PHAGOCYTIC ACTIVITY OF LEUKOCYTES DURING VITAMIN B_6 TREATMENT OF EXPERIMENTAL HEPATITIS

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Although much is still unknown about the assimilation, metabolism and body requirements of vitamin B_6, pyridoxine (a vitamin B_6 preparation) is already being widely used in the prophylaxis and treatment of many conditions in man and animals. Pyridoxine reduces the intoxications produced in animals by phosphorus, novarsenol and carbon tetrachloride [8, 9, 16 etc.]. Many clinicians [6, 10-14, etc.] have found that the preparation is effective in the treatment of certain diseases of the liver.

Experiments carried out by the authors on dogs [7] have shown that pyridoxine has a stimulating effect on bile secretion. It also has (particularly when combined with glucose) a favorable effect in dogs with toxic hepatitis. Vitamin B_6 administered to rats with experimental acute toxic hepatitis led to some improvement in the histological appearance of the liver with reduction of degenerative and necrobiotic processes [2].

Increasing the body resistance to certain diseases by means of nonspecific agents constitutes a very real problem. Several investigators [1, 3, 4, 5, 15, etc.] have shown recently that nonspecific preparations such as Dibasol, vitamin B_{12} can be used successfully to increase the body's defensive powers. Immunobiological reactions are thought to play a very important part in these phenomena.

In view of the wide clinical use of pyridoxine and the fact that it belongs to the same vitamin complex as B_{12}, it was decided to examine the effect this preparation had on the phagocytic activity of leukocytes in the course of acute experimental hepatitis.

METHOD

The experiments were carried out on 140 fully grown rats (mostly males), weighing 150-200 g. The animals were distributed in four groups. The first (22 animals) was the control group; the 10 healthy animals in the second group were each given a single injection of pyridoxine; the third (42 animals) was used for study of the course of untreated toxic hepatitis; and the 66 animals in the fourth group were given pyridoxine treatment for toxic hepatitis.

Acute toxic hepatitis was produced by subcutaneous injection of a 50 percent solution of carbon tetrachloride in oil, 0.2 ml initially and then 0.1 ml every four days. In the treatment group pyridoxine was injected subcutaneously in doses of 3 mg/100 g daily from the first day until death. The animals of the control group and the untreated group were given subcutaneous injections of normal saline (0.5 ml) at the same times. Rats were killed by decapitation 2, 4, 7, 10 and 20 days from the time of the last injection of carbon tetrachloride. The phagocytic activities of the leukocytes for Staph. aureus test strain No. 209 were examined repeatedly at the times stated. The phagocytic index, the percentage of active polymorphonuclear neutrophils which had phagocytized staphylococci, and the number of broken-down cells were determined in the usual way. Numerical results were processed statistically.

RESULTS

The phagocytic index for rats of the control group was 1.91 ± 0.13. No significant change in the index was produced by the single injection of pyridoxine (3 mg/100 g) given to the healthy rats of the second group. Phagocytosis was very markedly reduced [to 0.35 ± 0.04 ($P < 0.001$)] on the second day after the administration of

TABLE 1. Phagocytic Indices

Number of experiments	Group		Phagocytic index
18	Control (normal)		1.91 ± 0.13
10	Single pyridoxine injection		1.82 ± 0.14
8	Hepatitis-Untreated	2nd day after CCl_4	0.35 ± 0.04
8		4th day after CCl_4	0.56 ± 0.09
8		7th day after CCl_4	0.58 ± 0.08
8		10th day after CCl_4	0.77 ± 0.12
10		20th day after CCl_4	0.93 ± 0.09
10	Hepatitis-Treated	2nd day after CCl_4	0.79 ± 0.09
10		4th day after CCl_4	1.20 ± 0.11
11		7th day after CCl_4	1.46 ± 0.09
10		10th day after CCl_4	1.58 ± 0.08
10		20th day after CCl_4	2.04 ± 0.25

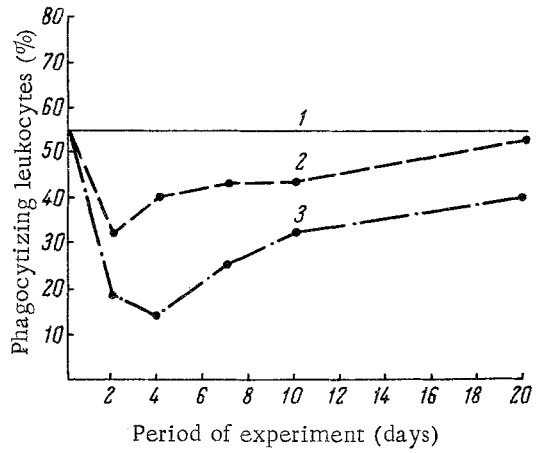

Fig. 1. Changes in phagocytic activities of leukocytes in rats with acute hepatitis given pyridoxine treatment. 1) Healthy animals; 2) animals with hepatitis given pyridoxine; 3) untreated hepatitis animals.

carbon tetrachloride. In the untreated animals the index rose only to 0.93 ± 0.09 in the course of the 20 days of the experiment. Recovery of their phagocytic function by the polymorphic neutrophils took place very slowly therefore after carbon tetrachloride.

The phagocytic index for the treated rats was down to 0.79 ± 0.09 on the second day. The difference between the indices for the treated and untreated rats was statistically significant, and the level of phagocytosis was generally 2-3 times higher in the former. At the end of the experiment, after 20 days of treatment, the phagocytic index had regained (or even passed) the level observed in these rats before the administration of carbon tetrachloride. Pyridoxine appeared, therefore, to have a favorable effect on the phagocytic function of leukocytes. Variation statistical treatment of the results confirmed the significance of the differences between phagocytic indices for treated and untreated animals ($P < 0.001$).

The loss of natural immunity and the depression of its cell mechanisms observed in rats with CCl_4-induced toxic hepatitis were thus corrected in significant extent by administration of vitamin B_6.

With the improvement of the phagocytic index in the treated rats, there was also a considerable increase in the number of active phagocytes (Fig. 1). Differences from the untreated hepatitis rats were significant at all times of examination (2nd, 4th, 7th, 10th and 20th days) ($P < 0.01$-0.001).

The leukocyte count was normal or high, but the percentage of active leukocytes sometimes tended to be low (e.g. on 4th day of illness). Neutrophil function was apparently restored more slowly than the neutrophil count.

No statistically valid changes in the percentage of active phagocytes were produced by the single injections of pyridoxine given to healthy rats (54.8 ± 2.28 percent as compared with 53.1 ± 17 percent for the control animals; $P = 0.5$).

The counts of broken-down leukocytes also revealed certain differences between the groups (Table 2). The single injections of pyridoxine to healthy rats did not produce any significant change in this respect.

TABLE 2. Percentages of Destroyed Leukocytes

Number of experiments	Group		Destroyed leukocytes (%)	P
18	Control (normal)		1.10 ± 0.44	
10	Single pyridoxine injection		0.80 ± 0.48	0.5
8	Hepatitis-Untreated	2nd day after CCl_4	13.25 ± 3.59	0.001
8		4th day after CCl_4	22.75 ± 2.60	0.001
8		7th day after CCl_4	11.00 ± 1.36	0.001
8		10th day after CCl_4	8.00 ± 2.20	0.001
10		20th day after CCl_4	1.60 ± 0.88	0.5
10	Hepatitis-Treated	2nd day after CCl_4	9.80 ± 2.16	0.5
10		4th day after CCl_4	2.20 ± 0.47	0.001
11		7th day after CCl_4	1.64 ± 0.75	0.001
10		10th day after CCl_4	1.20 ± 0.61	0.01
10		20th day after CCl_4	0.60 ± 0.31	0.5

In the hepatitis groups there were considerable increases in the numbers of destroyed leukocytes seen in smears in the course of the first 10 days after the injection of CCl_4. It was noted, when smears were being examined for phagocytic activity, that there were considerable numbers of destroyed leukocytes in the early stages of hepatitis in the untreated group (they numbered 22.75 ± 2.6 percent on the 4th day), although the total leukocyte counts for these animals were somewhat above normal at this time. The percentages of destroyed leukocytes were much lower in the treated hepatitis group.

Vitamin B_6 (pyridoxine) thus had a good effect on the cell factors of natural immunity, damaged by the toxic action of carbon tetrachloride. The evidence was a considerable rise of the phagocytic index, increase in the number of active phagocytes and marked reduction in the percentage of destroyed leukocytes in the treated animals.

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