These findings show that the lysolecithin formed in phospholipase-treated serum from the lecithin of the β-lipoprotein remained a part of the lipoprotein molecule, whereas the bulk of the liberated fatty acids left the lipoprotein to become bound to the serum albumin. The same, presumably, holds true for the products of the α-lipoprotein-bound lecithin, although the presence of lysolecithin in the α-lipoprotein was not directly demonstrated.

In a previous report[1] the shift towards the anode of both the α- and the β-lipoprotein fractions, consequent upon treatment of the serum with phospholipase A, was thought to be due to their association with excess fatty acids. However, the finding of only a small part of the liberated fatty acid in the β-lipoprotein fraction prompts the consideration that the shift of this fraction towards the anode may be due to the lysolecithin still present in the molecule. Similarly, it is possible that the shift towards the anode of the α-lipoprotein band in the paperelectropherogram of phospholipase-treated serum is due to the formed lysolecithin remaining bound to the α-lipoprotein molecule. Indeed, addition of lysolecithin to serum (1 mg to 1 ml) caused a shift of both the α- and the β-lipoprotein fractions towards the anode.

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Effect of ethionine treatment on esterification *in vitro* of free [4-^{14}C]cholesterol by rat plasma

The administration of ethionine rapidly produces a fatty liver in the fasted, female rat[1,2]. The pronounced increase in liver lipids is confined to the triglycerides[3–5] and is associated with a fall in triglycerides, phospholipids and cholesterol of plasma[4–6]. Ethionine treatment has been shown to depress the incorporation of radioactive amino acids into liver proteins[7–9] and plasma lipoproteins[9]. It has been suggested that an interference with formation of plasma lipoproteins in liver is the cause of both the fatty liver and the fall in plasma lipids[6,9]. Such a mechanism can account for the accumulation of glycerides in the liver (formed from free fatty acids mobilized from adipose tissue) and for the decrease of all lipids associated with plasma lipoproteins. But it does not account for the observation that the concentration of cholesterol esters in plasma is lowered to a much greater extent by ethionine treatment than are the concentrations of free cholesterol, triglycerides and phospholipids[5].

Recently we reported that evisceration in rats reduces the capacity of their plasma to esterify free cholesterol *in vitro*[10]. This was attributed to the removal of the source—probably the liver—of the plasma enzyme (or of heat-labile cofactors) responsible for esterification of the cholesterol. A reduction either in the capacity of the liver to synthesize this enzyme or in its release from liver to plasma could account for the disproportionate lowering of the plasma cholesterol esters observed in ethionine-treated rats. To test this possibility, we compared the esterification *in vitro* of labeled free cholesterol by plasma of ethionine-treated and normal rats. It is shown, in the experiments described below, that the capacity of rat plasma to esterify free cholesterol is reduced considerably by ethionine treatment.

200 mg of DL-ethionine (Nutritional Biochemicals Corp., Cleveland, Ohio) dissolved in 8 ml of 0.45 % NaCl were injected intraperitoneally into female rats that weighed from 155 to 180 g and had been fasted for 16 h. Control, fasted, female rats received 8 ml of 0.9 % NaCl intraperitoneally. 24 h later blood was withdrawn from the hearts into heparin-rinsed syringes, and centrifuged to separate the plasma.

TABLE I

ESTERIFICATION OF FREE [4-^{14}C]CHOLESTEROL BY PLASMA OF ETHIONINE-TREATED AND NORMAL RATS

[4-^{14}C]Cholesterol was added in the form of labeled β-lipoproteins (see text). Each flask contained 0.1 ml of this substrate. The figures in parentheses are the number of samples analysed.

Plasma from	*ml plasma incubated*	*Per cent [^{14}C]sterol in esterified form*	
		Range	*Average*
Normal controls	2	78.5–90.7 (4)	82.0
Ethionine-treated	2	15.7–49.0 (6)	32.8
Normal	1		
plus heated normal	1	46.5–56.0 (4)	51.5
Normal	1		
plus heated ethionine-treated	1	48.4–53.9 (3)	51.4
Heated normal	2		0.5 (2)
Heated ethionine-treated	1		0.5 (1)

The plasma was incubated with free [4-^{14}C]cholesterol-labeled β-lipoproteins at 37° with shaking for 22 h. The labeled substrate was prepared as follows: The β-lipoproteins were precipitated from rat serum with dextran sulphate by a modification[11] of BURSTEIN's[12] procedure. After centrifugation, the supernatant fraction was removed, and the β-lipoproteins were dissolved in a volume of 0.9 % NaCl equal to the original volume of serum. The precipitation was repeated and the β-lipoproteins were again dissolved in 0.9 % NaCl. [4-^{14}C]Cholesterol (purchased from Nuclear-Chicago Corp., and purified on silicic acid columns[13]) was dispersed on celite[14] and incubated with the β-lipoprotein preparation for 18 h at 37° with gentle shaking. The celite was separated from the cholesterol-labeled β-lipoproteins by centrifugation. We found that incubation of this cholesterol-labeled lipoprotein substrate with plasma resulted in greater esterification of cholesterol than did incubation of labeled cholesterol dispersed on celite directly with plasma as in our earlier study[10].

The lipids of the incubation mixture were extracted as previously described[15]. Free and esterified sterol fractions were separated on silicic acid columns[13] and assayed for ^{14}C as described earlier[10].

The results are shown in Table I. An average of 82 % of the labeled free cholesterol was esterified by plasma of the normal control rats (saline-injected). Only 33 % was esterified by plasma of the ethionine-treated rats. These averages were obtained from the results of experiments in which plasma from each of 6 ethionine-treated and 4 control rats was incubated in separate flasks.

To test for the presence of heat-stable inhibitors of cholesterol esterification in the plasma of ethionine-treated rats, the extent of esterification of the β-lipoprotein [4-^{14}C]cholesterol by 1 ml of normal rat plasma was determined in the presence of 1 ml of enzymically inactive plasma (heated for 1 h at 60°) obtained from either ethionine-treated or normal rats. The normal rat plasma (heated or unheated) was a pooled sample from several rats. The plasma samples of the ethionine-treated rats were obtained from individual rats. The esterification of cholesterol by normal rat plasma was the same in the presence of either of the heated plasma samples. Thus, the reduction in the esterification *in vitro* of free cholesterol by plasma resulting from ethionine treatment cannot be ascribed to the presence of a heat-stable inhibitor(s) of the reaction in plasma of the ethionine-treated rats. Most probably it resulted from a reduction in the plasma concentration of the esterifying enzyme synthesized in the liver. This might be brought about either by an inhibition in the synthesis of an enzyme in the liver or by an interference with release of the enzyme from liver into plasma. Since it has been reported that ethionine inhibits the adaptive synthesis of a number of enzymes in the liver[8,16-22], the former possibility seems the more likely.

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