LITERATURE CITED

1. P. D. Bonartsev, Zh. Nevropat. Psikhiatr., No. 7, 1061 (1976).
2. N. I. Kuznetsova and E. A. Sokolova, Abstracts of Proceedings of the 6th All-Union Congress of Neuropathologists and Psychiatrists [in Russian], Vol. 3, Moscow (1975), pp. 71-73.
3. R. R. Lideman and L. L. Prilipko, Dokl. Akad. Nauk SSSR, 192, 938 (1970).
4. A. G. E. Pearse, Histochemistry [Russian translation], Moscow (1962).
5. L. L. Prilipko and S. A. Vostrikova, Zh. Nevropat. Psikhiatr., No. 1, 86 (1976).
6. E. D. Carosella, K. Mochanko, and M. Braun, Cell. Immunol., 12, 323 (1974).
7. M. Jondal, G. Holm, and H. Wigzell, J. Exp. Med., 136, 207 (1972).
8. E. Hedfors, Clin. Exp. Immunol., 18, 379 (1974).
9. H. P. Lohrman, L. Novikovs, and R. G. Craw, J. Exp. Med., 139, 1553 (1974).
10. A. E. Powell and M. A. Leon, Exp. Cell Res., 62, 315 (1970).
11. J. Rabinowitz, Blood, 23, 811 (1964).
12. A. I. Rawson and T. C. Huang, Cell. Immunol., 17, 310 (1975).
13. J. D. Stobo, A. S. Rosenthal, and W. E. Paul, J. Immunol., 108, 1 (1972).

EFFECT OF AVITAMINOSIS B_6 IN MICE ON T-LYMPHOCYTE FUNCTION in vitro

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The effect of different degrees of avitaminosis B_6 in mice on the cytolytic activity of T lymphocytes, measured as the quantity of $Na_2Cr^{51}O_4$ released from lysed target cells, was studied on a model of the primary immune response in a mixed lymphocyte culture in vitro. Keeping animals for 3 weeks on a diet without pyridoxine did not affect the ability of the lymphocytes to proliferate in vitro or their cytolytic activity. In animals receiving a diet without pyridoxine for 45 days the content of pyridoxal-5'-phosphate in the spleen was 55% lower than in the control. Lymphocytes taken from these animals, when cultured in vitro, showed sharply weakened ability to incorporate [^{3}H]thymidine into DNA in response to the alloantigen. The cytolytic activity of these lymphocytes also was reduced. The ability of different forms of pyridoxine to restore the functions of T lymphocytes, when disturbed by avitaminosis B_6, was studied.

KEY WORDS: avitaminosis B_6; pyridoxine; pyridoxal; pyridoxal-5'-phosphate; cytolytic activity.

Mammalian lymphoid tissue is extremely sensitive to a deficiency of pyridoxine in the diet [7, 11]. Avitaminosis B_6, induced in experimental animals by restriction of the pyridoxine intake with the food or by injection of antagonists of vitamin B_6 causes a disturbance of both humoral [5, 8] and cellular [4, 10] immunity. Robson and Schwarz [9] showed recently that, during in vitro culture of lymphocytes taken from rats kept for 2 weeks on a diet deficient in vitamin B_6 the incorporation of [^{3}H]uridine into the DNA of the cells in response to an alloantigen is sharply depressed.

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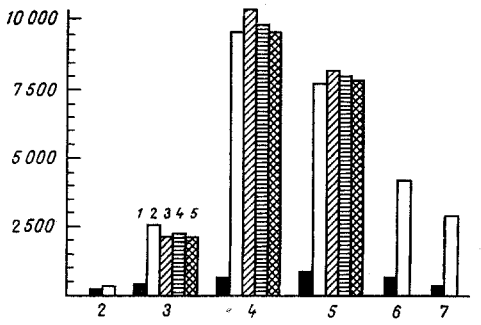

Fig. 1. Effect of pyridoxine deficiency in the diet on proliferation of lymphocytes stimulated in vitro. 1, 2) Monoculture of lymphocytes and lymphocytes of BALB/c mice kept on a diet with addition of pyridoxine stimulated in vitro, respectively; 3, 4, 5) lymphocytes of animals kept on vitamin B_6-deficient diet for 7, 14, and 22 days, stimulated in vitro. Ordinate, cpm/5 × 10^5 cells; abscissa, days of incubation of lymphocytes.

The effect of different degrees of avitaminosis B_6 in mice on proliferation and cytolytic activity of T lymphocytes, stimulated in a mixed culture in vitro was studied.

EXPERIMENTAL METHOD

Experiments were carried out on female BALB/c and C3H mice weighing 23-25 g. The BALB/c mice were kept on an artificial diet lacking in pyridoxine [1]. To speed up the development of avitamonisis B_6 in the mice of the experimental group isoniazid was injected intraperitoneally once a day in a dose of 80 μg per mouse for the first 3 days of the experiment.

To obtain stimulated lymphocytes in vitro the spleens were removed from BALB/c and C3H mice under sterile conditions and carefully homogenized in glass homogenizers, after which the number of living cells was counted. The lymphocytes from the C3H mice were irradiated in a dose of 1000 rad. Cells from the BALB/c and C3H (irradiated) mice were mixed in the ratio of 2:1 and poured into flasks (Sani Glas). The number of cells in 10 ml culture medium was 30 × 10^6. Carbon dioxide was blown through the culture medium and the flasks were incubated at 37°C.* The culture medium contained 100 ml of medium RPMI 1629, 20% of inactivated embryonic calf serum (ECS), 2 × 10^{-3} M L-glutamine, 5 × 10^{-3} M HEPES, 3 × 10^{-5} M 2-mercaptoethanol, and 100 units each of penicillin and streptomycin to 1 ml medium.

To determine the incorporation of [^{3}H]thymidine into DNA of the lymphocytes, 5 × 10^4-2 × 10^5 cells were incubated in 1 ml medium RPMI 1629 containing 5% ECS with 1 μCi [^{3}H]thymidine (specific activity 10 μCi/ml) for 6 h at 37° C. The cells were then washed twice with cold 0.9% NaCl solution and left to stand overnight in 10% TCA. The residue was transferred to a filter and washed with 5 ml 10% TCA. The filter was placed in flasks with 5 ml scintillation fluid (4.0 g PPO and 0.2 g POPOP in 1 ml toluene). For radiometry of the samples the Mark II beta counter was used. Each determination was carried out on two or three parallel samples. The ratio between the number of counts per minute in the experimental and control series was conventionally taken as the index of proliferation.

The cytolytic activity of the lymphocytes was determined by the method described in [6] using $Na_2{}^{51}CrO_4$ and with a monolayer culture of L cells as the target cells. Immune or normal lymphocytes were added to previously labeled L cells in the ratio of 10:1 in 0.5 ml of medium RPMI 1629 containing 10% ECS. Incubation continued for 18 h. Samples (0.4 ml) of supernatant were taken for counting. Radiometric measurements were made with a gamma counter from Nuclear Chicago. Each determination was carried out in five parallel tests. The percentage of specific cytolysis was calculated by the formula in [6].

The content of pyridoxal-5'-phosphase (PALP) in the mouse spleen was determined by the method described by the writers previously [2].

*Monocultures of lymphocytes of BALB/c mice or irradiated lymphocytes of C3H mice served as the control.

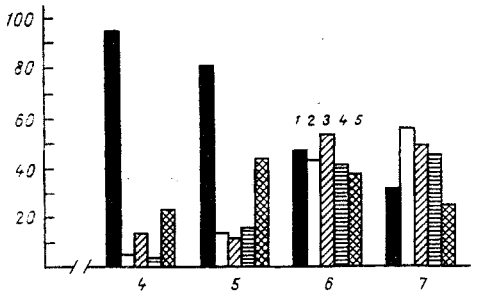

Fig. 2

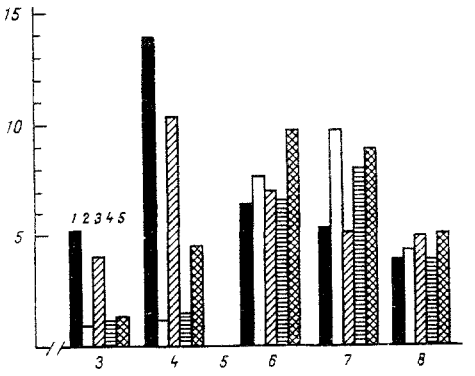

Fig. 3

Fig. 2. Effect of pyridoxine deficiency in the diet (45 days of avitaminosis) on cytolytic activity of lymphocytes stimulated in vitro. 1) Lymphocytes obtained from mice kept on diet with addition of pyridoxine; 2, 3, 4, 5) lymphocytes obtained from mice kept on vitamin B_6-deficient diet for 45 days; 3) 5 days before sacrifice animals received 50 μg PAL; 4, 5) PAL and PALP, respectively, added to system in vitro in a concentration of 2.5×10^{-4} M. Ordinate, specific lysis of L cells (in %); abscissa, days of incubation of lymphocytes.

Fig. 3. Effect of pyridoxine deficiency in the diet (45 days of avitaminosis) on proliferation of lymphocytes stimulated in vitro. Legend as in Fig. 2. Ordinate, index of proliferation of lymphocytes; abscissa, days of incubation of lymphocytes.

EXPERIMENTAL RESULTS

Lymphocytes from the spleens of BALB/c mice kept on a diet with the addition of pyridoxine (animals of the control group) proliferated in vitro in response to stimulation by irradiated cells from C3H mice. Simultaneously with a change in the cell composition of the population toward an increase in the number of large lymphocytes, there was a sharp increase in incorporation of [^{3}H]thymidine into the DNA of the cells. The index of proliferation of the lymphocytes on the 4th day of incubation was 8-12, after which it fell gradually (Fig. 1). In the monoculture, in which only cells from BALB/c mice were grown, the number of large lymphocytes also increased toward the fourth to fifth day of incubation and incorporation of the label into the cellular DNA increased correspondingly (Fig. 1).

Specific lysis of L cells, characterizing the cytolytic activity of the T lymphocytes, amounted to 1-3% during the first 3 days of culture of the lymphocytes in vitro. On the fourth day of incubation the stimulated lymphocytes, added to the L cells in the ratio of 10:1, induced 80-100% lysis of the target cells (Fig. 2). Lymphocytes from the monoculture added to the L cells in the above proportion virtually did not cause death of the target cells.

In the animals kept on a pyridoxine-deficient diet a progressive decrease in the weight of the spleen and a decrease in the PALP content in the organ was observed. By the 22nd day of the experiment the quantity of PALP in the spleen had fallen to 65-70%. Lymphocytes taken from BALB/c mice kept on a vitamin B_6-deficient diet for 1-3 weeks continued to respond normally in mixed culture in vitro to the alloantigen and proliferated at the same intensity as lymphocytes obtained from the control animals. The mean results of three series of experiments are given in Fig. 1. The indices of proliferation in the experimental tests did not differ statistically from the control values. The cytolytic activity of the lymphocytes obtained from animals with avitaminosis B_6 likewise remained unchanged during the first 3 weeks of the experiment.

The PALP content in the spleen of mice kept on a pyridoxine-free diet for 45 days fell to 45% of the control level. Some mice died by this time with clonic-tonic convulsions, a characteristic feature of severe pyridoxine deficiency. Lymphocytes taken from animals receiving an artificial pyridoxine-free diet for 45 days responded to the alloantigen, but the response was very weak. The peak of lymphocyte proliferation was shifted from the fourth to the seventh day of incubation and the incorporation of label into the DNA of the cells was only 50% of the control (Fig. 3). The peak of cytological activity of the lymphocytes also was shifted from the 4th to the 7th day of incubation and the degree of specific lysis of the L cells did not exceed 30-40% with a standard ratio of lymphocytes to target cells (Fig. 2).

Some of the animals kept on a vitamin B_6-deficient diet for 40 days were given an intraperitoneal injection of 50 μg pyridoxal (PAL) 5 days before sacrifice. Lymphocytes taken from these animals proliferated in mixed culture in vitro with almost the same intensity and at the same times as lymphocytes obtained from the control animals (Fig. 3). However, the cytolytic activity of the lymphocytes was sharply depressed at all times of incubation (Fig. 2). PAL added directly to the system in vitro in a concentration of 2.5×10^{-4} M restored neither the proliferative nor the cytolytic activity of the lymphocytes taken from animals with avitaminosis B_6 (Figs. 2 and 3). By contrast, PALP added to the system in vitro in a concentration of 2.5×10^{-4} M partly restored both the proliferative ability of the lymphocytes (Fig. 3) and their cytolytic activity (Fig. 2).

In the experiments of Robson and Schwarz [9], the proliferative and cytotoxic activity of the T lymphocytes of rats kept for 2 weeks on a vitamin B_6-deficient diet and receiving 4-deoxypyridoxine was considerably reduced. No disturbance of the functions of the T lymphocytes could be found during the first 3 weeks that the mice were kept on a pyridoxine-free diet, even through the PALP content in the spleen of these animals was reduced by 30%. The discrepancy between the results can perhaps be explained by a difference in the structure and physiology of the lymphocytes of rats and mice. The lymphoid system in mice is distinguished by the highest level of lymph production among mammals [3]; this, in turn, makes the lymphocytes of these animals more highly sensitive to a deficiency of vitamin B_6 in the diet. The possibility likewise cannot be ruled out that 4-deoxypyridoxine has a powerful toxic action on lymphoid tissue. Finally, another cause may be a difference in the experimental models used. Robson and Schwarz worked with lymphocytes from the thoractic duct of rats, whereas the source of the lymphocytes used in the present experiments was the mouse spleen. During preparation of the mixed culture and on the first days of culture in vitro, PALP liberated from the disintegrating cells may perhaps penetrate rapidly into the lymphocytes and abolish the specific disturbances caused by pyridoxine deficiency in the cells.

In the present experiments a disturbance of the proliferative and cytolytic activity of the T lymphocytes was observed only in animals kept on an artificial diet without pyridoxine for 5-6 weeks. PALP, added to the system in vitro under these circumstances partially restored the proliferative and cytolytic activity of the stimulated lymphocytes. This fact is important on its own account, for it leads to the conclusion that phosphorylated forms of pyridoxine can penetrate into lymphocytes.

LITERATURE CITED

1. Yu. V. Bukin and A. V. Sergeev, Biokhimiya, 33, 1092 (1968).
2. Yu. V. Bukin and A. V. Sergeev, Prikl. Biokhimiya, 6, 456 (1970).
3. V. A. Florensov, "The phylogeny and ontogeny of the hematopoietic function of the lymph nodes of vertebrates," Doctoral Dissertation, Irkutsk and Moscow (1964).
4. A. Axelrod, Am. J. Clin. Nutr., 24, 265 (1971).
5. A. E. Axelrod and J. Pruzansky, Vitamins and Hormones, 13, 1 (1955).
6. K. T. Brunner, I. Mauel, J. C. Cerottini, et al., Immunology, 14, 191 (1968).
7. S. D. Davis, T. Nelson, and T. H. Shepard, Science, 169, 1329 (1970).
8. M. Kumar and A. E. Axelrod, J. Nutr., 96, 53 (1968).
9. L. C. Robson and M. R. Schwarz, Cell. Immunol., 16, 135 (1975).
10. A. C. Trakatellis, W. R. Stinebring, and A. E. Axelrod, J. Immunol., 91, 39 (1963).
11. D. R. Weiz and J. F. Mueller, J. Clin. Invest., 30, 681 (1951).