ESTABLISHMENT OF FUNCTION OF THE BLOOD-TESTIS BARRIER IN GUINEA PIGS AND RATS DURING POSTNATAL DEVELOPMENT

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In guinea-pigs and rats rivanol, when injected parenterally, penetrates into the convoluted tubules of the testis during the first weeks after birth, but not in sexually mature animals. Stabilization of the function of the blood-testis barrier relative to rivanol is complete by the end of meiosis and beginning of spermatogenesis. Endogenous globulins are not detected outside the tunica propria of the convoluted tubules either in newborn or in sexually mature guinea-pigs and rats.

Recent work has shown the existence of a blood-testis barrier (BTB) which in its degree of resistance, selective permeability, and structural complexity closely resembles the blood-eye, blood-brain, and peripheral nervous barriers [1-5, 12, 16, 19].

Analysis of the literature shows that the main functions of the BTB are to isolate the autoantigenic cells of the spermatogenic epithelium in the late stages of development from the action of immunological factors, to protect cells undergoing meiotic division from mutagens of various types, and to maintain hormonal homeostasis.

Since spermatogenesis in the testis is established by the time that the animal reaches sexual maturity and since by this time the character of interaction between the testis and pituitary attains the characteristically adult pattern, it was decided to investigate the changes in permeability of the BTB taking place during postnatal development.

Only one investigation could be found in the literature in which the permeability of the BTB was studied relative to neutral acriflavine in rats in the early stages of postnatal development [13].

The object of the present investigation was to study the permeability of the BTB in guinea-pigs and rats relative to rivanol and to endogenous globulins in the course of postnatal development.

EXPERIMENTAL METHOD

The permeability of the BTB to rivanol was investigated in 30 noninbred male rats aged 4, 10, 13, 14, 15, 20, 21, 22, 23, and 24 days and 32 male guinea-pigs aged 4, 6, 8, 10, 11, 14, 17, 20, 22, 24, 30, 34, 47, and 60 days. The day of the animals' birth was taken as zero. An intravenous or intraperitoneal injection of 0.5-1% rivanol solution in physiological saline was given in a dose of 25 mg/kg body weight to the animals 1 h before sacrifice. Rivanol was detected in the testes in frozen sections through the organ 5-7 μ in thickness under the ML-2 luminescence microscope. The distribution of endogenous globulins in the testes was determined in frozen and dewaxed sections by a direct immunofluorescence method [7] in 34 rats aged 0, 1, 12, 14, 15, 19, 21, 23, 26, and 28 days and in nine guinea-pigs aged 0, 7, 10, 22, and 28 days. Rabbit sera against guinea-pig or rat globulins, labeled with fluorescein isothiocyanate, produced in the N. F. Gamaleya

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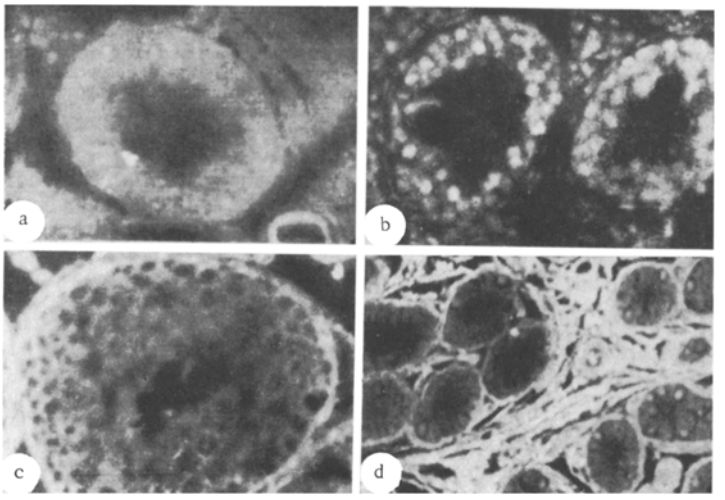

Fig. 1. Fluorescence in testes of animals studied: a, b) 1 h after injection of rivanol; c, d) endogenous globulins detected by a direct immunofluorescence method. a) testis of sexually mature rat, objective 20×, homal 3×; b) testis of rat aged 15 days, objective 40×, homal 3×; c) testis of sexually mature guinea-pig, objective 20×, homal 5×; d) testis of guinea-pig aged 10 days, objective 20×, homal 3×.

Institute of Epidemiology and Microbiology were used. All the animals were killed by decapitation. The specificity of fluorescence was verified by means of the criteria suggested by Coons and Kaplan [7]. The specimens were examined under the ML-2 luminescence microscope.

For histological study the testis was fixed in Carnoy's fluid and paraffin sections 5μ in thickness were stained with hematoxylin and eosin. The localization of the endogenous globulins and of rivanol in the testis and also in other organs of sexually mature guinea-pigs and rats was determined in control experiments.

EXPERIMENTAL RESULTS AND DISCUSSION

In the sexually mature guinea-pigs and rats rivanol did not penetrate through the wall of the blood vessels in the interstitial tissues of the testis (Fig. 1a) or into the brain tissue, whereas an intense green fluorescence of the dye was found in the cell nuclei of the parenchyma of the liver, spleen, kidney, and lung and the epithelium of the epididymis.

In young rats aged from 4 to 10 days a bright green fluorescence of rivanol was seen in the cell nuclei of the interstitial tissue, the epithelium of the convoluted tubules, and cells of the tunica albuginea testis. Microscopic examination of sections through the testes of rats aged 10 days showed that besides Sertoli cells the epithelium of the convoluted tubules also contained type A, intermediate, and type B spermatogonia.

Starting from the age of 17 days a gradual decrease in brightness of fluorescence was observed in all cells of the testis in rats (Fig. 1b). In the animals aged 20 days fluorescence of sections through the testis was weak but uniform. On the 21st-22nd day, fluorescence was completely absent in most of the area of the section, and weak fluorescence of the cell nuclei still persisted in the interstitial tissues and tubular epithelium only in regions next to the tunica albuginea. On the 23rd day of postnatal life rivanol fluorescence could no longer be seen in the rats' testes. This time coincided with the completion of meiosis and the beginning of spermatogenesis (Fig. 2). The distribution of rivanol in the testes of the guinea-pigs during the first days after birth was similar to that found in rats. In the testes of the animals aged 6-8 days, fluorescence was observed in the cell nuclei of the interstitial tissue, the tubular epithelium, and cells of the tunica albuginea. In guinea-pigs, however, the fluorescence was less bright than in rats. At this time Sertoli cells and type A intermediate, and type B spermatogonia were visible in the tubules of the testes. In the guinea-pigs (and in this respect they differed from rats) a marked decrease in the brightness of fluorescence of all the cells of the testis and differences in the intensity of fluorescence in the tubules

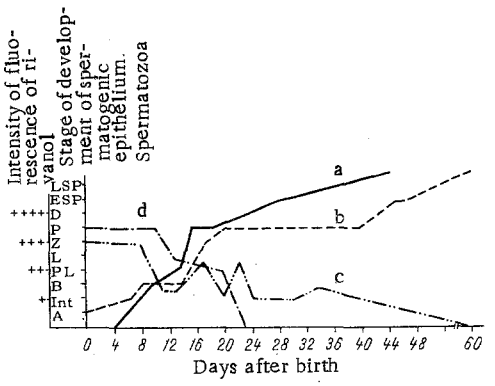

Fig. 2. Permeability of BTB to rivanol and establishment of spermatogenesis in guinea-pigs and rats in the early stages of postnatal development: stages of spermatogenesis (a) in rat and (b) in guinea-pig; fluorescence of rivanol in testes of (c) rat and (d) guinea-pig. A) Type A spermatogonia, Int) spermatogonia of intermediate type, B) type B spermatogonia, PL) pre-leptotene spermatocytes of the first order, L) leptotene spermatocytes, Z) zygotene spermatocytes, P) pachytene spermatocytes, D) diplotene spermatocytes, ESP) early spermatids, LSP) late spermatids.

in the same section through the testis were observed as early as on the 10th-11th day after birth. By this time spermatogenesis had proceeded to type B spermatogonia. Fluorescence remained similar in character in the testes of the guinea-pigs up to the age of 20 days, when spermatocytes appeared in the tubules in the pachytene stage. In the period from the 20th to the 47th day of life weak but uniform fluorescence of the testicular tissue was observed. By the 47th day, spermatids at different stages of development could be seen in individual convoluted tubules, but in most tubules spermatogenesis proceeded only as far as spermatocytes in the pachytene stage. In animals aged 2 months no rivanol could be found in the interstitial tissues and tubules of the testis, while spermatozoa were visible in the lumen of many convoluted tubules.

Endogeneous globulins did not penetrate into the convoluted tubules of the testes of the sexually mature guinea-pigs and rats but were localized in the interstitial tissue (Fig. 1c). Meanwhile, bright specific fluorescence of the tissue components of the kidney, spleen and lung was observed, whereas the cells of the brain tissue remained dark. In newborn guinea-pigs and rats and in the later stages of postnatal development, the tunica propria of the convulted tubules also was impermeable to endogenous globulins (Fig. 1d). No reference could be found in the literature to the study of the permeability of the BTB to endogenous globulins in sexually immature animals, whereas the reports of other investigations [4, 11, 12, 16] state that the tunica propria of the convoluted tubules is impermeable to endogenous globulins in sexually mature guinea-pigs and rats.

The results of the present experiments agree in their general form with those obtained by Kormano [13]. His investigations of the permeability of the BTB in sexually immature rats showed that neutral acriflavine, if injected subcutaneously, penetrates into the testis of newborn rats and of young rats aged 5 and 10-15 days, but is not found in the tubules of the testis in animals aged 20-25 days.

The results of the present investigation also show correlation between the degree of attainment of spermatogenesis in rats and guinea-pigs and the final stabilization of function of the BTB. The impression is obtained that the completion of meiosis (by the 47th day after birth in guinea-pigs and the 23rd day in rats) coincides in time with the completion of stabilization of BTB function (Fig. 2).

It must be emphasized, in conclusion, that the results of morphological investigations of the BTB are in agreement with the results of this investigation.

The BTB is known to consist of a combination of structures: the blood vessel wall, the tunica propria of the convoluted tubules, the Sertoli cells, the tunica albuginea testis, and the interstitial tissue [3]. Final completion of the formation of the morphological structure of the barrier is known to take place in animals and man during postnatal development. The myoid cells of the tunica propria of the convoluted tubules of the testis, which play an important role in barrier function [9], for instance, acquire the characteristic adult features in rats on the 22nd day [14] and in mice on the 19th day after birth [17]. Differentiation of the Sertoli cells is complete in mice at the 4th-5th week after birth [10] and in man at puberty [15, 20], or it coincides with the beginning of maturation of the spermatids in rats [6, 18].

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