THE CONCENTRATION OF CERTAIN MICROELEMENTS IN THE BLOOD AND ORGANS OF RABBITS WITH EXPERIMENTAL CIRRHOSIS OF THE LIVER

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Translated from Byullenten' Éksperimental'noi Biologii i Meditsiny, Vol. 57, No. 3,
pp. 62-67, March, 1964
Original article submitted December 15, 1962

As we know, the liver participates in all forms of the metabolism, and in addition, is the chief physiological depot for the microelements [2, 3].

We set out to reproduce cirrhosis of the liver in animals experimentally, and, with its progressive development, to study the concentration of certain microelements dynamically in the blood, and when the animals died, their concentration in the organs as well.

Such works are absent from the literature.

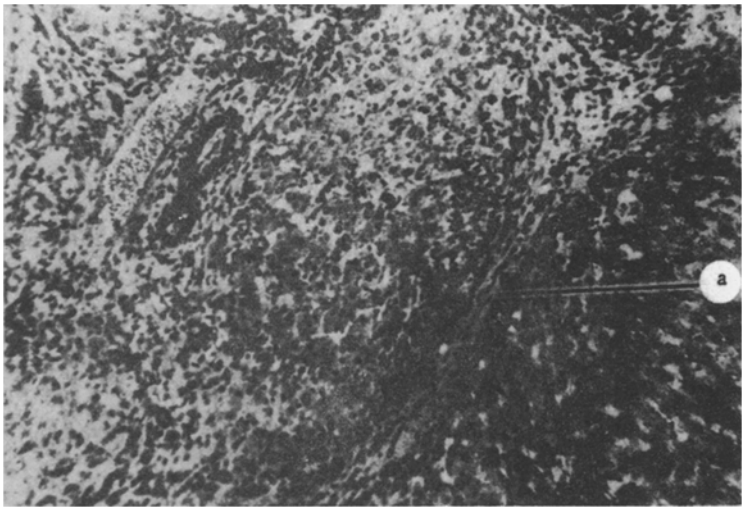
EXPERIMENTAL METHODS

Injury of the liver tissue in rabbits was caused by the injection of carbon tetrachloride, using the dosages proposed by L. S. Rubetskoi and R. N. Korotkina [4], but with some changes in the injection intervals. The rabbits were injected with the carbon tetrachloride twice a week, subcutaneously, using 0.2 ml of a 40% solution in apricot oil per kg of weight of the animals. The first course consisted of 14 injections, the second of 12, and all the following of 10; an interval of 1 month was allowed to elapse between courses. Throughout the entire period of the experiment, at certain time intervals (25, 45 days, 2, $2\frac{1}{2}$, 3, 4, 5, 6, 7, 8, 9, 10, $11\frac{1}{2}$, 12, 13 months, after initiation of the injections), we investigated the concentration of the microelements, copper, zinc, cobalt, and iron, as well as sugar, in the blood.

After the death of the animals, we investigated the concentration of the microelements in the liver, muscles, femur, brain, and spleen. In addition, we made a macroscopic study of the morphological changes in the liver, and also determined the changes in its structure visible in serial sections stained with hematoxylineosin. A total of 22 rabbits was used in the experiments, of which cirrhosis was induced in 10; in 7 control rabbits, we investigated the microelements in the blood without injection of the carbon tetrachloride, and in 5 control animals we determined the microelements in the organs. Male rabbits (weighing from 1.9 to 3 kg) were maintained on their normal diet, under the vivarium conditions.

The amount of microelements was demonstrated by the colorimetric technique, following the method of Babenko [1], making it possible to use a single sample for determining the following microelements: copper, zinc, cobalt, as well as iron. We performed a total of 693 determinations of the microelements in the blood and organs, and 118 determinations of the blood sugar concentration (the data in the tables and figures represents material gathered over 6 months, since after this period of time, due to the death of the animals, the number of observations was insufficient for calculating mean indices).

The results of the investigations were subjected to statistical analysis, with calculation of the mean error ($\pm m$).



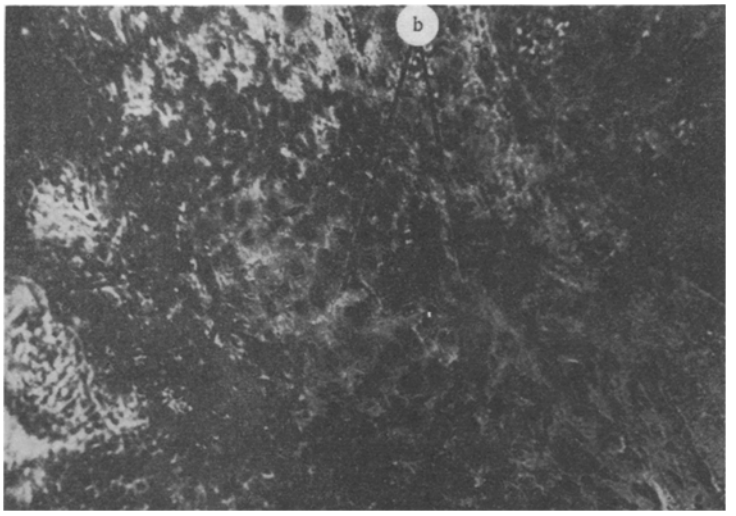


Fig. 1. a) Intense development of the interlobular connective tissue. Ocul. 10 ×, obj. 8 ×; b) false lobule, bounded by a layer of connective tissue. Ocul. 10 ×, obj. 40 ×; c) thickening of the fibrotic capsule due to connective tissue proliferation with scar tissue extension. Ocul. 10 ×, obj. 40 ×. Stained with hematoxylin-eosin.

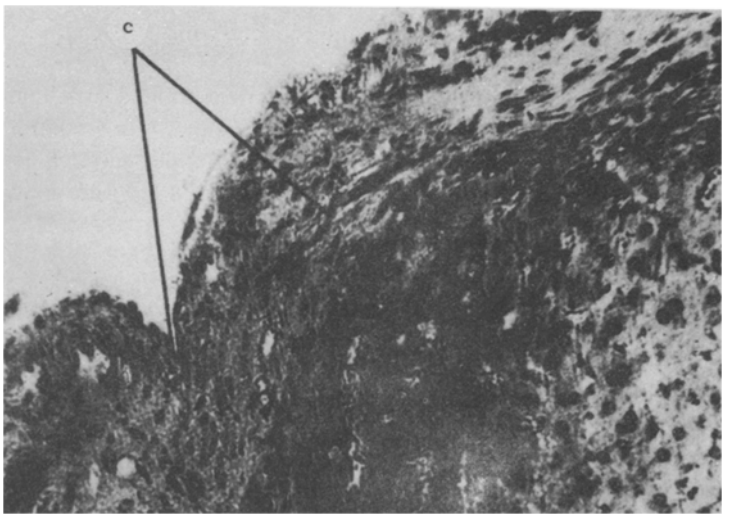


TABLE 1. Mean Data on the Concentration of the Microelements and Sugar in the Blood, in Normal and Experimental Rabbits

Index determined	Normal	In rabbits that received carbon tetrachloride, after							
		25 days	45 days	2 mos.	2½ mos.	3 mos.	4 months	5 months	6 months
Copper (in mg % in the ash)	16±1	19±2,5	20±3	19±2	17±3	17±5	27±3,6	20±1,7	25±2
Zinc (in mg % in the ash)	37±4	31,4	38±6	37±3	57±9	52±6	55±7	43±7	34±8
Iron (in % in the ash)	4,3±0,1	4,2±0,04	3,4±0,2	3,5 0,2	3,6±0,2	3,9±0,4	3,8±0,25	3,6±0,2	3,8±0,18
Cobalt (in micrograms % in the ash)	225±33	278±47	376±43	217±42	275±50	369±49	333±35	249±40	207±45
Sugar (in mg %)	103	110	115	109	112	118	109	112	117

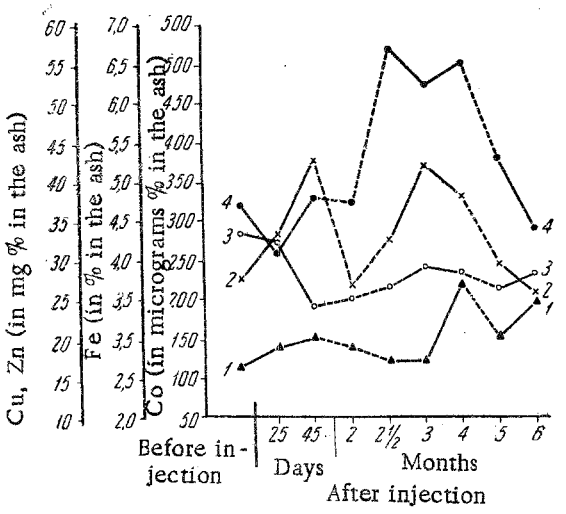


Fig. 2. Dynamics of the concentration of copper (1), cobalt (2), iron (3), and zinc (4) in the blood of rabbits, during the period of injection of carbon tetrachloride (straight line), and in the interval between injections (broken line).

EXPERIMENTAL RESULTS

Morphological investigation of the liver. The rabbits that received the carbon tetrachloride remained in satisfactory condition for a rather long time, and only shortly before their death did they begin to deteriorate and become sluggish.

On autopsy of the animals, attention was attracted, macroscopically, to the dense consistency of the liver (it crackled when cut into), its granular surface, and the decreased weight of the organ. Ascites was noted in 4 of the rabbits, that died after 7, 8 and 13 months. In 2 of the rabbits, on the surface of the liver, we observed numerous nodules, measuring 0.5-0.6 cm in diameter.

The histological investigations showed that, the outcome varied with varying degree of injury to the liver tissue, depending on the duration of injections with the toxic material. Only in one rabbit that received a single course of injections did the character of histological changes indicate dystrophy of the liver cells and early cirrhosis. In the remaining rabbits, prolonged application of small doses of carbon tetrachloride caused severe dystrophic changes in the liver, its "reorganization," and the development of cirrhosis, which, in the majority of cases, reached a pronounced level (Fig. 1).

Quantitative concentration of the microelements in the blood. As can be seen in Table 1 and Fig. 2, that amount of copper in the blood 25 days after initiation of the injections of carbon tetrachloride rose in comparison with the norm,* from 16 ± 1 to 19 ± 2.5 mg%, and by the end of the 1st course of injections, i.e., after 45 days, increased still more, consisting of an average of 20 ± 3 mg% in the ash. In the interval between the 1st and 2nd courses of injections, the concentration of copper returned to the starting level. Subsequently, the amount of copper increased without a tendency toward normalization. By the end of the 2nd course of injections, the concentration of copper in the blood was elevated up to an average of 27 ± 3.6 mg%, during the 2nd interval it fell to 20 ± 1.7 mg%, but after the 3rd course of injections it again increased to 25 ± 2 mg% in the ash.

* The mean indices for the concentration of microelements in the blood of the rabbits prior to injection of carbon tetrachloride were drawn from the results of their determinations in 17 animals.

TABLE 2. Mean Data for the Concentration of Microelements in the Organs of the Normal and Experimental Animals

Index determined	Animals	Liver	Muscles	Bone	Brain	Spleen
Iron (in % in the ash)	Control	2,1±0,12	0,26±0,02	0,04±0,007	0,37±0,05	
	Experimental	1,0±0,1	0,5±0,08	0,15±0,03	0,42±0,05	
Copper (in mg % in the ash)	Control	11,7±0,37	14±2,6	8±1	7±0,7	24±1
	Experimental	24±2	20±1,3	24±3	28±2	38±4
Zinc (in mg% in the ash)	Control	70±5	32±9	16±2	36±3	
	Experimental	120±11	120±14	33±5	65±9	
Cobalt (in micrograms% in the ash)	Control	194±34	225±43	280±37	326±31	
	Experimental	238±32	1 466±172	340±50	452±36	

Studying the concentration of zinc in the blood demonstrated a different picture. In the period of the 1st course of injections, the amount of zinc in the blood did not change in comparison with the starting level (in the normal animals, 37 ± 4 mg% in the ash). But by the end of the 1st interval between courses, i.e., 2½ months after initiation of the injections, the concentration of zinc in the blood rose in comparison with the starting figures by 54%, and remained at the elevated level during the 2nd course of injections. Then, beginning with the 2nd interval between courses, the amount of zinc began to decrease, and reached the starting figures by the end of the 3rd course of injections, consisting of an average of 34 ± 8 mg% in the ash.

After 45 days, i.e., after the 1st course of injections, the amount of iron in the blood decreased from 4.3 ± 0.1 in the normal animals to 3.4 ± 0.2% in the ash (see Table 1 and Fig. 2). Subsequently, the concentration of iron in the blood fluctuated slightly, but did not return to the initial level, and 6 months after the start of the experiment it was equal to an average of 3.8 ± 0.18% in the ash.

The curve reflecting the concentration of cobalt in the blood (see Fig. 2) had two peaks of increase and decrease. The maximum increase in the amount of cobalt in the blood (376 and 368 micrograms %), as compared with the original level (225 ± 33 micrograms %), fell in the periods of the 1st and 2nd courses of carbon tetrachloride injections, and the maximum decrease, correspondingly, in the two periods between courses. However, the following, 3rd, course of injections was accompanied by a subsequent drop in the blood cobalt level to a figure somewhat lower than the normal level; it was equal to an average of 207 ± 45 micrograms % in the ash.

During the entire period of the experiment, in parallel with determination of the quantitative concentration of microelements at certain time intervals, we also studied the blood sugar concentration. The mean indices for the normal level, drawn from the results of this determination in 17 rabbits (10 experimental and 7 control), ranged from 90 to 120 mg%. During the course of the 6 months of observations, the bloodsugar concentration did not range outside of the limits of normal fluctuation (see Table 1).

The concentration of microelements in the organs. The amount of microelements in the organs of the 10 experimental animals was compared with their concentration in the 5 control rabbits (Table 2).

If, for convenience of notation, we assume the concentration of microelements in the organs of the control animals to be 100% (Fig. 3), then it was found that, in the liver, the amount of copper rose by 105%, of zinc – by 71%, of cobalt – by 22%, and only the concentration of iron decreased – 52%.

A significant accumulation of cobalt was noted in the muscles; as compared with the concentration in the control animals, its amount in the experimental animals rose by 6½ times. The concentration of iron in the muscles increased by 92%, of copper – 42%, while the amount of zinc remained unchanged.

In the bones of the experimental animals, the microelements also underwent changes. Thus, the amount of iron rose by 275%, of cobalt – by 21%, the concentration of copper was tripled, and of zinc – doubled.

In the brain, the concentration of copper increased by 4 times, the amount of zinc – by 80%, of cobalt – by 38%; the concentration of iron remained almost unchanged.

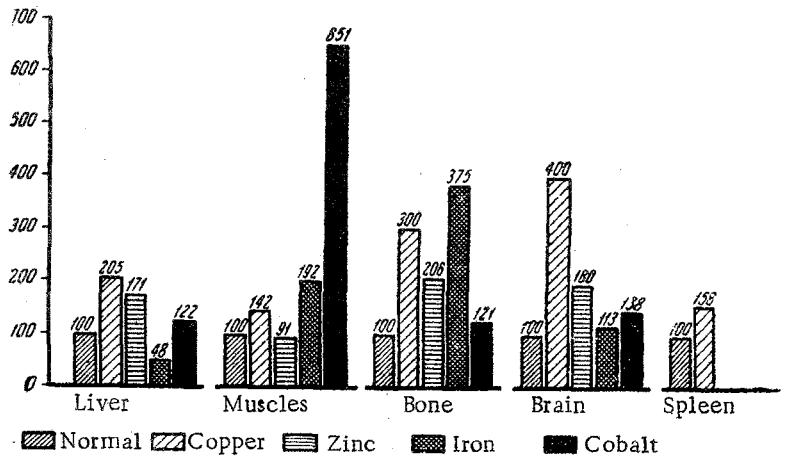


Fig. 3. The concentration of microelements in the organs of rabbits suffering from cirrhosis of the liver (in %).

In the spleen, the amount of copper rose by 58% in comparison with its concentration in the control animals.

It must be noted that during the dynamic investigation of the blood concentration of copper, zinc, cobalt and iron in the experimental animals, clearly manifested disturbances were demonstrated. The concentration of copper in the blood, upon the injection of carbon tetrachloride, increased earlier than the concentration of zinc. However the blood copper concentration began to rise starting with the 3rd month, and subsequently remained at the elevated level. The blood zinc concentration rose sharply after the 2nd month of observations, and remained at the high level up to the 4th month, when it then decreased. The amount of iron in the blood decreased. The changes in the quantitative concentration of cobalt in the blood were wave-like in character, with a tendency toward decreasing to a level below the initial figure by the 6th month of observation.

A comparison of the changes undergone by the copper, zinc, cobalt and iron concentrations in the organs of the rabbits leads to the conclusion that cirrhosis of the liver causes a distinct character of redistribution of each studied microelement among the tissues of the organism.

SUMMARY

The blood content of microelements (copper, zinc, cobalt and iron) was studied in rabbits with experimental cirrhosis of the liver; the content of these microelements was also studied in some of the organs of the animals which had perished.

With the development of cirrhosis of the liver there was a rise in copper and a reduction of iron in the blood. Zinc and cobalt content normalized after the periods of the rise. Cirrhosis of the liver caused a definite nature of microelements redistribution in the rabbit organs.

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