EFFECT OF SOME VITAMINS OF THE B GROUP ON THE COURSE OF VIRUS HEPATITIS IN ALBINO MICE

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The object of this investigation was to study the effect of comparatively high doses of thiamine (T) riboflavine (R), nicotinic (NA), pyridoxine (P) and mixtures of these substances on the course of experimental virus hepatitis in albino mice.

EXPERIMENTAL METHOD

The animals used in the experiment were about 1000 noninbred albino mice of both sexes, weighing 8-9 g, obtained from the nursery of the Academy of Medical Sciences of the USSR (Rappolovo). An acute liver lesion was produced by mouse hepatitis virus MHV-3 (Gledhill).* The lesion was produced by intraperitoneal injection of 0.2 ml of a suspension of lyophilized liver of infected animals in physiological saline. Mice of the appropriate control groups received an intraperitoneal injection of a suspension of lyophilized liver of healthy mice. A bacteriological control confirmed the absence of a contaminating flora in the suspension. Depending on the infecting dose (the dilution of the virus), from 10 to 100% of the animals died between the 3rd and 9th days after infection (the maximal number of mice died on the 4th-6th day). The clinical picture of the disease and the pathchistological changes in the liver and other organs corresponded fully to reports in the literature [2, 6, 9].

Working solutions of T. P, and NA were prepared in sterile physiological saline from ampule preparations. From crystalline R, a preparation was made up in 0.25% solution of DL-tryptophan in citrate-phosphate buffer (pH 6.8), containing 0.2% of the vitamin [8]. The preparation was passed through a Seitz filter and tested for sterility like the solutions of the other vitamins.

The vitamins were injected subcutaneously once daily in a volume of 0.1 ml (100 μg T, R, or P, 1 mg NA or a mixture of all four vitamins in the same doses). The mice received a diet consisting of oats, sunflower seeds, and fresh cows' milk. Particular attention was directed to choosing experimental and control groups identical in all respects. The animals of all groups were kept in identical conditions.

EXPERIMENTAL RESULTS

In the experiments of series I hepatitis was produced by a high dose of virus (dilution 10^{-2}), which led to death of 96% of the mice receiving 0.1 ml of physiological saline subcutaneously instead of the vitamins during the 9 days after infection. Each experimental group included 50 animals, and each corresponding control group (not infected, but receiving the same doses of vitamins) 25 animals. The vitamins in the doses mentioned above were injected from the day of infection for 9 successive days. None of the vitamins given in large doses, or the mixture of vitamins, lowered the mortality among the mice infected with a high dose of virus (mortality 96-100%). Death of the animals in all the experimental groups took place at the same time (90% of mice died from the 4th to 6th days after infection). The animals of all the control groups survived.

In the experiments of series II a smaller infecting dose of virus was used (in a dilution close to 10^{-4}). Each experimental group contained 30 mice and each corresponding control group 15 mice. The vitamins were injected from the second day after infection until the end of the experiment, which lasted

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14 days. Thiamine was associated with a more severe course of the hepatitis, as shown by death of 40% of the animals compared with 20% in the group of mice not receiving vitamins. However, statistical analysis of the results showed that the difference between these indices was not significant ($P = 0.1$).

A distinctly unfavorable effect on the course of virus hepatitis in the mice was observed after subcutaneous injection of large doses of R and NA (the mortality rates in these groups were 66.6 and 70% respectively, $P < 0.05$). An equally high mortality rate among the infected animals (73%) were also observed in the group of mice receiving the mixture of all four vitamins ($P < 0.05$), evidently as a result of the adverse effect of riboflavine and nicotinic acid. Injection of pyridoxine had no effect on the rate of survival of the infected mice. It was the same as in the animals receiving physiological saline instead of vitamins. All the animals in the corresponding control groups appeared perfectly healthy.

The results of these experiments demonstrated that parenteral injection of large doses of certain vitamins of the B group, especially R and NA, and to a lesser degree T, has a definite effect on mice infected with hepatitis virus. Analysis of the highly conflicting data in the literature suggests that administration of excessive doses of vitamins of the B group frequently lowers the resistance of experimental animals to virus diseases, but usually increases resistance to bacterial infections [7].

How can the mechanism of the negative effects of high doses of R and NA in acute virus diseases of the liver be explained? The development of acute hepatitis itself leads to a decrease in the level of flavines and pyridine-nucleotides in the liver and to an increase in the excretion of riboflavine and N_1-methylnicotinamide in the urine [3]. This is associated with profound disturbances of protein metabolism and, in particular, with disturbance of the synthesis and increased destruction of flavine and pyridine-nucleotide enzymes, the prosthetic groups of which contain R and NA. The "protein anchor" keeping these vitamins in the body is lost [4]. An endogenous protein deficiency essentially develops. In the conditions of a greatly reduced utilization of protein and predominance of catabolic over anabolic processes, some vitamins of the B group in excessive doses may prove toxic [1, 5], and attempts to correct the metabolic abnormalities by administration of large doses of vitamins may lead to still greater disorganization of the metabolic processes.

The possibility is not ruled out that during administration of large doses of R, NA, and to a lesser degree, of T favorable conditions are created for reproduction of the virus in the reticulo-endothelial cells and the hepatocytes, and this favors the development of a massive viremia and to a more malignant course of the disease.

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