

THE CONTENT OF COENZYME A IN THE LIVER IN CARBON TETRACHLORIDE POISONING

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Coenzyme A (Co A) occupies an exceptionally important place in the intermediate metabolism of many substances, and in recent years new metabolic processes have continued to be discovered in which it plays a part [2, 4,5,6,9]. There is little information in the literature, however, dealing with the changes in its concentration in the organs and, in particular, in the liver affected by various pathological processes [8, 10]. We accordingly considered it desirable to study the changes in the Co A content of the liver in animals poisoned with CCl₄.

EXPERIMENTAL METHOD

Carbon tetrachloride was injected into rats once or repeatedly. Young rats weighing 120-150 g, of both sexes and kept on a normal diet, were used for the experiments. The CCl₄ was injected subcutaneously in a dose of 0.2 ml/100 g body weight. When only one injection was given, the Co A content was determined 3, 6, 12, and 24 hours after the injection, and when the animals received 3 or more injections (15-18 injections on alternate days) they were sacrificed 24 hours after the last injection. Approximately 1 g of cooled liver was rapidly homogenized in a porcelain crucible, three times its volume of distilled water was added, and the mixture was brought quickly to the boil and centrifuged at 100 rpm, after which the concentration of Co A in the centrifugate was determined. Since an aqueous extract of liver does not contain acetylation apoenzyme, this apoenzyme was extracted from the liver of a healthy pigeon in the form of an autolyzate of an extract of an acetone powder [7]. This apoenzyme preparation does not contain Co A, and without addition of the latter it is practically inactive.

TABLE 1. Concentration of Co A in Rats' Liver at Various Intervals after a Single Subcutaneous Injection of CCl₄, Calculated per 1 g Liver Protein, as a Percentage of its Concentration in the Liver of Control Rats

	After 3 h	After 6 h	After 12 h	After 24 h
	118	119	118	79
	116	120	118	86
	132	126	129	71
	130	121	114	79
	126	118	122	87
	116		97	76
				93
$M \pm m$	123 \pm 2.95	120.6 \pm 1.2	116.3 \pm 4.4	81.6 \pm 2.76
P	<0.001	<0.001	<0.01	<0.001

Since the apoenzymes extracted in different experiments possessed different activity, each was taken in an amount which, in conjunction with the same quantity of Co A, would acetylate 50-60% of the substrate.

The Co A content was determined as the amount of p-aminobenzoic acid (PABA) acetylated by extracts of the test tissue after incubation in the presence of the potassium salt of ATP, cystein, sodium acetate, and apoenzyme. Each experiment was accompanied by a corresponding control.

Into a centrifuge tube was placed 0.2 ml of the test solution, 0.3 ml of acetylating mixture (consisting of 10 ml of 0.004 M PABA solution, 8 ml of 0.05 M solution of the potassium salt of ATP, 2.5 ml of a 1 M solution of CH_3COONa , and 10 ml of a 0.2 M solution of sodium citrate), 0.08 ml of a freshly prepared 1 M solution of NaHCO_3 , 0.1 ml of a 0.1 M solution of cystein hydrochloride (also freshly prepared), 0.2-0.3 ml of apoenzyme solution (depending on the activity of the extract), and water to a total volume of 1.5 ml. The tube was incubated at 37° . The incubation time was varied to suit the activity of the apoenzyme.

The reaction was topped by the addition of 1 ml of a 15% solution of cold trichloroacetic acid. The mixture was centrifuged and the concentration of PABA in 1 ml of centrifugate was estimated by the colorimetric method of Bratton and Marshall [3] as modified by O. N. Sytinskaya [1].

In the meantime the protein content in the same tissue was determined colorimetrically by the biuret reaction. The Co A concentration in the tissue was expressed as a weight of acetylated PABA (in μg), calculated per 1 g liver proteins.

EXPERIMENTAL RESULTS

The results obtained are shown in Tables 1 and 2.

According to Unno [10], the Co A concentration in the liver falls in CCl_4 poisoning. Severi and Fonnesu [8] also observed a decrease in the Co A content of the liver during CCl_4 poisoning.

It will be clear from the results shown in Tables 1 and 2 that the Co A concentration in the liver affected by CCl_4 poisoning may be raised or lowered depending on the length of time elapsing after injection of CCl_4 and on the number of injections. During the first 12 hours after a single injection the Co A concentration in the liver rose. As the liver damage progressed, this increase gave way to a decrease in the Co A concentration, reaching 20% 24 hours after the injection.

TABLE 2. Concentration of Co A in Rats' Liver after Triple or Multiple Subcutaneous Injections of CCl_4 , Calculated per 1 g Liver Protines, as a Percentage of its Concentration in the Liver of Control Rats

	After triple injections	After multiple injections
	83	135
	70	148
	76	159
	80	118
	64	119
	87	100
	84	97
		130
		143
$M \pm m$	77.7 ± 3.1	127.6 ± 7.0
P	<0.01	<0.01

After triple injections of CCl_4 the Co A concentration also fell appreciably (by 20-25%). After multiple injections (15-18) the Co A concentration rose by 25-30% over its normal value.

By analogy with what happens to other enzymes when the liver is damaged, the initial increase in the Co A concentration after a single injection may be explained by the liberation of a larger quantity of Co A after damage to the cell structures with which it is connected. The subsequent fall in its concentration is evidently associated with destruction of Co A.

So far as the increased concentration of Co A after prolonged liver damage is concerned, this is probably mainly due to the ability of the tissues to react and adapt themselves to the noxious agent, to their ability to repair the damage, and so on.

SUMMARY

Following subcutaneous administration of CCl_4 to rats (0.2 ml per 100 gm of body weight) a rise of the Co A content in the liver is seen for the first 12 hours which becomes already manifested after a lapse of 3 hours. With the development of hepatic affection the Co A content tends to decrease by 20% in 24 hours in comparison with the normal value. After a triple CCl_4 administration (in the same dose) the Co A level drops in the affected liver (by 23%); after 15-18 times administration, it goes up and exceeds the initial level by 25-30%.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
