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Studies on the protein synthesis in poisoning. II. Incor-poration of C14-2-glycine into albumin and other protein fractions by liver slices from normal guinea pig and those injected with CCL4*

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Abstract

Protein synthesis of the liver in both normal and CCl4 intoxicatedguinea pigs has been examined in vitro by incubating liver slices with C14-glycine. It has been demonstrated that normal liver slices synthesize albumin in vitro, which in turn incorporates with Cl4-glycine and is finally liberated into the medium very rapidly. On the other hand, immunized lymph nodes, kidney, and spleen do not show any C14-glycine incorporation into albumin. The liver slices of CCl4. intoxicated animal revealed a marked decrease in C14-glycine incorporation into albumin. Observation on the subcellular fractions proved that the incorporation of C14-glycine into microsome fraction is severely arrested, and oxygen consumption of liver slices is only slightly reduced. With the observation on the liver slices incubated with DNP, the author attributes the effect of CCl4 on protein synthesis to the decreased ATP formation by the action of CCl4 as an uncoupler for oxidative phosphorylation.

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STUDIES ON THE PROTEIN SYNTHESIS IN POISONING

II. INCORPORATION OF C^{14}-2-GLYCINE INTO ALBUMIN AND OTHER PROTEIN FRACTIONS BY LIVER SLICES FROM NORMAL GUINEA PIG AND THOSE INJECTED WITH CCl_4

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In the previous paper[1] the author reported that CCl_4 injection inhibits the protein synthesis in mice as revealed by observing the incorporation of C^{14}-glycine into the subcellular fractions in liver and serum albumin *in vivo*.

By using the immunological method, PETERS[2-6] has successfully extracted the labeled serum albumin from the liver slice incubated with C^{14}-glycine, proving that the serum albumin is actually synthesized in liver cells.

The present paper describes the incorporation of C^{14}-2-glycine into protein of each subcellular component and serum albumin of liver slices from the animals with CCl_4 poisoning.

MATEREALS AND METHODS

Four guinea pigs of the same litter served as materials. They were divided into two groups of two animals each. The animals belonging to the first group were injected with CCl_4 subcutaneously, 1.5 ml. once. Those of the second group were pair fed control without treatment. Each animal was sacrificed by drawing blood from heart 21 hours after the final CCl_4 injection with one pair fed control.

Fresh liver was cut into slices by STADIE type slicer and washed in the modified Krebs-Ringer phosphate solution in order to remove the existing albumin. The modified Krebs-Ringer phosphate solution was prepared by mixing stock solutions of 110 ml. of 0.9 per cent NaCl, 4 ml. of 1.15 per cent KCl, 1 ml. of 3.82 per cent $MgSO_4$, and 6 ml. of 0.1 mole of sodium phosphate buffer (pH 7.6).

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The washed liver slices (0.5 g of wet weight) from both normal and poisoned guinea pigs were incubated with C^{14}-2-glycine (5 μc) respectively in Warburg vessels containing the modified Krebs-Ringer phosphate solution.

Two hours after incubation, slices were homogenized with 4 volumes of isotonic sucrose at 0 °C for 5 minutes and the subcellular components were fractionated by the method of SCHNEIDER[7].

The washing, planting and counting of the radioactivity of subcellular fractions were conducted by the methods exactly the same as in the previous paper.[8] Specific activity is expressed as the ratio of radioactivity of glycine to dry weight of protein.

For the estimation of net synthesis of serum albumin in liver slices, the quantitative precipitin curve between guinea-pig serum albumin and antiserum was drawn by the method of KABAT, MAYER and HEIDERBERGER[9], as the standard curve. Slices were homogenized and centrifuged at 10,000 × g for 60 minutes. The clear supernatant containing albumin was separated and divided into two parts, and to each of them antiserum was added, under the same condition as that in the determining of the standard curve. After 24 hours the antibody precipitates were centifuged for 5 minutes and washed 3 times with cold saline solution. By using about one half of the precipitate the amount of antibody precipitate was determined by Folin reagent[10]. The other half of the washed antibody precipitate was further washed 5 times with 5 per cent TCA, poured into count dish with ether-aceton, evaporated to dryness under the infra-red lamp and radioactivity was counted in a gas flow G. M. counter. The anti-albumin used in this experiment was prepared as follows. Guinea-pig serum albumin was obtained from guinea-pig serum by starch block electrophoresis employing the method of KUNKEL[11] as described in the previous paper[1]. Rabbits were injected with alum precipitated albumin[12] at 2 day intervals for 30 days. Dose of a single injection was 10 mg. Blood was taken by heart puncture after an interval of one week, and the serum served as antibody.

Next, using the rat liver slices incubated with C^{14}-glycine identically the same as in the case of the guinea-pig liver slices, supernatant centrifuged 10,000 × g from homogenate was prepared for the electrophoretic analysis[13] as described in the previous paper[1] with Holt's buffer[14]. After developing the proteins the paper was stained with brom-phenol blue, $HgCl_2$, and acetic acid solution, adjusted to the final concentration of 0.05, 1.0, and 2.0 per cent respectively. After washing with 2.0 per cent of acetic acid and drying, the paper was divided into 2 parts cutting longitudinally. One of them was used for the estimation of stained protein by densitometer. The other half was cut into small pieces 1 cm in width each, transversely. From each piece eluated by washing with 0.1 N NaOH, the eluated protein was precipitated by addition of serum protein as

carrier, and thereafter equal volume of 20 per cent TCA. Washing, planting and counting of precipitate were measured by the method described in the previous paper.[8]

Besides these, with the purpose to confirm the absence of serum albumin synthesis of lymph node[8], 3 adult male rabbits were sensitized by giving 8 mg. alum precipitated crystalline ovalbumin subcutaneously at one foot pad once every other day, 14 times for the period of 4 weeks. A few days after the last injection the animals were sacrificed and popliteal lymph nodes from all the three animals were excised. These were put together and minced. The cell minces were incubated with C^{14}-1-glycine for 2 hours at 38°C and paper electrophoresis was performed.

RESULTS

The net synthesis of serum albumin in the liver slices from control animals estimated by using the standard curve appearing in Fig. 1, increased with the lapse of incubation time, reaching a level more than twice the original level after 2 hours, while in the course of CCl_4 poisoning, the net synthesis of serum albumin was found to be very low even after 2 hour incubation (Fig. 2).

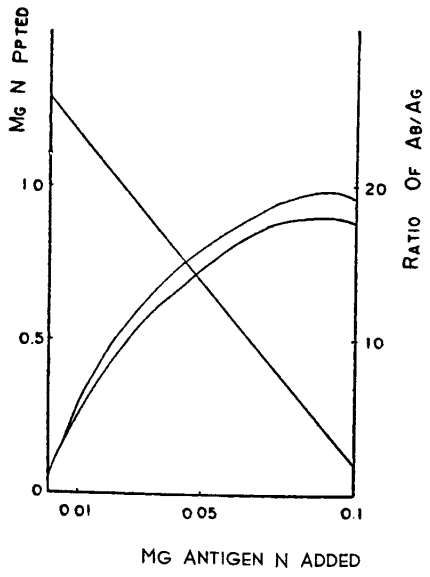

Fig. 1. Quatitative precipitin curves between guinea-pig serum albumin and rabbit antibody.

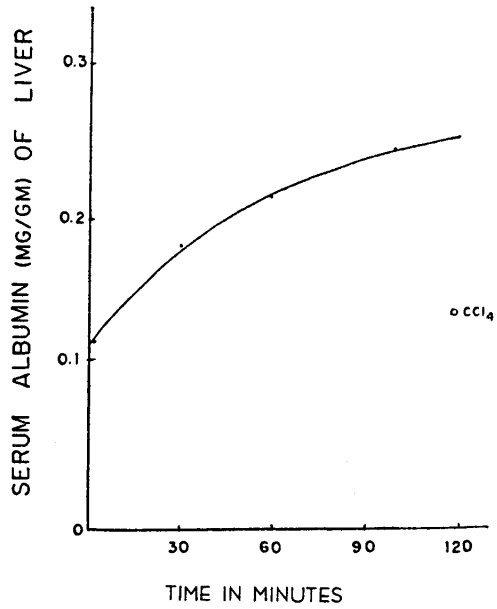

Fig. 2. Increase in the amount of serum albumin content of normal and CCl_4 injected guinea-pig liver slices. —normal liver slices. ∘ CCl_4 injected liver slices.

Observation on the incorporation of C^{14}-glycine into subcellular components and serum albumin obtained from the incubated liver slices also showed a suppressed incorporation rate, especially marked in nuclear, microsomal and albumin fractions (Table 1), and a slight suppression in the fractions of mitochondria and soluble protein. That is, the specific activity of subcellular components of liver from the animals introduced CCl_4 was 22.1 per cent of that of control in nucleus, 75.9 per cent in mitochondria, 39.3 per cent in microsomes, 82.7 per cent in soluble protein, and 25.7 per cent in serum albumin respectively.

Table 1. Radioactivities (c. p. m/mg) of Serum Albumin and Subcellular Components from the Liver Slices of Normal and CCl_4 Intoxicated Animals, incubated with C^{14}-2-Glycine for Two Hours at 38°C.

		Spec. activity		Average	Pois/Nor*
Nucleus	Normal	276.0,	316.0	291.0	22.1%
	Poisoned	49.1,	80.4	64.5	
Mitochondria	Normal	455.0,	380.0	417.5	75.9%
	Poisoned	348.0,	282.0	315.0	
Microsomes	Normal	776.0,	765.0	770.5	39.3%
	Poisoned	225.0,	381.0	303.0	
Sol. Protein	Normal	348.0,	308.0	328.0	82.7%
	Poisoned	269.0,	274.0	271.0	
Albumin	Normal	6550.0,	5510.0	6030.0	25.7%
	Poisoned	1700.0,	1540.0	1620.0	

* Ratio of poisoned slice to normal one.

The oxygen consumption of liver slices from the animals poisoned with CCl_4 was only 78. 1 per cent of pair fed control, i. e. the respiration was affected rather less than what was expected from the decrease in the incorporation of C^{14}-glycine into protein (Fig. 3).

From the findings showing a high specific activity of albumin (Table 1), it is obvious that serum albumin is synthesized in liver. This fact has again been reconfirmed by observing the incorporation of C^{14}-glycine into serum albumin by paper electrophoresis.

As can be seen in Fig. 4 a, the ratio of specific activity of the serum albumin released into the medium from the rat liver slices incubated with C^{14}-glycine is markedly high compared with that of other proteins determined by paper electrophoresis. The soluble proteins remaining in the liver slice do not demonstrate any albumin fraction nor radioactivity, suggesting that synthesized albumin is easily liberated from the slice (Fig. 4 b). DNP (5×10^{-4}M) added to

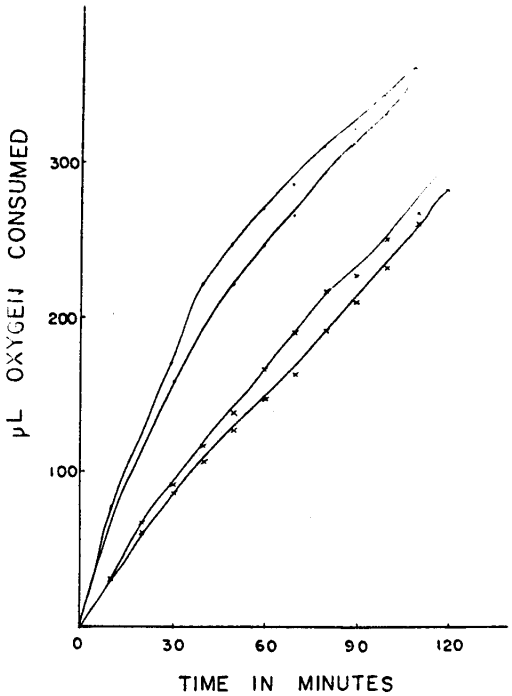

Fig. 3. Rate of oxygen uptake of normal and CCl_4 injected liver slices (500 mg. of weight). ·—·—·— Normal liver slices. -×-×-×- CCl_4 injected liver slices.

the medium inhibited markedly the incorporation of C^{14}-glycine into albumin and other proteins (Fig. 4 c).

Similar observations on the supernatants from kidney and spleen of normal adult rat and immunized lymph nodes proved that the labeling of serum albumin can not be attained, suggesting that the serum albumin is synthesized solely in the liver cells. In the case of protein fractions from the popliteal lymph nodes of sensitized rabbits, the radioactivities by paper electrophoresis showed a high specific activitity in the γ-globulin fraction which will be antibody for the ovalbumin antigen (Fig. 4 d, e), but not in serum albumin.

DISCUSSION

As reported precisely in the previous paper, in the peripheral blood of mice injected with CCl_4 the amount of serum albumin decreases, while β-globulin increases. The increase in β-globulin may be correlated with the severe fatty degeneration of liver by CCl_4 intoxication, and decrease in albumin with the suppressed albumin synthesis in the degenerated liver. To clarify whether these

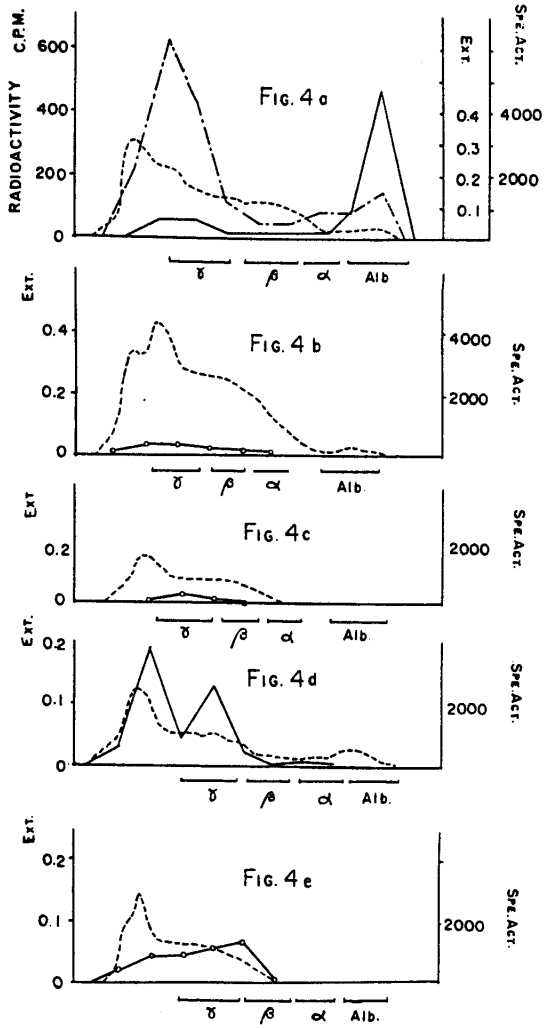

Fig. 4. Incorporation of C^{14}-1-glycine into albumin and soluble protein of rat liver slice and soluble protein of immunized lymph nodes of rabbit (by paper electrophoresis, slices and cell minces were incubated at 38°C for 2 hours) ---------- Optical densities. -·-·-·- Radioactivities. ——— Ratio of specific activities. (Radioact./ Opt. dens.)

Fig. 4-a. Albumin and other soluble protein released from liver slice into medium during incubation.

Fig. 4-b. Protein remaining in the liver slice.

Fig. 4-c. Protein released into medium containing DNP, 5×10^{-4}M.

Fig. 4-d. Protein fractions released from cell minces of immunized lymph nodes into medium during incubation.

Fig. 4-e. Protein fractions remaining in the cell minces of immunized lymph nodes.

suppositions are correct or not, *in vitro* experiment for serum albumin production in liver cells from the animals of CCl_4 intoxication may give a clear-cut answer.

By incubating liver slices of normal animals with C^{14}-glycine, it has been definitely proven that serum albumin is selectively synthesized *in vitro*, showing a marked incorporation of C^{14}-glycine as revealed by both immunological and paper electrophoretic methods. Lymph node, kidney, and spleen showed no synthesis of albumin. These results show that the production of albumin is done solely in liver cells.

The similar experiment repeated with the liver slices of guinea pigs injected with CCl_4 proved again a positive synthesis of albumin but a remarkable suppression comparing to the normal level, indicating that CCl_4 arrests directly the serum albumin synthesis in liver, resulting in the decrease of albumin in blood sera.

Observation on the subcellular components revealed that CCl_4 suppresses the C^{14}-glycine incorporation into microsomes and nuclei. The suppressed incorporation into nuclear fraction may or may not be correlated with the suppressed incorporation in microsome, however, the decreased incorporation into microsomal protein will be correlated directly to the decreased synthesis in albumin. In this suppression of microsomal synthesis of protein, the suppressed activity of pH-5 enzyme or the system concerned with ATP synthesis should be responsible. Observations on oxygen consumption of CCl_4 poisoned liver proved that O_2-consumption is not so severly arrested, but it is thought that oxidative phosphorylation is possibly arrested independently of the normal functioning of electron transport system as pointed out by HABA[15]. The similar experiment by using DNP instead of CCl_4 resulted in the suppression of glycine incorporation into albumin giving actually the same results as in CCl_4 intoxication. This suggests that CCl_4 will act as uncoupler of P*i* in the formation of ATP and results in the suppression in protein synthesis.

CONCLUSION

Protein synthesis of the liver in both normal and CCl_4 intoxicated guinea pigs has been examined *in vitro* by incubating liver slices with C^{14}-glycine.

It has been demonstrated that normal liver slices synthesize albumin *in vitro*, which in turn incorporates with C^{14}-glycine and is finally liberated into the medium very rapidly. On the other hand, immunized lymph nodes, kidney, and spleen do not show any C^{14}-glycine incorporation into albumin.

The liver slices of CCl_4 intoxicated animal revealed a marked decrease in C^{14}-glycine incorporation into albumin. Observation on the subcellular fractions proved that the incorporation of C^{14}-glycine into microsome fraction is severely

arrested, and oxygen consumption of liver slices is only slightly reduced. With the observation on the liver slices incubated with DNP, the author attributes the effect of CCl_4 on protein synthesis to the decreased ATP formation by the action of CCl_4 as an uncoupler for oxidative phosphorylation.

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