EFFECT OF BIOMYCIN AND LEVOMYCETIN ON THE DEVELOPMENT OF NUTRITIONAL DISORDERS OF THE LIVER IN RATS

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It has been found that chronic nutritional disorders of the liver in experimental animals, similar to those in man, can be prevented by antibiotics [7, 11]. This preventive effect has not been observed in other studies[1, 4].

The present work is a study of the effect of Soviet antibiotics, biomycin (similar to the foreign aureomycin) and levomycetin (chloramphenicol), on the occurrence of nutritional (choline-deficient) fatty dystrophy of the liver in rats.

EXPERIMENTAL METHODS

The trials were on 30 white rats 20-30 days old of average initial weight 45 g. Seven animals were given a complete diet (control group), 7 a so-called "cirrhogenic" diet, 8 the same diet with biomycin, and 8 with levomycetin. The complete diet consisted of starch 40, sugar 30, casein with no vitamins 10, beef fat 15, salt mixture 4% and crystalline vitamins: ascorbic acid 100, vitamin B_1, 6, B_2 12, B_6 6, B_{12} 30, calcium pantothenate 30, nicotinic acid 60, inositol 30, vicasol 15, folic acid 30 mg; choline 2 and methionine 2 g. This amount of vitamins was added to 1.5 kg of the feed described above. Fish fat, 3 drops daily per rat, was given as a source of vitamins A and D. Each rat was given 1 mg vitamin E daily. To convert this diet into a "cirrhogenic" one, vitamin B_{12}, choline, methionine and folic acid were excluded. The feed was put out on the calculation that a small amount would remain for the next feeding. The feed of the 3rd and 4th groups were supplemented with antibiotics, 25 mg biomycin and 25 mg levomycetin daily, respectively. The animals were allowed tap water.

Each rat was kept in an individual cage with a mesh base to prevent coprophagy, which is very important in such trials. For the first 2 weeks all rats were given the complete diet, and then were transferred to the diet for the appropriate experimental group. For 2 weeks after this the feed of the rats of the experimental groups was supplemented daily with 3 drops of a 20% choline solution to prevent death from kidney necrosis due to choline deficiency. The trial lasted 9 months. The condition of the rats was evaluated by observing outward appearance, activity, weight changes and by histological examination of the liver. One rat from each group was killed in the 2nd, 6th and 7th months of the trial and the remainder at the end of the 9th month. The liver was weighed, and liver tissue after wetting in paraffin was stained with hematoxylin-eosin and by the method of van Gieson, and another part on fat with sudan III.

EXPERIMENTAL RESULTS

During the whole trial period the rats of the first group appeared well, were active, ate their feed willingly, grew well and gained considerable weight. The average initial weight of the animals in this group was 37 and the final weight 223 g, the proportional index of the final to the initial weight being 6. The average liver weight was 8 g. On histological examination, the structure of the liver tissue was normal at all stages of the trial.

By the end of the experiment the animals of the 2nd group lagged behind those of the first in weight and growth. Despite their initial average weight being higher, 50 g, the final weight reached only 180 g and the proportional

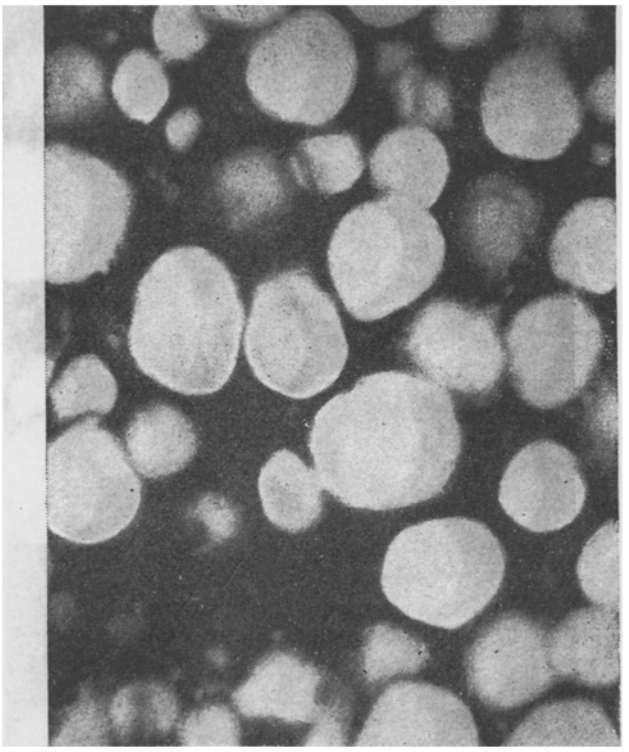

Fig. 1. Microphotograph of liver tissue of rat on a "cirrhogenic" diet. Sharp definition of fatty dystrophy, with here and there fatty "cysts", and increase in number of lymphoid cells; disordered lobular-trabecular structure. Staining according to van Gieson. Magnif. 7 × 40.

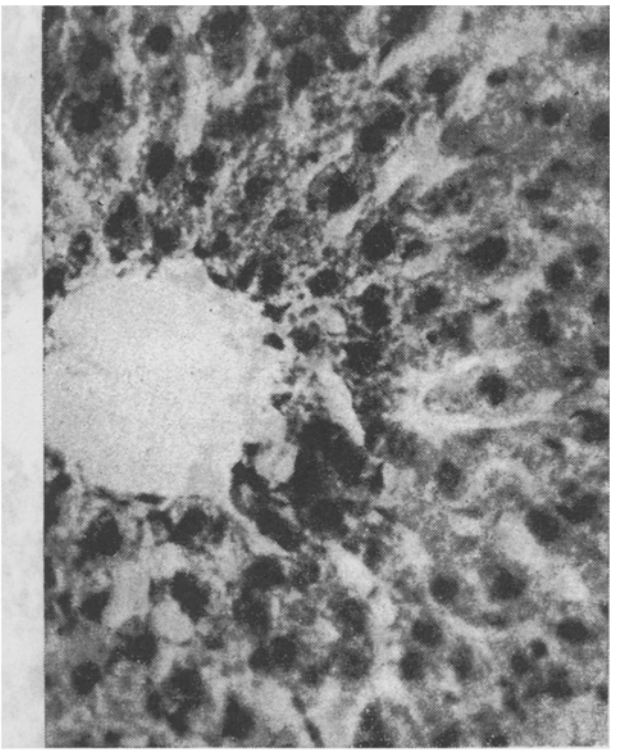

Fig. 2. Microphotograph of liver tissue of rat given biomycin and a "cirrhogenic" diet. Signs of indistinct protein dystrophy. Staining according to van Gieson. Magnif. 7 × 40.

index of the final to the initial weight was only 3.6. On dissection even in the 2nd month the liver appeared yellowish and by the end of the experiment the granularity of its surface was seen. Its average weight was 7 g. Histologically, all the rats of this group showed fatty dystrophy of the liver cells, increasingly progessively becoming sharply defined in the 9th month (Fig. 1). Figure 1 shows that the liver tissue was subject to almost total fatty dystrophy with formation of individual fatty "cysts". The usual lobular-trabecular structure was not discernible and an increase in the number of lymphoid cells was observed.

The rats of the 3rd group looked well, ate their feed willingly and were active during the whole experimental period. Their average initial weight was 42 and final weight 223 g, and the proportional index of final to initial weight was 5.3. On dissection attention was drawn to considerable enlargement of the caecum and also the thorough yellow staining of the rat tissues (effect of biomycin). Macroscopically, the liver appeared normal, with a light or dark brown coloration and a smooth surface. Its average weight was 7 g. Histological examination showed indistinct signs of protein dystrophy somewhat greater than normal, granularity of the protoplasm and vague cell margins (Fig. 2), but fatty dystrophy was absent or minimal.

The rats of the 4th group appeared worse than the animals of the other groups, grew and gained weight poorly and their activity was feeble. Their average initial weight was 47 and final weight 183 g. The proportional index of final to initial weight was 3.8, i.e., almost the same as in the animals of the 2nd group. On dissection, the liver appeared yellowish and its average weight was 6.5 g. Histologically, all showed fatty dystrophy the degree of which depended on time of dissection, while at all stages of dissection the fatty dystrophy in the rats of this group was even more pronounced than in the animals of the 2nd group. Figure 3 (liver tissue in the 9th month of the trial) shows that the fatty dystrophy was total, all the liver tissue was streaked with "fatty bands", there were fat droplets between the cells also, and the accumulation of lymphoid cells was more pronounced than in the 2nd group.

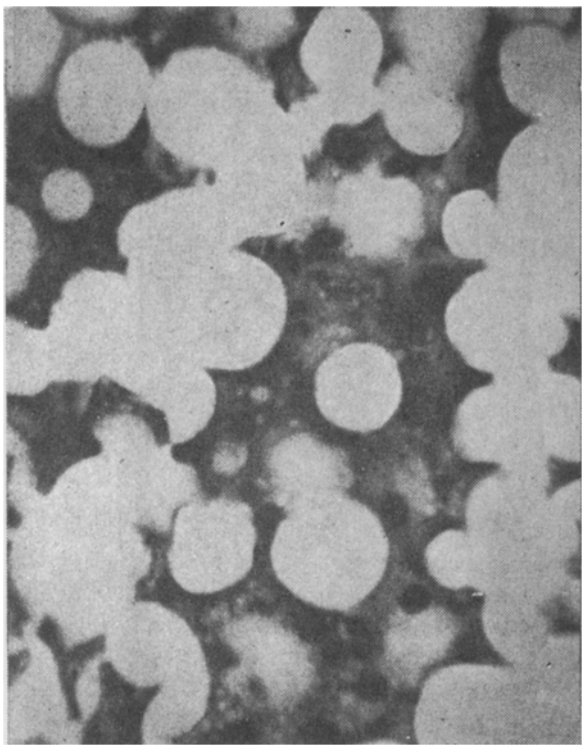

Fig. 3. Microphotograph of liver tissue of rats given levomycetin and a "cirrhogenic" diet. Total fatty dystrophy; bursting of most liver cells with formation of fatty clumps; considerable clusters of lymphoid elements; lobular-trabecular structure not discernible. Staining according to van Gieson. Magnif. 7 × 40.

Thus, in our experiments biomycin prevented the choline-deficient fatty dystrophy of rat liver. The outward appearance, weight gains and condition of the internal organs of the rats of the biomycin group were almost the same as in the control animals. A preventive effect, similar to that found by us, has been found with aureomycin [10, 11], which, as is well-known, is similar to the Soviet biomycin. At the same time, in other trials [1] biomycin, which has a generally favorable effect (improving weight gains and viability experimentally), had no influence on the dystrophic effect of a choline-deficient diet, from which it may be concluded that the antibiotic in question has no preventive action. However, in this case the effect of antibiotics was studied in acute experimental conditions (for 2 weeks) which, in our opinion, makes the trials difficult to compare.

The prevention of fatty dystrophy of the liver by biomycin may seem paradoxical as the opinion is widespread that this antibiotic itself causes similar changes. However, this point of view is based on observations on patients with various liver disorders [14] and therefore requires more critical evaluation. It has been shown experimentally that even prolonged administration of considerable doses of tetracycline antibiotics has no toxic effect on the organism, and causes no liver changes [1, 8, 13, 14]. It has been demonstrated [2], that in rats fatty dystrophy of the liver does not occur, even after 60 days of tetracyline administration at the rate of about 100 mg daily.

In our trials levomycetin could not prevent choline deficient fatty dystrophy of the liver and, what is more, it intensified it somewhat. In trials by other workers [1, 11] this antibiotic also did not prevent pathological changes due to choline deficiency.

It has been shown in a number of works [6, 7, 11, 12] that pathological changes in the liver due to choline deficiency may also be prevented by other broad-spectrum antibiotics (terramycin, streptomycin and neomycin). It has been demonstrated [10, 11] that these antibiotics contain S amino acids, choline, folic acid and vitamin B_{12} in insufficient amounts to have a preventive effect. Careful study of the liver and other internal organs revealed no infection the suppression of which could also be explained by the action of the antibiotics. This suggests the idea that the action of a choline-deficient diet is mediated through the normal intestinal microflora and the action of the antibiotics consists in changes of this flora. Depending on the character of the effect of the antibiotic on the flora, pathological changes in the liver are prevented or are intensified or there is no effect at all. A number of workers [3, 5, 9] have studied the intestinal microflora of rats given antibiotics and a choline-deficient diet. It has been shown that it is to a certain extent possible to speak of a specificity of changes in the intestinal microflora under the influence of various antibiotics. The results of a direct study of the ability of intestinal bacteria in rats to synthesize vitamin B_{12} [5] showed that when levomycetin is given, strains of bacteria with a low vitamin-synthesizing ability begin to predominate. However, also when the animals are given biomycin such strains appear in considerable numbers and it is therefore difficult to explain the differences in the action of the antibiotics. Apparently, it is all a question of the differing effect of the antibiotics on the synthetic aspects of the activity of the intestinal microflora which are important for the macroorganism.

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