DATA FROM A STUDY OF THE EFFECT OF VITAMIN B_{12} ON THE COURSE OF EXPERIMENTAL INFECTION AND THE IMMUNOLOGICAL REACTIONS OF THE ORGANISM

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Data exist in the literature indicating that preliminary injection of such preparations as dibasol, vitamin B_{12}, gentian, eleuterocock, etc., reduces the harmful effects of various agents on the organism [1,5,7,12,15]. Especially interesting are the reports which present data on increases in the organism's resistance to various infections under the influence of these preparations.

Encouraging results from preliminary injection of dibasol were obtained by A. M. Kapitanenko, using salmonellae [10], by I. M. Ivanushkin, using pneumococcal infections [9], by S. N. Teplova and N. V. Radkevich, using experimental Friedlander's infections [13], and by S. A. Burov and P. I. Remezov, using certain viral diseases [2]. We were unable to find similar reports on the effect of vitamin B_{12} on the course of these or other infectious diseases within the available literature.

In earlier investigations devoted to this subject, we noted that a repeated prophylactic injection of vitamin B_{12} into white mice, with subsequent inoculation of the animals, using cultured Friedlander's bacillus, significantly reduced the mortality of the experimental animals.

Naturally, the results obtained prompted us to carry out further supplementary investigations of the effect of vitamin B_{12} on the course of experimental Friedlander's infection; it was of particular interest to determine the optimal interval for injecting the aforementioned preparation.

EXPERIMENTAL METHODS AND RESULTS

For this purpose, two series of experiments were set up, using 348 white mice of the same line, weighing 18—21 grams, initially maintained under identical conditions for 7 days. In the first series of experiments we used 170 white mice, divided into four groups. The mice in the first group served as the control. For 12 days, these animals were injected subcutaneously with physiological saline. The animals in the other groups were injected daily with vitamin B_{12}, using a dosage of 5 micrograms/kg, over a course of 4, 8 and 12 days. At the end of the vitamin injections to the mice of the experimental groups, and the physiological saline to the mice of the control group, all the animals were inoculated with LD_{50} of a culture of Friedlander's bacillus (Table 1).

From the data in this table, it is apparent that the duration with which the experimental animals are injected with the vitamin B_{12} substantially influences their resistance to actual infection.

Taking these results into consideration, we concluded that the 8-day injection of white mice with vitamin B_{12} is the optimal duration for injections of this preparation, and results in the minimum mortality of experimental animals.

In the second series of experiments that were set up, we studied the effect of preliminary vitamin B_{12} injection on the duration of life of the mice following their subsequent inoculation with 6 LD_{100} of cultured Friedlander's bacillus. We used 178 white mice in the experiment, divided into four groups. The animals of the first group received 0.2 ml of physiological saline per injection, over a period of 12 days, while the animals of the other three groups were injected with vitamin B_{12}, using a dosage of 10 micrograms/kg, over the course of 4, 8 and 12 days. Following inoculation of all the animals with the above indicated dose of cultured Friedlander's bacillus, we observed

death of all the animals, both control and experimental. However, the death of the animals occurred at different intervals, as shown in Table 2.

TABLE 1. The Effect of the Duration of Vitamin B_{12} Injections on the Survival of White Mice Inoculated with Cultured Friedlander's Bacillus

Group of mice	Number	Type of preparation and duration of injections	Survival of the white mice following inoculation			
			1st day	2nd day	3rd day	4th day
First	49	12 days, physiological saline	29	26	26	26
Second	41	4 days, vitamin B_{12}	34	31	28	28
Third	41	8 days, vitamin B_{12}	36	35	33	33
Fourth	39	12 days, vitamin B_{12}	28	26	25	25

Table 2 shows that the duration of life of the mice that received vitamin B_{12} prior to the inoculation of Friedlander's bacillus was higher than it was in the animals of the control group, which received physiological saline in place of the vitamin B_{12}, and this, in turn, is evidence of an increase in the resistance of the experimental animals to Friedlander's infection secondary to injecting this preparation. In addition, the death of the mice that received vitamin B_{12} for 8 days occurred significantly later than in the other experimental groups, which again serves as proof that this is the optimal duration for injections of the indicated vitamin.

In addition to this, in the process of the experiments we studied the changes in weight of the white mice, before and after the inoculation, under the influence of vitamin B_{12}.

The literature contains evidence that vitamin B_{12} potentiates the growth of young animals [14].

According to our data, in the period prior to the inoculation the mice that gained the greatest amount of

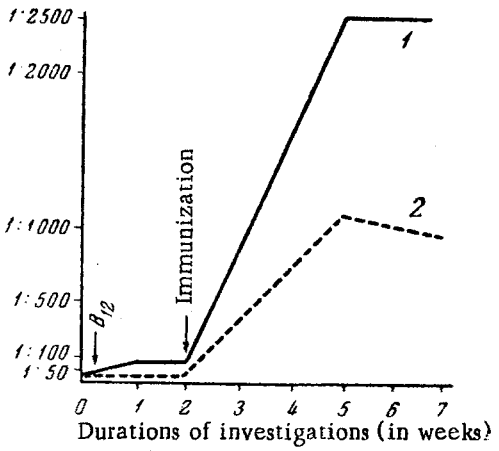
TABLE 2. The Effect of Vitamin B_{12} on the Course of Experimental Friedlander's Infection

Group of mice	Number	Type of preparation and duration of injections	Time of death of the animals following inoculation							
			1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
First	44	12 days, physiological saline	21	20	3	-	-	-	-	-
Second	44	4 days, vitamin B_{12}	13	26	3	2	-	-	-	-
Third	45	8 days, vitamin B_{12}	3	26	9	4	1	1	1	-
Fourth	45	12 days, vitamin B_{12}	2	27	10	5	1	-	-	-

weight were the ones that received the preparation for 8–12 days, while after the inoculation, especially in the first four days, when, according to the data in the literature, the weight loss associated with pneumococcal infection is greatest, the mice in these groups showed much smaller drops in weight than the control animals, which received physiological saline in place of the vitamin B_{12}.

On the basis of the preceding, we concluded that preliminary injection of vitamin B_{12} causes in the organism of white mice resistance to subsequent inoculation with cultured Friedlander's bacillus, which is reflected both in a lowering of the mortality rate of the experimental animals, and in an increase in the duration of their survival. Prophylactic application of vitamin B_{12} for a period of 8 days appears to be the optimal duration for injecting this preparation into white mice when dealing with Friedlander infection.

Having established that vitamin B_{12} increases the resistance of the organism to Friedlander infection, we became interested in the possible mechanisms behind the "defense" action of this preparation. In the available literature on this question, we found the work of Vincenzo [17], in which the author showed that injecting rabbits with vitamin B_{12} stimulates the bactericidal properties of the blood serum; we also encountered the report of Liotta and Lo Polito [16], in which the authors demonstrated that injection of vitamin B_{12} into rabbits results in a more rapid accumulation of antistreptolysins in the blood of the experimental animals.



The effect of vitamin B_{12} on the process of antibody formation in rabbits. 1) Titer of antibodies in the experimental rabbits; 2) titer of antibodies in the control rabbits.

In investigations performed earlier, we determined certain mechanisms for the "defense" action of vitamin B_{12}. Thus, it was established that vitamin B_{12} causes the elevation of cellular (phagocytic activity of leukocytes and degree to which the phagocytic process is fulfilled) and humoral (stimulation of the reticulo-endothelial system, enhancement of the bactericidal and leukocytolytic properties of the blood serum) defense reactions of the organism. In addition to the studied defense factors of the organism, major interest was directed toward investigating the effect of vitamin B_{12} on the production of antibodies, one of the defense powers of the macroorganism specifically directed against microorganisms.

To elucidate the effect of vitamin B_{12} on the production of antibodies, experiments were set up on 22 rabbits, weighing 2.1–2.9 kg, divided into two equal groups. The rabbits of the experimental group were injected with vitamin B_{12} subcutaneously for 7 days, using a dosage of 5 micrograms/kg, while the animals in the control group received physiological saline instead of vitamin B_{12} for the same period of time.

To determine the antibody titer, the conventional Vidal reaction was set up, by which it was possible to determine the typhoid and B–paratyphoid agglutinins.

The figure shows that before the experiment the titers of the typhoid and B–paratyphoid agglutinins in all the animals were approximately equal. After injection of the experimental animals with vitamin B_{12}, the titers of the normal antibodies in these animals rose, while in the rabbits that received physiological saline in place of the vitamin B_{12}, for the same period of time, the changes in these indices were small, and physiological in nature.

Thus, it is obvious that preliminary injection of vitamin B_{12} into rabbits over the course of 7 days, using a dosage of 5 micrograms/kg, stimulates the production of normal typhoid and B–paratyphoid antibodies in experimental animals.

A week later, again determining the titer of the typhoid and B–paratyphoid agglutinins, we established that in the group of rabbits that received vitamin B_{12} there were no changes in the levels of these antibodies. The same thing was noted in the group of control animals, which received physiological saline in place of the vitamin B_{12}. Immediately after this, all the rabbits were simultaneously immunized with 1 ml of tetravaccine, containing typhoid, B–paratyphoid, and dysentery (Flexner C and Sonne) antigens. Figuring that with single dose immunization

the antibody titer attains its maximum on approximately the 20th day and stays there for several days [8], we took a subsequent sample of blood for determination of the titer of the agglutinins under study 3 weeks after the immunization. In this case, as would be expected, we observed an increase in the antibody titer both in the experimental and in the control rabbits. However, the level of the rise in agglutinins in the animals that received the vitamin B_{12} previously was considerably higher than in the control animals, which were injected with physiological saline. This, in turn, permitted us to conclude that preliminary injection of vitamin B_{12} into immunized rabbits leads to a more intense increase in the antibody titer in experimental animals, as compared with animals which are injected with physiological saline in place of this vitamin for the same period of time.

Further observations showed that after 2 weeks, a decrease in the level of the studied antibodies is noted, but the titer of the typhoid and B–paratyphoid agglutinins in the rabbits that received vitamin B_{12} remains at a higher level than in the control animals.

Thus, vitamin B_{12} stimulates the production of antibodies both in normal and in immunized rabbits.

SUMMARY

Data are presented on the possibility of increasing the organic resistance of white mice to Friedlander bacillus infection by means of vitamin B_{12}. It is considered that one of the possible mechanisms in the protective action of this preparation is the intensified production of both normal and immune antibodies.

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