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In vitro esterification of cholesterol by plasma: the effect of evisceration*†

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SUMMARY

The capacity of plasma of eviscerated rats to esterify free cholesterol was compared with that of plasma of normal control rats by incubating the plasma of these rats with free cholesterol-4-C^{14} dispersed on Celite and by determining the percentage of labeled sterol in the esterified form at the end of 18 hr. Cholesterol esterification was severely restricted in incubation mixtures prepared with plasma of the eviscerated rats. The restriction could not be accounted for by the presence of inhibitors nor by the absence of heat-stable activators. On the basis of these as well as other observations, it is concluded that the reduced capacity of the plasma of eviscerated rats to esterify cholesterol resulted from removal of the source (probably the liver) of the *plasma* enzyme or of heat-labile cofactors responsible for the esterification, or both.

The observation that partially hepatectomized or eviscerated rats are unable to replace lost plasma cholesterol esters (1) has led to the view that the liver is the *direct* source of plasma cholesterol esters. However, since plasma contains an enzyme that esterifies free cholesterol (2), it is conceivable that the inability of the liverless rat to restore to normal its cholesterol ester level results from a loss of the plasma enzyme that esterifies the cholesterol. In order to throw more light on this problem, we have compared the *in vitro* esterification of C^{14}-labeled free cholesterol by plasma obtained from normal rats with that by plasma obtained from rats subjected to evisceration.

EXPERIMENTAL METHODS

Labeled Cholesterol. Cholesterol-4-C^{14} purchased from Nuclear Chicago Corporation was purified on silicic acid columns (3) before use.

Procedure for Evisceration. Male Long-Evans rats were used. The entire liver, gastrointestinal tract, pancreas, and spleen were removed by the two-stage operation described by Ingle (4). The first stage was performed when the rats weighed 170–190 g, the second when they weighed 250–270 g. As soon as the circulation of the liver was tied off during the second stage, an infusion of glucose and insulin was begun through either a femoral vein or artery (glucose: 48 mg/100 g body weight/hr; insulin: 0.17 unit/100 g body weight/hr). The infusion solution was prepared in physiological saline and injected at the rate of 1.2 ml/hr. The infusion was continued for 11 hr after completion of the operation, at the end of which time 2–3 ml of blood was obtained either by allowing blood to drain from the arterial catheter into a centrifuge tube containing one drop of heparin or by withdrawing blood from the abdominal aorta into a heparinized syringe. Rats that served as controls were subjected to a sham operation that consisted of laparotomy and the infusion, for 11 hr, of the glucose–insulin solution.

Incubation Procedure. The labeled cholesterol was dispersed on Celite in a 25-ml Erlenmeyer flask, as described by Avigan (5). The cholesterol–Celite proportion was 2.5 μg of the former to 100 mg of the latter, rather than the 1 mg to 100 mg proportion used by Avigan. The low concentration of cholesterol was used because little or no esterification occurs with the higher concentration. Incubation of plasma with the cholesterol dispersed on Celite was carried out (as described by Avigan) for 18 hr, after which the incubation mixture was filtered through a fine-sintered glass funnel under reduced pressure.

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† The term "evisceration," as used here, refers to excision of the entire liver, gastrointestinal tract, pancreas, and spleen.

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TABLE 1. ESTERIFICATION OF FREE CHOLESTEROL-4-C^{14} BY PLASMA OF TOTALLY HEPATECTOMIZED-EVISCERATED RATS AND OF NORMAL RATS

Flask No.	Incubation Mixture: Plasma from: Eviscerated Rat No.	Eviscerated Rat Unheated	Normal Rat No.	Normal Rat Unheated	Normal Rat Heated	Celite*	Remarks	Sterol-C^{14} in Esterified Form
		ml		*ml*	*ml*	*mg*		%
1	1	2.0				100		1.9
2			6†	2.0		100		33.6
3	2	2.0				100		5.7
4	2	0.5	7	0.5		0	Plasma from flask 3 reincubated	33.6
5	2	0.5	7		0.5	0	" " " " "	6.8
6	3	2.0				100		8.3
7	3	0.5	8	0.5		0	Plasma from flask 6 reincubated	52.3
8	3	0.5	8		0.5	0	" " " " "	8.7
9			9†	2.0		100		55.5
10	4	2.0				100		5.5
11	4	0.5	10	0.5		0	Plasma from flask 10 reincubated	25.7
12	4	0.5	10		0.5	0	" " " " "	5.2
13	5	0.5	11	0.5		50		23.5
14	5	0.5	11		0.5	50		2.5

* The isotopic cholesterol was dispersed on the Celite (see text).
† Sham-operated (see text).

Extraction of Plasma Lipids and Separation of Free and Esterified Sterol. The plasma lipids were extracted as described by Lossow *et al.* (6), and the esterified and free sterol fractions were separated by silicic acid chromatography (3) under the conditions previously described (6).

C^{14}-Assay. Dried lipid samples were dissolved in 15 ml of toluene containing 45 mg of 2,5-diphenyloxazole and 1.5 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene, and assayed for C^{14} in a TriCarb liquid scintillation spectrometer (Packard Instrument Co., LaGrange, Ill.).

RESULTS

As shown in Table 1, 44.5% (average) of the labeled sterol incubated with plasma obtained from two sham-operated control rats (flasks 2 and 9) was esterified. The labeled ester found in the incubation mixtures prepared with plasma of four eviscerated rats averaged 5.4% of that added (flasks 1, 3, 6, and 10).

A test was made for the possible presence of inhibitors of cholesterol esterification in the plasma of eviscerated rats. Three mixtures (flasks 3, 6, and 10) obtained from eviscerated rats and incubated for 18 hr were filtered to remove the Celite, and 0.5 ml of each filtrate was reincubated for another 18 hr with 0.5 ml of fresh, normal plasma (flasks 4, 7, and 11). Another 0.5-ml sample was reincubated at the same time with 0.5 ml of fresh, normal rat plasma that had been heated for an hour at 60° (flasks 5, 8, and 12). Reincubation of the plasma samples from eviscerated rats with fresh, normal, unheated rat plasma resulted in a five- to sixfold increase in esterification of the labeled sterol, but reincubation with heated normal plasma produced no further increase. Further proof that inhibitors of cholesterol esterification are not responsible for the marked reduction in esterification of free cholesterol by plasma of eviscerated rats is provided by the experiment in which 0.5-ml samples of plasma freshly drawn from an eviscerated rat were incubated with 0.5 ml of freshly-drawn, normal, unheated plasma (flask 13) and with heated plasma (flask 14). Esterification in the former case was about nine times that of the latter.

The reduction in esterification of cholesterol observed in the experiments with plasma obtained from eviscerated rats might be accounted for by the absence of activators of esterification. The failure of the addition of heated, normal rat plasma to augment cholesterol esterification by eviscerated rat plasma rules this out.

DISCUSSION

Sperry (2) was the first to demonstrate the presence of an enzyme in plasma that esterifies free cholesterol. He observed that incubation of human plasma for 72 hr resulted in a fall in free cholesterol with no change in

total cholesterol. He also found that bile acids inhibited this system (7, 8), and suggested that the low ratio of cholesterol ester to free cholesterol in plasma of patients with liver disease might be explained by inhibition of cholesterol esterification by elevated plasma bile acid levels. A number of investigators, employing Sperry's system, have observed that plasma of patients with liver disease has a restricted capacity to esterify free cholesterol (9–12). In those studies, plasma samples were not tested for the presence of inhibitors of cholesterol esterification. However, although the concentration of bile acids in plasma of patients with liver disease is higher than normal (13), it is considerably lower than the concentration at which Sperry (7, 8) demonstrated inhibition of cholesterol esterification.

Evisceration should not increase the level of bile acids in plasma, and this was confirmed by determination of the plasma bile acids in two of our eviscerated rats by the procedure of Kier (14). In our experiments, the severe restriction in the capacity of plasma of the eviscerated rat to esterify free cholesterol could not be accounted for by the presence of inhibitors of esterification or by the absence of heat-stable activators. The restriction, therefore, was apparently due to a reduction in the concentration of the enzyme that esterifies free cholesterol or of heat-labile cofactors in the plasma of these rats, or both. This could have resulted from removal of either the liver, pancreas, spleen, or intestines. But since it has been reported that partial hepatectomy or evisceration-functional hepatectomy destroys the ability of the intact rat to restore the experimentally reduced levels of cholesterol ester in plasma, whereas removal of the entire gastrointestinal tract, pancreas, and kidneys failed to do so (1), it seems probable that the liver was the responsible organ.

Our results, then, suggest that one factor responsible for the failure of an animal to maintain a normal plasma cholesterol ester level after removal of its liver is a reduced capacity of its plasma to esterify free cholesterol. Since the activity of the enzyme in plasma that esterifies free cholesterol relative to that in liver is not known, however, the quantitative importance of the enzyme in plasma cannot be evaluated at this time.

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