(2) Fluoride is known to be a powerful inhibitor of lipase[8,4]; for this reason our experiments have been carried out with fluoride 0.05 *M*.

(3) For short chain fatty acids (butyric), we have extracted the enzymes from a liver acetone powder by phosphate buffer 0.0128 *M* pH 7.7; it was demonstrated that fluoride completely inhibits any lipase activity in such conditions[5].

Apart from the above evidences, we usually have 2 controls in our determinations, i. e. without substrate and without ATP, in order to ascertain that hydroxamic acid formation we measure is merely due to presence of ATP.

Results. The force feeding of CCl_4 resulted in (a) an increase of total liver fat at 3–4 h, according to RECKNAGEL[1] and (b) a diminished activation of palmitic acid which appears to be almost contemporary to the fatty infiltration and in any case is already statistically significant 5 h after poisoning (Table).

CCl_4 added *in vitro* inhibited the activation of palmitic and butyric acid (Fig.).

Discussion. The experimental work reported here supports the view that the impairment in the ability to activate fatty acids is a direct result of CCl_4 hepatotoxicity. From other experiment[5], we have evidence that the activation takes place in the mitochondrial and microsomal fractions[10]. It is well known that an activation is the preliminary operation for fatty acid oxidation and for synthesis of phospholipids and triglycerides[11–14].

The fact that this activation is very precociously impaired shows that an enzymic lesion has set in just in the period when liver fat is rising. This lesion seems to represent one of the points of the pathological sequences correlated to the early increase in liver fat, which intervenes long before any demonstrable mitochondrial damage as defined[1,2] in terms of ATP ase transformation, loss of pyridine-nucleotide-dependant oxidative function, uncoupling of oxidative phosphorylation and impairment in the ability to reaccumulate potassium.

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Istituto di Patologia generale, Università di Padova (Italy), May 23, 1960.

Zusammenfassung

Bei CCl_4-Vergiftung zeigt die Leber eine vermehrte Aktivierung der Fettsäuren, die sich fast gleichzeitig mit der fettigen Infiltration kundgibt. Die Abnahme der Aktivierung äussert sich bereits 5 h nach der Vergiftung in statistisch signifikanter Weise. Zugabe von CCl_4 hemmt *in vitro* die Aktivierung von Palmitin- und Buttersäure.

The Histochemistry of the Dehydrogenase Systems in the Aortae of Rabbits with Experimental Atherosclerosis

In a previous work[1] we have studied alkaline and acid phosphatases, non-specific esterase and succinic dehydrogenase (the latter enzyme by means of blue tetrazolium and neotetrazolium) in the aortae of atherosclerotic rabbits and rats. Recently developed methods for succinic dehydrogenase[2], DPN-diaphorase[3], TPN-diaphorase[4], and the dehydrogenases linked to the latter based on the use of 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (= nitro-BT) as the chromogenic electron acceptor gave the possibility of investigating the above-mentioned enzymes also in the vascular wall.

The present communication deals with results of a histochemical investigation of succinic dehydrogenase, DPN-diaphorase, TPN-diaphorase, lactic dehydrogenase, malic dehydrogenase, glutamic dehydrogenase, and glucoso-6-phosphate dehydrogenase in the aortae of 9 rabbits with cholesterol atheromatosis (produced by addition of 1 g cholesterol to the daily diet for 4 months) and 5 control animals. Directions given by PEARSE[5] have been adhered to. The tetrazolium used was nitro BT.

Activity of the enzymes mentioned in normal aortae can be detected chiefly in the muscle cells of media (in form of small granules-mitochondria) in decreasing order as follows: DPN-diaphorase, lactic and malic dehydrogenases, TPN-diaphorase, succinic dehydrogenase; the activity of glutamic and glucoso-6-phosphate dehydrogenases was negligible. The activity of the endothelium and cellular elements of the intima is weaker, and in some cases cannot be detected in the sections at all. In adventicial fibrocytes a more pronounced activity could be ascertained.

In rabbits with experimental atherosclerosis, enzyme activities in media and adventicia had mostly the same localisation and intensity as in the control samples.

An interesting pattern could be observed in the plaques. DPN-diaphorase (Fig. b), lactic dehydrogenase (Fig. c), malic dehydrogenase, and TPN diaphorase are here localised in the macrophages (lipophages), and in the fibrocytes ('Faserzellen' Benninghoff) in the form of small granules penetrating into all cellular processes. Preparations stained with Oil Red O showed that the localisation of enzymes activities were not always identical with the localisation of lipids. In the same elements succinic dehydrogenase (Fig. a) could be detected, but the corresponding activity was much slighter. A minute activity was shown by glucoso-6-phosphate (Fig. f) but only in some of the plaques. Similarly, glutamic dehydrogenase activity (Fig. e) which was the slightest of all the enzyme systems investigated, was weakly positive only in some cases in the surface parts of the plaques. It must be emphasized that the stain intensities of all the enzymes studied showed differences, which were most apparent upon comparing small plaques with large ones. In the small plaques with a small number of lipophages, reaction was very intense in nearly all cellular elements. In the large plaques, where substantial amounts of cholesterol could be detected, activity was only in the lipophages and fibrocytes at the surface and in depth close to the media. In the center of the plaques which showed the most intense reaction for cholesterol, enzyme reactions were negligible (Fig. b). Above the small plaques and in the neighbourhood of some larger plaques, reactions were rather pronounced also in the endothelia. Similar reactions are also shown by plaques on the semilunar valves (Fig. d).

The reported findings of high activity of the dehydrogenase systems in the small plaques present, as well as the previously reported activities of alkaline and acid phosphatases, and non-specific esterase, further evidence

Further work on the dehydrogenase systems in plaques in rabbit, rats, and humans is in progress and will be reported in detail elsewhere.

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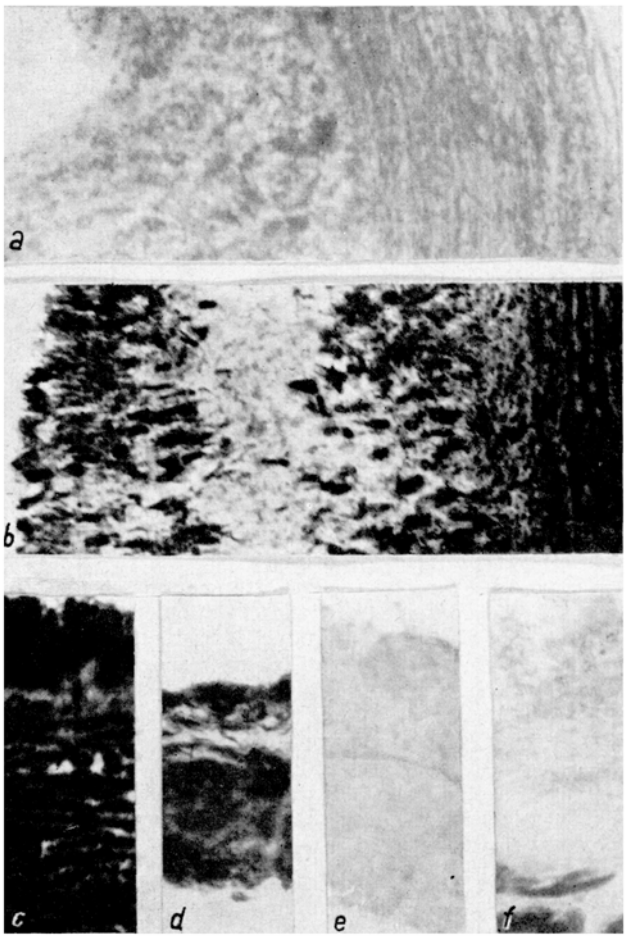

All microphotographies refer to the aortae of atherosclerotic rabbits. a) Succinic dehydrogenase. Slight positive reaction in muscle cells of media (right half of the Figure) and in the macrophages (lipophages) and fibrocytes (left half of the Figure) of intima. b) DPN-diaphorase. Strong positive reaction in macrophages and fibrocytes on surface of the plaque (left quarter) and in muscle cells of media (right quarter). In the center of the plaque (second quarter) negligible reaction. c) Lactic dehydrogenase. Intense reaction in the plaque (top) and in muscle cells of media. d) Lactic dehydrogenase in the plaque (bottom) on the semilunar valve (compare with Fig. c). e) Glutamic dehydrogenase. Reaction is almost negative. f) Glucoso-6-phosphate dehydrogenase. Weakly positive reaction in some cellular elements of the plaque, a stronger one in adjacent myocardium (bottom).

in favour of the concept of relatively high metabolic activity of the intima in the initial stage of the atheromatous lesions.

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Zusammenfassung

In Aorten von Kaninchen mit experimenteller Atherosklerose wurden die folgenden Dehydrogenasen untersucht: DPN-Diaphorase, Lactat- und Malat-Dehydrogenase, TPN-Diaphorase, Succinat-, Glucoso-6-phosphat- und Glutamat-Dehydrogenase. Die Aktivität der fünf erstgenannten war in den Endothelien, Makrophagen (Lipophagen) und Fibrozyten der verdickten Intima und kleiner atherosklerotischer Beete deutlich erhöht. Die Befunde stehen im Einklang mit der Ansicht, dass die Stoffwechselaktivität der Intima am Anfang der experimentellen Atherogenesis erhöht ist.

The Effect of Litter Rank on the Development of Mouse Lymph Nodes

Evidence has been obtained that maternal age influences the occurrence of mongolism in human beings[1] and that the incidence of spontaneous[2-4] and induced[5] tumors in mice varies with litter rank. The mechanism by which these effects are produced is unknown. The present studies, however, indicate that in young mice of certain strains lymph node size changes significantly in successive litters. Since lymphatic tissue is an important part of host defense, the possibility exists that the influence of litter rank in tumor growth might be mediated through an alteration in lymph node development.

Female mice were used throughout. They were weaned at 5 weeks, thereafter housed 5–12/cage, kept on racks in an air-conditioned room, and fed water, and Purina Laboratory Chow *ad libitum*. At sacrifice, by cervical dislocation, two elbow and two inguinal nodes were removed, trimmed of extraneous tissues, blotted, and the combined weights determined on a Roller-Smith torsion balance.

In the first experiment the lymph node and body weights of 6–7 $^1/_2$ week old second and fourth litter low-tumor C57BL/Sp mice were compared. Mean body and lymph node weights (standard error in parentheses) for 7 second litter mice were 15·9 (0·3) g and 11·6 (0·6) mg respectively, while those for 7 fourth litter mice were 14·0 (0·5) g and 8·3 (0·5) mg respectively. Highly significant ($P < 0{\cdot}01$) decreases in both body and lymph node weights of fourth litter C57BL/Sp mice were observed. On the other hand, similar measurements on first and fourth litter low-tumor $C3H_f$/AnSp mice showed no significant litter rank difference.

In the second experiment (Table I) body and lymph node growth were investigated in C57BL/Sp mice of first through fourth litters by comparing their weights in mice 3 and 6 weeks of age. No significant differences were observed at 3 weeks. Significantly lower body and lymph node weights of mice of the third and fourth litters were found at 6 weeks ($P < 0{\cdot}001$ and $< 0{\cdot}05$ respectively). Smaller percent increases in lymph node weight during the 3–6-week interval were found in mice of second and third litters than in first litter mice. In marked contrast, no such trend was observed in the body growth of successive litters.

Recently[6] it was observed that in foster-nursing mice from strains characterized by large lymph nodes to mothers possessing small nodes the young developed smaller lymph nodes than non-foster-nursed controls. Conversely, foster-nursing mice with normally small nodes to mothers with large lymph nodes resulted in large nodes in the young. These results indicated that a maternal-infant relationship in mice during the nursing period, presumably mediated *via* the milk, is important in lymph node development.

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