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Abstract

The incorporation of C14-2-glycine into the subcellular fractions of liver, kidney and serum proteins was observed in mice receiving CCl4 injections. The results showed a marked inhibitory effect of CCl4 on incorporation of C14-glycine into each subcellular fraction of the liver, but not of the kidney. The inhibition of the C14-glycine incorporation was most marked in mitochondria, moderate in soluble protein and minimal in microsomes, in the groups of mice given two injections of CCl4. In the animals given CCl. injection, serum albumin is decreased with the decreased incorporation of C14-glycine into the albumin but β-globulin fraction is increased. The former will be the result of the decreased albumin synthesis in the poisoned liver and the latter will be correlated with the fatty degeneration of liver.

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STUDIES ON THE PROTEIN SYNTHESIS IN POISONING

I. THE INHIBITORY ACTION OF CCl_4 ON THE INCORPORATION OF C^{14}-2-GLYCINE INTO THE PROTEIN OF MOUSE LIVER*

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Carbon tetrachloride[1–4] is widely used in some industries, e. g. as solvent in dry cleaning. But, as is well known, CCl_4 is a toxic substance especially for liver parenchymal cells[5]. CHRISTIE and JUDAH[6] ascribed the primary locus of the action of the substance to the membrane of liver mitochondria, and DIANZANI[7–9] showed that CCl_4 results in the uncoupling of oxidative phosphorylation with the loss of pyridine nucleotide and the lowering of the content of adenosine triphosphate in mitochondria. RECKNAGEL and ANTONY[10] showed the activity of magnesium-activated ATP-ase is elicited after the administration of CCl_4, while the activity of dinitrophenol-activated ATP-ase is decreased. Thus, it is very probable that in CCl_4 poisoning the formation of ATP will be lowered resulting in the disturbances in protein synthesis.

Very little information is, however, available as to the protein synthesis in the case of CCl_4 intoxication. The report of CAMPBELL *et al.*[11] is only one concerned with this problem, showing the reduction in protein and nucleic acid of rat liver after receiving one injection of CCl_4.

In this paper the results of the *in vivo* observations on the effects of CCl_4 on the incorporation of C^{14}-2-glycine into the proteins of liver and kidney is presented.

MATERIALS AND METHODS

Sixteen inbred mice (Cb strain) were used. They were divided into two groups. Those belonging to the first group were given subcutaneous injection of CCl_4, twice every other day, 1.5 ml/kg per injection. The day after the final injection of CCl_4, C^{14}-2-glycine (5 μc.) was injected into the peritoneal cavity of mice. Forty-five minutes after the final C^{14}-glycine injection, mice were

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sacrificed by decapitation. Other animals belonging to the second group were injected with CCl_4 six times every other day during 12 days, and C^{14}-2-glycine was injected two days after the final CCl_4 injection. Pair fed control (same indred mice) were injected with C^{14}-2-glycine with the same radioactivity as the poisoned groups. The liver or kidney from the animals was homogenized by Waring blender. After homogenization, the nuclear, mitochondrial and microsomal fractions were separated by the method of SCHNEIDER[12]. Each cellular fraction was washed 3 times successively with 7 per cent trichloroacetic acid (TCA) solution by repeated centrifugation. Thereafter, each precipitate was washed with cold 95 per cent ethanol once, and twice with hot ethanol-ether, then dispersed in 3 ml of 5 per cent TCA and heated for 15 minutes at 90 °C to remove nucleic acid. The residual precipitate was washed twice with 5 per cent of TCA and then with pure ethanol. The residual pellets of protein were homogenized in a petroleum ether-ether-aceton solution (6 : 3 : 1). This suspension was poured into stainless steel discs and evaporated to dryness under infrared rays. Then the dried protein was equiblirated in air at least for one hour, the weight of protein on the disc was determined and its radioactivity was measured by gas flow G. M. counter.[13]

For the determination of radioactivity in serum fractions, just before the sacrifice of mice, the blood was withdrawn by heart puncture in each mouse. The serum separated served for the electrophoresis. Filterpaper electrophoresis was conducted by GRASSMAN and HANNIG's[14] method. As the medium HOLT's buffer[15], a mixture of sodium veronal, sodium-acetate and acetic acid, at pH 8. 6 and ionic strength of 0. 045, was used. The migration time varied from 5 to 8 hours. After drying the paper was cut into 2 parts along the long axis; one half was stained with the solution of brom-phenol blue, $HgCl_2$ and acetic acid adjusted to the final concentration of 0. 05, 1. 0 and 2. 0 per cent respectively. Any excessive brom-phenol blue can be removed by 2 per cent acetic acid. Drying again the paper, the stained proteins were estimated by densitometer with automatic recorder. The other half of the paper, after staining and washing as with the former half, was cut transversely into several pieces of 1 cm wide each. The radioactivity of each piece was established by a gas flow G. M. counter.

For the measurement of change in the relative concentration of serum fractions of poisoned mice, five mice were given subcutaneous injection of CCl_4 (1. 5 ml/kg per injection), 3 times a week for 15 times. The blood was obtained from the tail of mice, before and after 10 and 15 injections. Filter paper electrophoresis was conducted by the same methods described above. Relative concentration of each fraction was measured by staining the proteins with 0. 2 per cent amido-black 10 B (Merk) dissolved in the methanol and glacial acetic acid solution. After washing paper strips with 2 per cent acetic acid solution and

drying, the stained proteins were estimated by densitometer.

For the precise analysis of radioactivity of serum albumin, guinea pigs were given one injection of CCl_4, (1. 5 ml/kg) after 24 hours C^{14}-2-glycine was injected (10 μc.), the blood was obtained by heart puncture 45 minutes later, and a pair belonging to the same group were put together. Control were treated identically same as in the mouse control. With the serum obtained from these blood samples electrophoresis was carried out on starch block prepared by the method described by KUNKEL[16]. The protein of each elute was precipitated and washed with TCA and radioactivity of each precipitate was measured by a gas flow G. M. Counter.

RESULTS

In the case of the mouse liver given two injections of CCl_4, the specific activity of C^{14}-2-glycine in each cellular fraction is 32.5 per cent of that of control in nuclear fraction, 75.0 per cent in mitochondrial fraction, 78.0 per cent in microsomal fraction respectively, and that of serum 66.7 per cent (Fig. 1a), all being the average values of two animals.

In the other set of experiments the symptoms varied from case to case through each animals receiving the same dosage of CCl_4. In severe cases the incorporation of C^{14}-2-glycine into cellular fractions is extremely suppressed (Fig. 1b, solid columns); 8.1 per cent of that of control in mitochondria, 26.9 per cent in microsomes, 20.0 per cent in soluble protein, while in the moderately affected cases (hatched columns) 41.1 per cent in mitochondria, 80.6 per cent in microsomes, 90.9 per cent in soluble protein of the control respectively.

The results also indicate that the inhibiton of C^{14}-glycine uptake into protein of liver cellular fractions is most marked in mitochondria, followed by soluble protein and microsomes, although the incorporation itself is greatest in microsomes, moderate in mitochonrdria and minimal in soluble protein, the order being the same as in the normal liver.

In the cases of the mouse liver two days after 6 injections of CCl_4 the specific activity of cellular fractions is 64.8 per cent of control in microsomes, 71.8 per cent in nucleus, 83.5 per cent in mitochondria (Fig. 2a), all being the average of three animals. The data show a relatively marked decrease in the C^{14}-glycine incorporation into microsomes. The incorporation rate of C^{14}-glycine into each cellular component of kidney in the same group of mice proves to be 84.0 per cent in the nucleus, 92.0 per cent in soluble protein, 95.5 per cent in mitochondria, and 96.0 per cent in microsomes (Fig. 2b), showing a much less inhibitory effect of CCl_4 on the incorporation of C^{14}-glycine into subcellular fractions of kidney than that of liver.

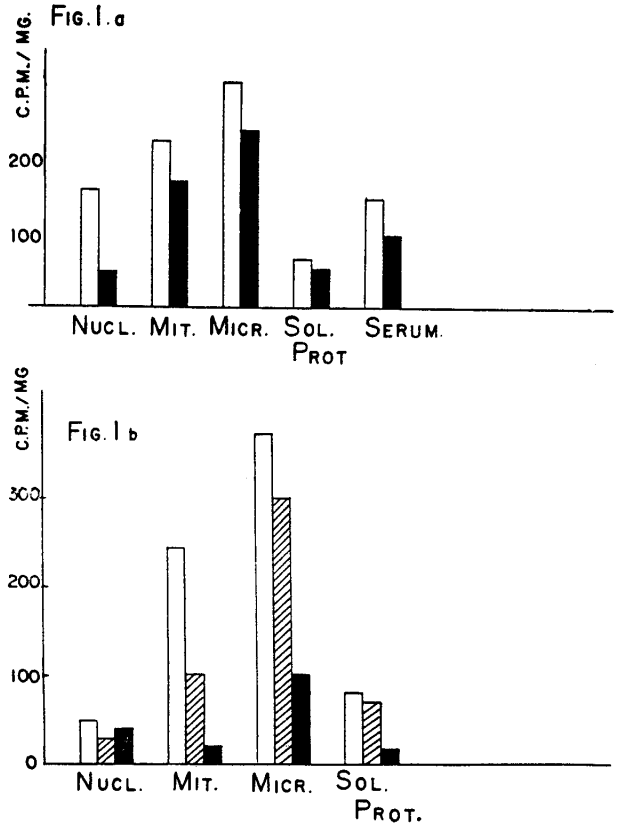

Fig. 1. Rate of incorporation of C^{14}-2-glycine into liver fractions of the mice given 2 injections of CCl_4.

Fig. 1-a. White columns are specific activities of normal control and black columns are those of poisoned mice.

Fig. 1-b. White columns are specific activities of normal control, hatched columns are those of moderate cases, black columns are those of severe cases.

In the serum fractions CCl_4 showed a moderate inhibitory effect on the C^{14}-glycine incorporation into protein. Table 1 shows variations in the amount of serum fractions of the mice injected with CCl_4 several times every other day. The increase in β-globulin is recognized after 10 injections. This increase in β-globulin becomes marked after 15 injections. The increase in γ-globulin is also demonstrated. Albumin is decreased from 45.5 per cent to 35.1 per cent after 10 injections, and further decreases to 33.6 per cent after 15 injections. The paper electrophoretic pattern of the typical case is shown in Fig. 3 a.

The decrease in albumin is distinct by the test on C^{14}-glycine incorporation of serum of mice after two injections of CCl_4 (Fig. 3 b), showing a reduction in

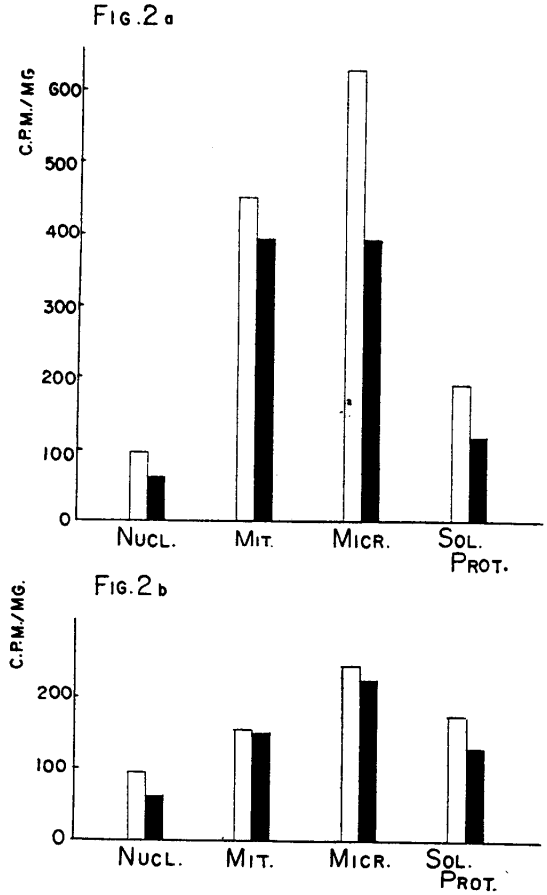

Fig. 2. Rate of incorporation of C^{14}-2-glycine into liver and kidney fractions of the mice given 6 injections of CCl_4. White columns are specific activities of normal control and black columns are those of the poisoned mice. Fig. 2-a represents liver fractions and Fig. 2-b. kidney fractions.

Table 1. Change in serum fractions in mice after subcutaneous injection of carbon tetrachloride. (Each value shows the amount of the fraction appeared on the paper of electrochromatography)

	Albumin	α_1-Glob.	α_2-Glob.	β-Glob.	γ-Globulin
Before inj.	45.5±2.81	11.4±0.89	7.4±0.92	18.7±1.96	17.0±2.13*
After 10 injs.	35.1±4.11	10.0±0.77	9.1±1.55	29.3±3.73	16.5±2.09
After 15 injs.	33.6±3.10	6.9±1.04	7.0±0.60	31.0±3.83	21.6±0.83

* $m \pm \sigma$ (Relative percentage)

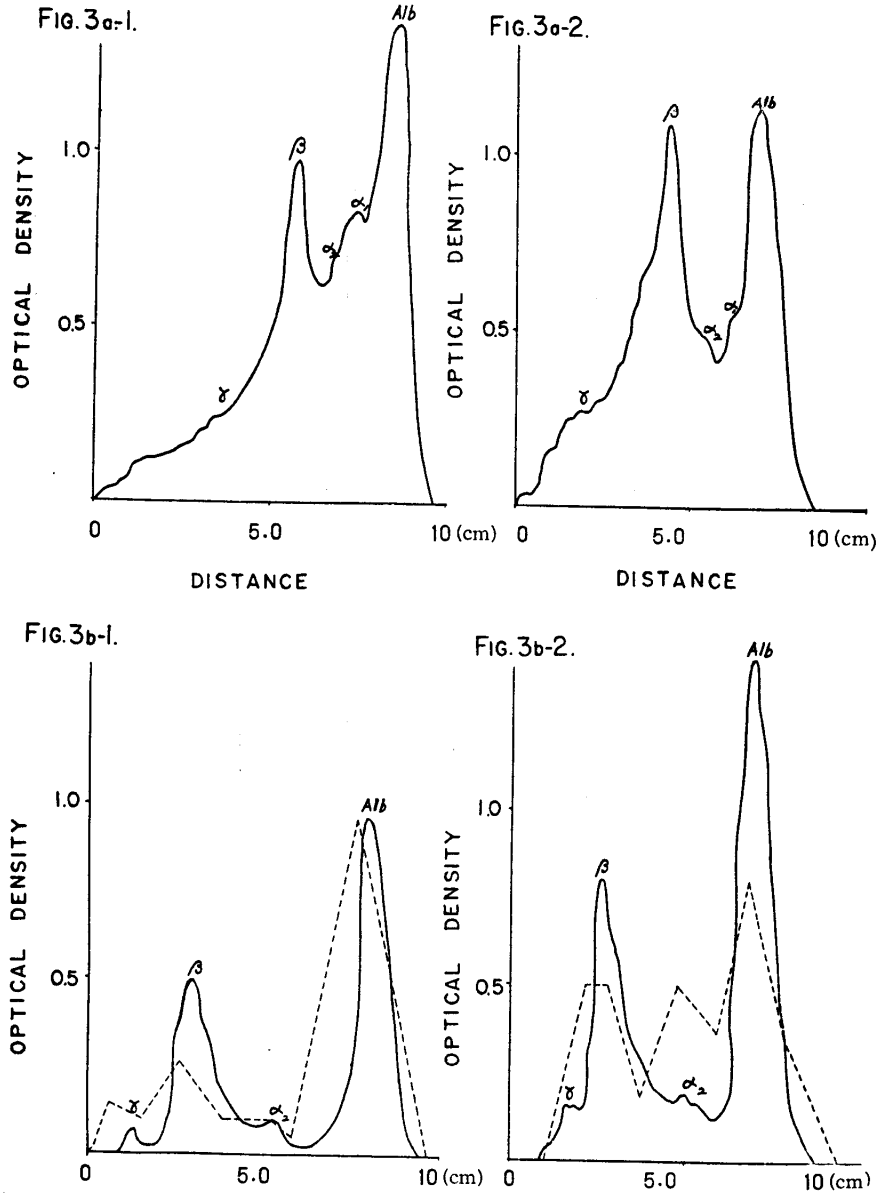

Fig. 3-a. Paper electrophoretic patterns of normal and CCl_4 injected mouse sera. Fig. 3-a-1. Serum of mouse before injection. Fig. 3-a-2. Serum of mouse given 10 injections of CCl_4.

Fig. 3-b. Incorporation of C^{14}-glycine into serum fractions of normal and CCl_4 injected mice. Fig. 3-b-1. Normal control. Fig. 3-b-2. Mouse serum given 2 injections of CCl_4. ——— Optical density of BPB. ····· Radioactivities.

radioactivity of albumin fraction compared with pair fed control. In the experiment of zone electrophoresis the ratio of the specific activity of albumin fraction of guinea pigs after one injection of CCl_4 (1.5 ml/kg) is 68 per cent of that of the pair fed control.

DISCUSSION

The injection of CCl_4 brought about a marked inhibitory effect on the incorporation of C^{14}-glycine into subcellular fractions, most markedly in the mitochondrial fraction. This result is not inconsistent with the findings of CHRISTIE and JUDAH[6] in that the primary locus of the action of CCl_4 is the membrane of the liver mitochondria. However, in every one of our experiments a fair degree of inhibitory effect of CCl_4 was also observed on C^{14}-glycine incorporation in microsomes. If it is accepted that CCl_4 results in the uncoupling effect on oxidative phosphorylation, this effect of CCl_4 in microsomes can reasonably be understood. This reduction in glycine incorporation into microsomes may be related with the reduced serum albumin synthesis which has been demonstrated in this experiment, and it can be deduced from the fact that the liver microsomes are the site of the serum albumin synthesis as PETERS[17] recognized. Besides these, a marked increase in β-globulin fraction has been demonstrated. This increase in serum β-globulin, lipoprotein fraction, in CCl_4 poisoning may have a correlation with fatty degeneration of liver which is general in CCl_4 poisoning. Furthermore, inhibitory effect of CCl_4 on incorporation of C^{14}-glycine into subcellular fraction has been seen on nuclear fraction and supernatant, that is, all cellular fractions in liver. This may be explained by the decrease of ATP, because the results reveal a weaker inhibition of protein synthesis in kidney than that in liver in the present experiment, which correspond with those where the decrease of ATP in kidney is less than that of liver, as described by DIANZANI[9]. Moreover, MCLEAN[18] stated that microsomal and mitochondrial proteins can be synthesized by the addition of pH-5 enzyme independently. From the above results it is possible that the protein synthesis of cellular components (microsomes, mitochondria, and nucleus) are affected independently by the decrease in ATP.

SUMMARY

The incorporation of C^{14}-2-glycine into the subcellular fractions of liver, kidney and serum proteins was observed in mice receiving CCl_4 injections.

The results showed a marked inhibitory effect of CCl_4 on incorporation of C^{14}-glycine into each subcellular fraction of the liver, but not of the kidney. The inhibition of the C^{14}-glycine incorporation was most marked in mitochondria, moderate in soluble protein and minimal in microsomes, in the groups of mice

given two injections of CCl_4. In the animals given CCl_4 injection, serum albumin is decreased with the decreased incorporation of C^{14}-glycine into the albumin but β-globulin fraction is increased. The former will be the result of the decreased albumin synthesis in the poisoned liver and the latter will be correlated with the fatty degeneration of liver.

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