested that most of the differences observed in energy metabolism before and after maturation involve the seminiferous tubules rather than the interstitium (Leiderman & Mancini, 1968; Leiderman, 1969; Free, 1970). Since no effort was made in this study to separate these tissues, any suggestion at this stage concerning the cellular localization of the $(\text{Ca}^{2+}-\text{Mg}^{2+})$ -ATPase would be speculative. Further experimental work is now in progress to clarify this point.

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