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Abstract

In connection with the cloudy swelling of the liver cell seen in the CCl4 intoxication the author observed the oxygen consumption rate of the liver slices at frequent intervals within 20 hours of CCl4 intoxication in rats. Unexpectedly, the oxygen consumption did not decrease by CCl4, intoxication in the stage where the cloudy swelling can be seen, especially in the media added with succinate. This finding suggests that the energy produced by respiration is not concerned with the swelling phenomenon in the case of CCl4 intoxication, differing from the supposition on the experiment of protozoa by using cyanide and others.

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MORPHOLOGY OF MITOCHONDRIA AND CELL RESPIRATION

III. BIOCHEMICAL STUDIES ON CELL RESPIRATION OF THE RAT LIVER IN CARBON TETRACHLORIDE POISONING

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In the previous papers the author studied the process of cell degeneration using the liver cell of rats given carbon tetrachloride (CCl_4) revealing that the activities of cytochrome c oxidase and succinic dehydrogenase of the liver do not decrease within twenty hours after the CCl_4 administration, whereas the swelling of both cytoplasm and mitochondria was marked in this stage as observed by light and electron microscopes[1]. This fact is inconsistent with the view presented by ROBINSON; i. e. the swelling of cell is caused by the inhibited oxygen consumption as the cell maintains its normal volume by the incessant pumping out of water, the energy of which is given by respiration[2]. Actually, it is proved that the cells swell easily by the action of some agent like cyanide, the arrest of the cytochrome c oxidase system, even in the isotonic solution. It is well known fact that the enzyme system concerned with respiration is mainly located in mitochondria[3,4,5,6]. From the chemical and morphologic observations GREEN demonstrated that the unmolested double membrane structure of mitochondrial cristae is essential for the function of the mitochondrial enzyme system, even the mitochondria themselves are cut into small segments[7]. Morphologic structure of the mitochondria of liver in CCl_4 intoxication showed a well maintained double-layer structure of mitochondrial cristae, and this will be the main reason why the degenerated cells still keep a high level of the activity of respiratory enzymes as stated in the previous report. The data suggested that the swelling of liver cells in CCl_4 intoxication should be caused by a mechanism other than the lowered activity of respiratory enzymes and the lowered oxygen consumption. But before drawing this conclusion, it seems to be necessary to confirm that the persistent activity of the enzyme system demonstrated by the histochemical and chemical methods means actually the unmolested oxygen consumption of living liver cells. For the purpose to confirm this the author

measured the oxygen consumption of the tissue slices by Warburg's method from the liver of rats treated with CCl_4 in the similar way as in the cases of the previous experiment[1].

MATERIALS AND METHODS

Eighty hybrid male adult rats weighing 100—150 g. served as materials. Seventy of them were given 0.25 ml. of CCl_4 per 100 g. of body weight by oral administration using a fine gum catheter. Other ten animals were fed controls. Ten animals each were sacrificed by decapitation at 1.5, 5, 6, 10, 17, 20, and 22 hours after the CCl_4 administration and their livers were removed immediately. These fresh livers were sliced at once with a razor, less than 0.5 mm. thick and about 0.5 to 1.5 cm^2 in size. The oxygen consumptions of these tissue slices were measured by Warburg's manometric method[8,9,10] using the apparatus of Shinko Kagaku Co. Two to three pieces of them were put into the main chamber of Warburg's vessels, 18 ml. in volume, and floated in 10 ml. of Ringer's solution. Three tenths ml. of 20 per cent potassium hydroxide solution was poured into the "inset", which had a rolled filter paper for the enlargement of the CO_2 resorption space.

In one material the experiments were made in two series two in each, two supplemented with sodium succinate as the substrate and other two without substrate. The substrate in Ringer's solution, 0.3 ml., was in the "side". These operations were done in the cold room at 4 °C. Then the troughs were connected with the manometers, whose capillaries were filled with Brodie's fluid. The spaces between the vessels and manometers were filled with oxygen. These were set on incubating bath, 37.5 ± 0.2 °C, and shaken, 65 shakes per minutes and 6 cm. in amplitude. After 30 minutes the substrate, sodium succinate, was added to the tissue slices in the main chamber, 3×10^{-4} *M*ol. in final concentration. As the control a manometer connected to the respiration-trough with the reacting media only served the purpose. Oxygen consumption rates were recorded at each 10 minutes for 1—2 hours. After the observation in one group the slices were put into a small glass vessel, dried at about 120 °C for 2 hours and measured in dry weight. In other group the slices with the reacting media were transferred into a glass homogenizer and homogenized treating gently. The numbers of nuclei in the homogenate were calculated.

RESULTS

In the tissue slices without any substrate the oxygen consumption of the liver decreased gradually within 4 to 6 hours but it reached a constant level which was maintained till 17 hours after the CCl_4 administration and then de-

creased slowly till 20 hours reaching the level of about 70 per cent of the original value though the oxygen consumption rate gave a fairly varied values from case to case (Fig. 1).

Little difference was observed between those values of Qo_2 calculated from the dry mass of the tissues and the cell number or the number of nuclei, but some delicate changes seem to be demonstrated in the latter, a rather marked fall in the early stage and a slow decrease in the latter stage (Fig. 1).

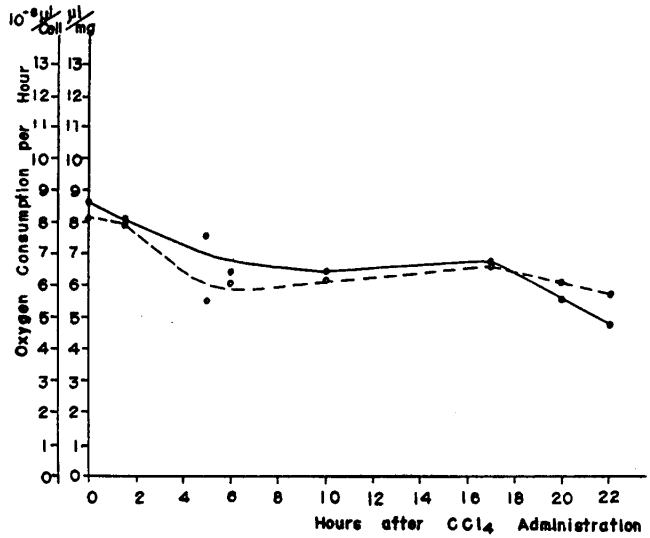

Fig. 1. Oxygen consumption of the rat livers after CCl_4 administration. Measured without addition of succinate. Solid line and black dots show the oxygen consumption per mg. of dry weight and the broken lines and the circles show the values per cell. Each of them is the mean value of ten animals.

In the cases added the substrate, sodium succinate, to the reacting media a considerable increase in oxygen consumption was observed in the early stage of CCl_4 intoxication i. e. it measured by about 50 per cent after about 1.5 hours and then decreased to the original level 5 to 6 hours after the CCl_4 administration. This level persisted for about 11 hours and then decreased gradually reaching the level of about 85 per cent of the original (Fig. 2). The result showed a good coincidence with the changes in succinic dehydrogenase activity presented in the previous paper[1].

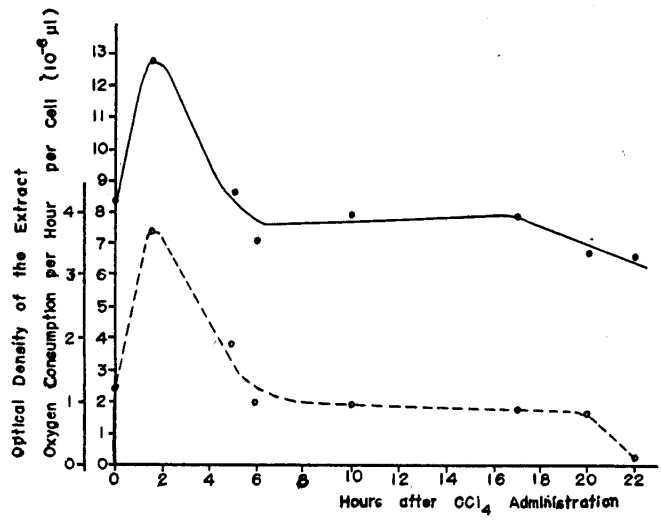

Fig. 2. Oxygen consumption of the rat liver of CCl_4 intoxication and the succinic dehydrogenase activity of the liver. Solid line and black spots show the oxygen consumption of the liver slices in the media containing succinate. Broken lines and circles show the succinic dehydrogenase activity measured by using neotetrazolium (Method: see the previous paper).

DISCUSSIONS

In the field of pathology the cell degeneration is one of common phenomena but the changed metabolic process correlating with the morphologic change of the cell is not yet established. Concerning the cloudy swelling the process of the increase in cell volume is generally thought to be the loss of the regulating mechanism of the cell volume by pumping out water from the inside of cell[2,11,12]. It is a well-known fact that if the cells die, they swell rapidly even in the isotonic media[11]. ROBINSON[2] asserted the energy required for the pumping water out of the cell is given by the respiration and linked to the ATP formation. Many agents acting to suppress the cell respiration like cyanide, sodium azide, etc. act as to bring about the cell swelling when they are added to the media. ROBINSON[2] stated CCl_4 will also act as to suppress the respiration and the cell swelling. This may be true in the case of some protozoa, but in the case of CCl_4 intoxication of the animals the mechanism of the cell swelling of the liver will be due to some other mechanism. As presented in this paper no decrease in the oxygen consumption of the liver tissue was observed in the stage of cloudy swelling induced by CCl_4 intoxication. Succinic dehydrogenase activity demonstrated in the liver of CCl_4 intoxication closely correlated with the respiration of the tissue elucidating that the enzyme activity demonstrable by the chemical

or histochemical method will directly show the respiratory activity of the cell.

Probably the swelling of the cell in CCl_4 intoxication would be due to the mechanism having no relation with the respiration. It may be due to the changed permeability of the cell membrane induced by CCl_4, which is a non-polar lipophilic substance and may result in the changed characteristics of the lipid membrane of both cytoplasm and mitochondria though the true mechanism is at present unknown.

SUMMARY

In connection with the cloudy swelling of the liver cell seen in the CCl_4 intoxication the author observed the oxygen consumption rate of the liver slices at frequent intervals within 20 hours of CCl_4 intoxication in rats. Unexpectedly, the oxygen consumption did not decrease by CCl_4 intoxication in the stage where the cloudy swelling can be seen, especially in the media added with succinate. This finding suggests that the energy produced by respiration is not concerned with the swelling phenomenon in the case of CCl_4 intoxication, differing from the supposition on the experiment of protozoa by using cyanide and others.

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REFERENCES

1. HABA, K. : Morphology of mitochondria and cell respiration. I. Morphologic studies on the rat liver and its mitochondria in carbon tetrachloride poisoning. Acta Med. Okayama, 14, 227, 1960; II. Histochemical study on the liver with experimental carbon tetrachloride poisoning. Acta Med. Okayama, 15, 153, 1961.
2. ROBINSON, J. R.: Secretion and transport of water. Symposia of the Society for Experimental Biology. VIII. Active transport and secretion, 42, Cambridge Univ. Press, London, 1954.
3. BENSLEY, R. R. and HOERR, N. L. : Studies on the cell structure by the freezing-drying method. V. The chemical basis of the organization of the cell. Anat. Rec. 60, 251, 1934; VI. The preparation and properties of mitochondria. Anat. Rec. 60, 449, 1934.
4. HOGEBOOM, G. H., and SCHNEIDER, W. C. : Physical state of certain respiratory enzymes in mitochondria. J. Biol. Chem. 194, 513, 1952.
5. HOGEBOOM, G. H. CLAUDE, A. and HOTCHKISS, R. D.: The distribution of cytochromoxidase and succinoxidase in the cytoplasm of mammalian liver cell. J. Biol. Chem. 165, 615, 1946.
6. ODA, T. : Cytochemical and biochemical studies on the terminal electron transport system. I. Analytical studies on the colorimetric estimations of the activities of the succinic dehy-

drogenase system and cytochrome oxidase. Symposia Cell. Chem. **8**, 157, 1958. II. Cytochemical demonstration of the succinic dehydrogenase system and cytochrome oxidase in mitochondria with the electron-microscope. Symposia Cell. Chem. **8**, 173, 1958.

7. GREEN, D. E. : Mitochondrial electron transport system. Symposia Cell. Chem. **8**, 145, 1958.
8. WARBURG, O. : The metabolism of tumours. Constable, London, 1930.
9. YOSHIKAWA, H. and *et al.*: Warburg's manometer. Kagaku no Ryoiki, Suppl. **13**, Nankodo, Tokyo, 1954. (in Japanese).
10. FUJITA, A. : Manometric method and its application in the field of medical and biological studies. Iwanami Shoten. Tokyo, 1949. (in Japanese).
11. OPIE, E. L. : An osmotic system within the cytoplasm of cells. J. Exp. Med. **87**, 425, 1948.
12. OPIE, E. L. : The movement of water in tissues removed from the body and its relation to movement of water during life. J. Exp. Med. **89**, 185, 1949.