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On the mechanism of the lipolytic action of the lipaemia-clearing factor

In a study of the composition of the mixture of glycerides formed during the heparin-induced lipaemia-clearing reaction[1] it was found that the reactions triglycerides → diglycerides and diglycerides → monoglycerides proceeded at rather similar rates. *In vitro* as well as *in vivo* there was during the earlier phase of the reaction a considerable accumulation of monoglycerides, *i.e.* the reaction monoglyceride → glycerol proceeded relatively slowly. This suggested that the clearing factor might have a specific action on the α-ester bond in the glyceride molecule. If that was so the resulting β-monoglyceride would not be so readily attacked and thus the monoglyceride concentration would increase. A further investigation of this possibility is presented in this paper.

Rat chylomicrons, obtained after cannulation of the thoracic duct[2], were flotated in a Spinco ultracentrifuge and washed twice in slightly buffered saline. The purified chylomicrons were incubated with post heparin clearing factor, prepared from human plasma according to NIKKILÄ[3], in the presence of 5% human serum albumin. 10 mg 1-^{14}C-oleic acid dissolved in 5% serum albumin solution were added to the reaction mixture that had a final volume of 2,000 ml, the final concentration of glyceride being 2 mg/ml. The reaction was carried out at 37° in a phosphate buffer, pH 7.5, and ionic strength 0.05.

The optical density at 700 mμ was registered and at different time intervals 100 ml of the incubation mixture were extracted with 2 l alcohol-ether (3:1). After filtration, the extracts were concentrated to a small volume under reduced pressure at 60° C. The residue was repeatedly extracted with light petroleum ether, the combined extracts dried with sodium sulphate and taken to dryness *in vacuo*. The phospholipids were separated off on a silicic acid column[4], and to obtain complete separation the phospholipids were afterwards precipitated with acetone and $MgCl_2$ in the cold. The glycerides and the free fatty acids obtained from the silicic acid columns were separated on columns of the ion-exchanger IRA-400[5]. Tri-, di- and monoglycerides were separated chromatographically on a silicic acid column[6], using 10 g silicic acid for about 100 mg glyceride mixture. The glycerides were saponified and the fatty acids separated from the unsaponifiable matter by extraction[5].

Radioactivity was determined after mounting 1 mg of fatty acid on aluminium planchettes. At least 1,000 counts were counted.

The specific activities of the phospholipid fatty acids were in all fractions less than 1% of the specific activities of the free fatty acids. The traces of activity found in these fractions were probably contaminants from the other lipid fractions.

In Fig. 1 (upper part) the course of the hydrolysis can be followed from the decrease in optical density and the release of fatty acids. After about 6 h, the reaction had reached a state of equilibrium where the optical density remained practically constant and no additional amounts of fatty acids were released. The specific activity curves for the free fatty acids and glyceride fatty acids are seen in the lower part of the same figure. As is apparent, there was a rapid incorporation of the labelled free acid into the fatty acids of the glycerides simultaneous with a dilution of the labelled free acid with acids released from the glycerides. After a few hours the specific activity curves for the free fatty acids and the glyceride fatty acids are roughly parallel, indicating that an equilibrium has been reached. Here, however, there is still a difference in the specific activities of the two fractions, the specific activity of the glyceride fatty acids only reaching around 60% of that of the free fatty acids. The specific activity of the glyceride fatty acids relative to that of the free fatty acids is seen in the small figure included in the lower part of Fig. 1. This difference in the specific activity of the glyceride fatty acids and the free fatty acids indicates that only part of the glyceride fatty acids are exchangeable.

In Table I the specific activity of the fatty acids of the different glyceride fractions at 15 h are given in per cent of the specific activity of the free fatty acid at the same time. The fatty acids of the tri-, di- and monoglycerides reached a percentage specific activity of about 70, 40 and 20, respectively.

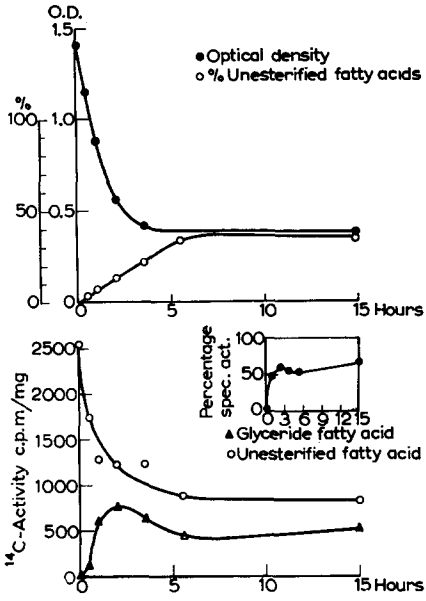

Fig. 1. For explanation see text.

TABLE I

SPECIFIC ACTIVITY AND PERCENTAL SPECIFIC ACTIVITY OF THE DIFFERENT GLYCERIDE FRACTIONS AFTER 15 h INCUBATION

	Specific activity c.p.m./mg	*Specific activity in per cent of the specific activity of the free fatty acids*
Triglycerides	573	68
Diglycerides	348	41
Monoglycerides	170	20
Free fatty acids	841	(100)

From these results it is quite evident that *in vitro* there was a rapid exchange between the albumin-bound unesterified fatty acids and the glyceride fatty acids of the chylomicrons. Whether or not this exchange is due to the formation of new glyceride ester bonds or to a transacylation is impossible to say from these experiments. However, the fact that the hydrolysis of the triglycerides does not proceed to completeness in spite of the presence of an active enzyme system and potent fatty acid acceptors[1] suggests that the exchange might be explained, at least partly, by a resynthesis.

The data in Table I show that the triglycerides had completely exchanged approximately two thirds and the diglycerides one half of their fatty acids. These results can be interpreted in the following way. The triglycerides have two fatty acids which are in equilibrium with the free fatty acids. These fatty acids would then have the same specific activity as the free fatty acids, *i.e.* a percentage specific activity of 100. The remaining fatty acid is inactive. Similarly, the diglycerides have one equilibrated and one inactive fatty acid. This indicates that the inactive fatty acid is in the 2-position, since the two fatty acids in the 1-position in the triglycerides with all probability must be considered equal. If this assumption is correct, the diglycerides formed are of the 1,2-configuration. This implies that the clearing factor preferentially attacks the glyceride ester bonds in the 1-position. Quite similar results have been obtained for pancreatic lipase[7]. Furthermore, in that case it was proved that the diglycerides formed had the 1,2-configuration[8].

No unequivocal interpretation can be given for the relative specific activity figure obtained for the monoglyceride fatty acid (see Table I). One possibility is that the enzyme has a low but certain activity against the fatty acid in the 2-position of the diglyceride. Another possibility is that the monoglyceride formed initially is of the 2-configuration but then is slowly isomerized to the 1-position. The fatty acid in the 1-position will then be exchanged with the free fatty acids.

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