GLUTAMATE IN POST-NATAL RAT TESTIS

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Summary. Glutamate concentration and total content were determined in the post-natal rat testis by an enzymatic method. The concentration of glutamate in the rat testis was found to be higher than had been reported in ram testicular fluid, but about the same as in human seminal plasma. A significant increase in concentration occurred between 15 and 20 days of age. It coincided with the onset of fluid secretion by the seminiferous tubules and spermatogenesis.

According to several authors (Mann, 1964; Setchell, 1967), there is a high concentration of free glutamic acid in the mammalian testis and seminal plasma. That considerable amounts of certain amino acids are secreted by the ram testis into the testicular fluid was reported by Setchell, Hinks, Voglmayr & Scott (1967). These authors collected testicular fluid by cannulating the ductuli efferentes of conscious rams and compared its composition with that of testicular lymph, blood plasma, epididymal plasma and seminal plasma. They found that the concentration of glutamic acid in testicular fluid is about ten times higher than that in testicular lymph or in blood from the internal spermatic vein.

In the present work, the level of glutamate in the developing post-natal rat testis was followed up in order to relate it to the general morphological and functional development of the testis.

Forty, male, Sprague-Dawley rats were used, four animals in each group. The animals in these groups were 0, 5, 10, 15, 20, 25, 30, 40, 50 and 60 days old, respectively. They were anaesthetized by an intraperitoneal injection of 50 mg/kg pentobarbitone sodium (Nembutal, Abbot). Both testes were rapidly extirpated 10 min after the barbiturate injection, immediately frozen in liquid nitrogen, and stored at −75 °C. Samples of 5 to 10 mg were cut from these testes at −20 °C, weighed, and extracted as follows (Nelson, Lowry & Passonneau, 1966). The tissue was crushed with nylon rods in a small glass tube after addition of 20 μl of methanol–0.05 n-HCl, at −20 °C in a 50% ethanol dry-ice bath. The tube was transferred to an ice bath at 0 °C and 200 μl of 0.3 n-perchloric acid with 1 mm EDTA was added. After mixing and crushing, the contents were centrifuged at 8000 rev/min for 20 min at +2 °C. The supernatant fluid (193 μl) was neutralized with 2.5 M-KHCO₃ (20 μl), and stored at

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−75° C. Glutamate was measured fluorometrically using 1 ml of the reagent containing 100 mm-tris-HCl buffer (pH 8.4), 0.1 mm-ADP, 0.3 mm-NAD⁺, 0.02% bovine serum albumin, and 50 µg glutamate dehydrogenase (in glycerol) (principally according to Lowry, Passonneau, Hasselberger & Schulz, 1964; Folbergrova, personal communication).

The concentrations and total contents of glutamate in the post-natal rat testis are presented in Text-fig. 1. From 0 to 15 days of age, the concentration was at a relatively stable level (mean 2.22 m-moles/kg). Between 15 and 20 days, it increased significantly (P<0.01) and at 25 days, the increase became highly significant (P<0.001) when compared with the 15-day level. The concentration remained at this high level up to the adult age (mean 4.02 m-moles/kg).

The glutamate concentration in whole rat testis as reported in the present study (4.02 m-moles/kg) is higher than that reported by Setchell et al. (1967) in ram testicular fluid (1.82 µ-moles/ml), but lower than in bull testis (1000 µg/g = 6.80 µ-moles/ml) (Melampy, Cavazos & Duncan, 1955). In ejaculated human seminal plasma, the glutamic acid concentration (179.7 mg/100 ml = 12.2 µ-moles/ml), as determined chromatographically by Krampitz & Doepfner (1962), is twice as high as that reported in ram seminal plasma (5.16 µ-moles/ml) by Setchell et al. (1967).

Age differences in the testicular glutamate concentrations are probably related to differences in the spermatogenic activity per unit of tissue. The most likely rôle of glutamate is its involvement, together with certain other amino acids of the testicular fluid such as aspartate and glycine, in the synthesis of purine and pyrimidine bases, thus creating favourable conditions for nucleic acid synthesis within the seminiferous tubules. A beneficial effect on the sperm maturation process in the epididymis, like that exerted by some other amino
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acids (Mann, 1964), has also been suggested by Setchell et al. (1967). Glutamate does not penetrate the blood–testis barrier, as Setchell et al. (1967), and Setchell Voglmayr & Waites (1969) demonstrated by infusing [U-14C]glutamate and measuring its activity in the tubular fluid in the ram. However, after infusion of [U-14C]glucose, they found a high radioactivity in the glutamate of tubular fluid. This suggests that glutamate is synthesized in the mature seminiferous tubule.

The significant increase in the glutamate concentration of rat testis between the ages of 15 and 20 days occurs just after the maturation of the boundary tissue (Leeson & Leeson, 1963; Niemi & Kormano, 1965). At the same age, there is a rapid progress in spermatogenesis, the second layer of spermatocytes is formed (Kormano, 1967a), and the histochemical and permeability characteristics (Kormano, 1967b) attain the mature pattern. The simultaneous development of glutamic acid synthesis and of active nucleic acid reproduction in the division of secondary spermatocytes accords well with the suggestion that glutamic acid is concerned in the synthesis of purine and pyrimidine bases required for nucleic acid synthesis (Setchell et al., 1967). The tubular lumen forms a considerable proportion (about 17%) of the contents of the rat testis, and this luminal fluid space increases during puberty (Kormano and Suvanto, unpublished observations). Therefore, the increase in the concentration of glutamate may, in part, be due to the increase of tubular fluid. Since, however, the increase of glutamate concentration of the whole testis observed in the present work coincides roughly with the beginning of fluid secretion (Setchell, 1968), we cannot decide as yet whether it is due only to the beginning of the process of fluid secretion or to the activation of separate synthetic mechanisms.

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REFERENCES

