It will be clear from Table 2 that in rats of the 4th to 6th generations there was a significant increase with age in the frequency of development of manifest and latent diabetes. During a study of dependence of the frequency of development of diabetes mellitus (both latent and manifest together) in the offspring of the rats with alloxan diabetes on the presence or absence of hypoglycemia in them at the age of 1-3 months showed that in a group of 100 rats of the 1st to the 3rd generations which had hypoglycemia at the age of 1-3 months, the frequency of development of latent and manifest diabetes mellitus at the age of 4-6 months was 26.0 ± 4.4%, whereas in the group of 124 animals which did not have hypoglycemia it was 8.8 ± 2.6% ($p < 0.001$). In a group of 47 rats of the 4th-6th generation which had hypoglycemia at the age of 1-3 months, the frequency of development of diabetes was 59.5 ± 7.1%, whereas in a group of 77 rats which did not have hypoglycemia it was 18.2 ± 4.4% ($p < 0.001$). The type of GTT of the mother rat of the previous generation had a significant influence on the frequency of disturbance of tolerance of the diabetic type in the offspring (Table 3).

Thus an increase in the frequency of development of diabetes was found in consecutively later generations of rats which were offspring of female probands with alloxan diabetes, and this increase was particularly marked in the presence of disturbances of GTT of the diabetic type in the mother and the presence of hypoglycemia in the offspring at the age of 1-3 months. The results of investigations which showed that removal of 95% of the pancreas from female probands and from the offspring of six successive generations causes the development of diabetic GTT in the 7th generation of offspring [3] are evidence of the role of metabolic disturbances in the development of diabetes mellitus in the offspring. However, this does not rule out the possibility that alloxan may injure the genetic apparatus.

LITERATURE CITED

1. V. G. Baranov and I. M. Sokoloverova, Probl. Éndokrinol., No. 2, 50 (1985).
2. V. G. Baranov, I. M. Sokoloverova, A. I. Nikitin, and R. F. Onegova, Byull. Éksp. Biol. Med., No. 3, 34 (1974).
3. I. Foglia, cited by G. Spergel et al. [5].
4. G. Spergel, L. Levy, and M. Goldner, Metabolism, 20, 401 (1971).
5. G. Spergel, T. Khan, and M. Goldner, Metabolism, 24, 1311 (1975).

IMMUNOENZYME DETECTION OF BRAIN NS-2 ANTIGEN AS THE CRITERION OF ALTERED PERMEABILITY OF THE BLOOD-BRAIN BARRIER AFTER γ-IRRADIATION IN MICE

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KEY WORDS: blood-brain barrier; brain NS-2 antigen; γ-irradiation of mice.

A leading place in the mechanism of radiation damage to the living organism is occupied by disturbance of the functions of the blood-brain barrier (BBB) [1, 5, 11, 13]. The function of this anatomo-physiological system is nowadays estimated by studying changes in its permeability for low-molecular-weight substances of varied origin [3, 7, 14]. As a rule these substances are foreign for the animal and may have an independent action on the BBB, which modifies its permeability and sharply reduces the possibility of using these substances as markers of the functional state of the BBB. Meanwhile the question of the use of specific brain substances to assess the functional state of the BBB has been inadequately studied [2, 9].

The aim of this investigation was to conduct an immunoenzyme study of changes in permeability of BBB for species-specific mouse brain protein NS-2 in the early stages after acute γ-irradiation.

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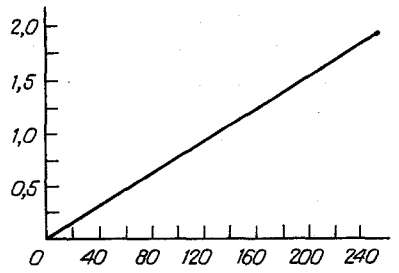

Fig. 1. Calibration curve for immunoenzyme determination of mouse brain NS-2 antigen. Abscissa, concentration of antigen (in ng/ml); ordinate, optical density at 450 nm (in relative units).

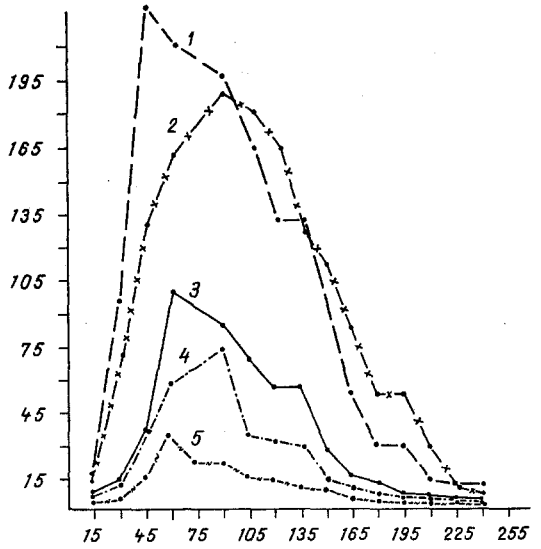

Fig. 2. Dependence of maximal concentrations of NS-2 antigen in blood serum on time after irradiation with various doses. Abscissa, concentration of NS-2 antigen (in ng/ml); ordinate, time after irradiation (in min). Irradiation in a dose of: 1) 10 Gy, 2) 7 Gy, 3) 5 Gy, 4) 3 Gy, and 5) 1 Gy.

EXPERIMENTAL METHOD

Experiments were carried out on 400 (CBA × C57BL)F_1 mice with an average weight of 18-24 g, subjected to a single whole-body γ-irradiation on the Luch (^{60}Co) apparatus in doses of from 1 to 10 Gy, with a dose rate of 0.36 Gy/min. The test object was blood serum from control and irradiated mice. Permeability of BBB was studied by an immunoenzyme method. Antisera to specific NS-2 antigen were obtained by immunizing chinchilla rabbits with a purified preparation of this antigen, obtained by the method described previously [9]. Antibodies to NS-2 were isolated from monospecific antisera on an immunosorbent prepared on the basis of CNBr-sepharose 4B (Sigma, USA) and a purified preparation of the antigen [10]. Isolation was carried out on a 1.6 × 40 cm chromatographic column (LKB, Sweden), filled with the immunosorbent. For this purpose 10 ml of monospecific antiserum to NS-2 with an initial titer of 1:32 was mixed with an equal volume of 0.05 M Na-phosphate buffer, pH 7.4, containing 0.1 M NaCl. The resulting mixture was recirculated through the column with immunosorbent for 3 h at 4°C. The adsorbed antibodies were eluted with 0.05 M glycine-HCl buffer, pH 2.2, and immediately alkalified with dry Tris (Merck, West Germany) to pH 8.8. The isolated antibodies were dialyzed against distilled water and concentrated on an XM 100A membrane (Amicon, USA).

The immunoglobulin concentrations in the fraction of isolated antibodies was determined by Ouchterlony's double immunodiffusion method, using donkey antiserum against rabbit immunoglobulins (Behringwerke, West Germany). The concentration of specific antibodies to NS-2 was determined by the immunodiffusion method in the modification in [4].

Immunoenzyme assay was carried out in polystyrene plates (Dynatek, Switzerland) by the method in [15]. The polystyrene plates were activated with a 0.003% solution of antibodies in 0.05 M carbonate buffer, pH 9.6, at 4°C for 18 h. Conjugation of the antibodies with horseradish peroxidase (type VI, from Sigma) was carried out by the periodate method [12]. A 0.08% solution of 5-aminosalicylic acid (Merck) was used as the substrate. To plot calibration curves a purified preparation of NS-2 was used. The results were recorded on a multichannel Titertek Multiscan MC spectrophotometer (England) at 450 nm.

EXPERIMENTAL RESULTS

The immunoenzyme system for detection of NS-2 in biological fluids, developed in the process of the experiment, enabled it to be detected between concentrations of 2 and 250 ng/ml. Optimal working with this system was obtained by the use of antibodies in a concentration of 30 μg/ml and with a dilution of the conjugate of 1:60, to activate the polystyrene plates. A graph showing dependence of the NS-2 concentration in the test sample on optical density at 450 nm, plotted on the basis of the results of 15 experiments, is given in Fig. 1. Statistical analysis of the standard deviation of points, which did not exceed 1.2%, demonstrated the reliability and high reproducibility of the developed system. The immunoenzyme system for detection of NS-2 antigens was used to study the permeability of BBB in unirradiated mice of the control group and also in animals subjected to acute γ-irradiation.

Further investigations showed that the level of NS-2 antigen in the blood serum of 200 unirradiated mice was constant at 4-8 ng/ml. The results of an immunochemical study of the NS-2 concentration in the blood serum of the irradiated mice are given in Fig. 2. Analysis of the data show that an increase in permeability of BBB began 15 min after irradiation. Later the concentration of specific NS-2 antigen rose steadily for 45-60 min and having reaches a maximum, began to fall steadily

It will be noted that strong dependence exists between the maximal NS-2 concentration and the adsorbed dose (Fig. 2). For a definite dose of irradiation, other experimental conditions being the same, there was a corresponding characteristic peak concentration of NS-2 in the blood serum.

In our view the increase in the rate of fall of the NS-2 concentration under the influence of decreasing doses is an interesting fact, for it confirms the presence of highly developed mechanisms of self-regulation of BBB permeability.

The view is nowadays held that the permeability of BBB is unchanged in the early stages after irradiation [6]. The authors cited observed an increase of permeability 10-20 h after irradiation. Considering the results given above, we must evidently agree with the view [3] that the early stage of changes in BBB permeability was not observed by these workers because the method of investigation which they used was defective.

The use of the immunoenzyme method of detection of brain NS-2 antigen thus not only reveals changes in BBB permeability in the early stages after irradiation, but also enables their time course to be studied. Taking into consideration the facts that the concentration of eliminated antigen rises with an increase in the absorbed dose and the rate of fall of the concentration increases with a decrease in the dose, it is our opinion that this method can be used for indirect assessment both of the degree of morphological and functional disturbances of BBB and of the mechanisms of its self-regulation in the early stages after irradiation.

LITERATURE CITED

1. A. E. Ivanov, N. N. Kurshakova, and V. V. Shikhodyrov, The Pathological Anatomy of Radiation Sickness [in Russian], Moscow (1981).
2. G. V. Morozov, V. M. Morkovkin, V. P. Chekhonin, and Z. I. Kekelidze, Problems in Emergency Psychiatry [in Russian], Moscow (1985), pp. 110-112.
3. I. B. Ushakov, Byull. Éksp. Biol. Med., No. 6, 697 (1986).
4. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 101 (1961).
5. C. Blomstrand, B. Johansson, and B. Rosengren, Acta Neuropath. (Berlin), 31, 97 (1975).
6. N. Enolo and M. Sakko, Radiat. Res., 61, 87 (1975).
7. M. E. Greig and W. C. Holland, Science, 110, 237 (1949).
8. O. S. Iorgensen and E. Bock, Scand. J. Immunol., 4, 25 (1975).
9. D. Juan, E. S. Vitetta, and M. Schachner, J. Immunol., 118, 551 (1977).
10. I. Kohn and M. Wilchek, Biochem. Biophys. Res. Commun., 107, 878 (1982).
11. V. Nair and I. D. Mackie, Radiat. Res., 58, 378 (1974).
12. P. K. Nakane and A. Kawao, J. Histochem. Cytochem., 22, 1084 (1974).
13. Y. Olson, I. Klatzo, and A. Carsten, Neuropath. Appl. Neurobiol., 1, 59 (1975).
14. L. I. Roth and C. F. Borlow, Science, 134, 22 (1961).
15. A. Voller, D. E. Bidwell, and A. Bartled, Bull. WHO, 53, 55 (1976).