Secretion of high density lipoprotein by the isolated perfused alcoholic rat liver[1]

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Summary. Chronic alcohol feeding of a low fat diet for 5 weeks led to a slightly raised though statistically non-significant high density liproprotein cholesterol/apoB containing lipoprotein cholesterol ratio in both the fasting rat serum as well as the secretory products of the isolated perfused liver.

Previously published epidemiological studies have convincingly established that there is a very strong negative correlation between plasma HDL cholesterol levels and the probability (risk) of developing coronary heart disease (CHD)[2-4]. The molecular mechanisms underlying this protective capacity of HDL are not entirely clear. However, 2 propositions[5,6] have been made: a) HDL may facilitate reverse cholesterol transport from cells of the artery wall and b) HDL may inhibit the uptake of LDL by cells of the artery wall. Plasma HDL levels may be influenced by dietary carbohydrates and alcohol, by physical activity, some lipid-lowering drugs and endocrine factors such as sex steroids and insulin[7]. Alcohol appears to play a deleterious role with respect to cardiac muscles[8], while several recent epidemiological studies suggest a protective role for alcohol via elevation of HDL on the development of CHD[9-11]. It was therefore decided to examine the capacity of an isolated perfused rat liver to secrete HDL cholesterol subsequent to chronic administration of alcohol in a totally liquid low fat (5%) Metrecal diet.

Materials and methods. Male Sprague-Dawley rats originally weighing 150-175 g were fed a 37% alcohol low fat (5%) totally liquid Metrecal diet for 5 weeks as previously described[12,13]. Livers from control and alcoholic rats fasted 20-24 h were perfused with recirculation for 3 h according to Lee and Hosein[13]. Serum samples were obtained from the inferior vena cava prior to protal vein cannulation.

The lipids were extracted from the serum and cell-free perfusate with chloroform-methanol (2:1) and washed by the method of Folch et al.[14]. Cholesterol was determined according to Zlatkis et al.[15]. ApoB-containing lipoproteins (VLDL + LDL) were isolated by ultracentrifugation as follows: the density of the liver perfusate or rat serum was raised to 1.09 g/ml[16,17] using solid sodium bromide. The samples were centrifuged in a Beckman SW 56 rotor at 100,000 × g for 23 h. The float containing VLDL and LDL was quantitatively removed by suction and dialyzed extensively against saline-EDTA, pH 7.4 containing 0.02% sodium azide. The total lipids of the apoB-containing liproproteins were extracted[14] and the cholesterol content determined[15]. The HDL cholesterol value was calculated by difference (total cholesterol minus apoB-containing lipoprotein cholesterol).

Results. Rats maintained on the 37% low fat alcohol diet gained weight at a slower rate than their sucrose pair fed controls[12]. As a result, both the body weight and wet liver weight at the time of sacrifice were lower in the experimentals compared to the controls[13]. Alcoholic rats regularly consumed 15-18 g alcohol per kg b.wt per day; during withdrawal, they usually displayed vivid signs of tremor, rigidity and generalized hyperexcitability[12,13].

The effect of chronic ethanol administration on the fasting serum levels of cholesterol, as well as lipoprotein cholesterols is shown in table 1. Serum cholesterol level seems to be slightly elevated in animals treated chronically with alcohol and this increase appears primarily in the HDL fraction resulting in a slightly raised HDL/(VLDL + LDL) ratio. However, no statistical significance (2-tailed Student's t-test) was found for any of the parameters measured.

The influence of chronic alcohol feeding on the capacity of an isolated perfused rat liver to secrete cholesterol as HDL and apoB containing lipoprotein is shown in table 2. The rate of total cholesterol secretion remains similar in both the control and alcoholic livers. However, the alcoholic liver appears to secrete slightly more HDL and slightly less apoB containing lipoprotein, thus resulting in a tendency towards higher, though statistically non-significant HDL/(VLDL + LDL) ratio as compared to the control liver.

Discussion. Increased lipids in both plasma and the liver subsequent to chronic alcohol consumption have been amply documented[8]. It is known that increased alcohol intake increased HDL levels in humans[18] and that elevated HDL levels, in particular a high HDL to LDL ratio, are advantageous with respect to CHD[18].

In animal studies, the effect of alcohol on HDL levels and the risk of CHD is much less definitive. Chronic alcohol feeding to rats is known to cause an accumulation of esterified cholesterol in the blood[19,20]. The incorporation of dietary ^{3}H-palmitate or i.v. administered ^{14}C-lysine into rat serum HDL appeared not to be impaired after prolonged alcohol ingestion[21].

The results described in this brief communication show that chronic ethanol feeding of a low fat diet does not alter the capacity of an isolated perfused rat liver to secrete cholesterol containing lipoproteins. A slightly raised HDL/(VLDL + LDL) ratio was observed after alcohol feeding in

Table 1. Lipid profile in fasting rat serum after chronic alcohol administration

	Ethanol-fed experimentals	Sucrose-fed controls
Total cholesterol (mg%)	58 ± 7 (9)	45 ± 5 (8)
HDL-cholesterol (mg%)	46 ± 7 (7)	30 ± 3 (5)
(VLDL + LDL)-cholesterol (mg%)	13 ± 3 (7)	9 ± 2 (5)
HDL/(VLDL + LDL) ratio	4.2 ± 0.7 (7)	3.7 ± 0.6 (5)

Rats were fed for 5 weeks on a 37% alcohol low fat liquid Metrecal diet and the serum samples obtained after a 20-24-h fast as described in the text. Each value represents the mean ± SEM for the number of observations indicated inside the parentheses.

Table 2. Cholesterol output from the isolated perfused rat liver

	Ethanol-fed experimentals	Sucrose-fed controls
Total cholesterol (μg/g liver/h)	54 ± 4 (11)	53 ± 7 (10)
HDL-cholesterol (μg/g liver/h)	32 ± 4 (11)	27 ± 4 (10)
(VLDL + LDL)-cholesterol (μg/g liver/h)	22 ± 2 (11)	26 ± 3 (10)
HDL/(VLDL + LDL) secretion rate ratio	1.6 ± 0.3 (11)	1.07 ± 0.13 (10)

Livers from 20-24-h fasted rats were isolated and perfused as described in the text. Perfusate lipids were quantitated after 3-h perfusion. Each value represents the mean ± SEM for the number of observations indicated inside the parentheses.

both the fasting rat serum as well as the liver perfusate, though statistical significance was not achieved. We have observed an increase HDL cholesterol level in the serum of rats fed ethanol although the values were not statistically different from the controls. This is at variance with the work of Hirayama et al.[21] who found significantly higher levels of HDL in fasting rat serum after chronic alcohol feeding. However, their diet has a considerably higher fat content (35%) compared to ours (5%).

In view of the possible beneficial effects of an increased HDL/LDL ratio towards CHD[18], further studies into the role of alcohol on the metabolism of HDL and LDL are indicated. These will include variables such as duration, frequency and amount of alcohol ingested as well as dietary manipulation (e.g. changing protein or fat content).

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Effect of ricin, of its subunits and of modeccin on cAMP level in Yoshida ascites cells[1]

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Summary. The B chain of ricin strongly decreases the PGE_1-enhanced level of cAMP in Yoshida ascites sarcoma cells, whereas the A chain is ineffective. Modeccin does not have any effect.

Ricin (R-60), from the seeds of *Ricinus communis*[2] and modeccin, from the roots of *Adenia digitata*[3,4] are highly toxic lectins which share the property of inhibiting protein synthesis in vitro. Both toxins consist of 2 unequal subunits[5,12]; the A chain is responsible for the inhibitory effect, and the B chain for cell penetration through specific binding to galactosyl receptors on the cell surface. The 2 toxins differ in haemagglutinating capacity[6,7], are not immunologically closely related, and produce different lesions in rats[9,10]. Nomoto et al.[11] found that ricin lowers the level of 3'-5'-cyclic-adenosine-monophosphate (cAMP) in Yoshida ascites sarcoma cells (YS cells) in vitro, after preincubation with prostaglandin E_1 (PGE_1), through modification of adenylate cyclase activity, with an increase of the K_m for ATP. This effect of ricin was suppressed in the presence of lactose.

We report here that the isolated B chain of ricin is responsible for the lowering of the cAMP level, whereas the A chain has no effect. Modeccin has no effect on the cAMP level in YS cells.

Materials and methods. Ricin (R-60) and its A and B chains were prepared as described by Nicolson et al.[12]. The A chain contamination of the isolated B chain was 1% maximum, as judged from the effect on protein synthesis by a lysate of rabbit reticulocytes. Modeccin was prepared as described previously[5]. PGE_1 was a kind gift of the Upjohn Spa (Caponago, Milan), cyclic 8-^{3}H AMP (spec. radioact. 27 Ci/mmole) was purchased from The Radiochemical Centre, Amersham, Bucks. U.K., cAMP and bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA, theophilline and activated charcoal from Merck, Darmstadt, West Germany. YS cells were a kind gift of Professor Olivotto, Florence, and were transplanted into anesthetized Wistar rats, weighing 120–150 g. All chemicals used were of analytical grade.

Determination of cAMP level in vitro. Before utilization, cells were washed 3 times in NaCl 0.9% and were suspended in Krebs-Henseleit Ringer bicarbonate (KH) medium[13] at a final concentration of 15×10^6 cells/ml. The reaction mixture contained 5 mM theophilline and cellular suspension in KH medium, and was preincubated for 15 min at 30 °C with shaking. Incubation was started with or without (control) PGE_1 (5 µg/ml) at 30 °C with shaking, and after 15 min ricin or modeccin (1 µg/ml) or the separated A and B chains (0.5 µg/ml) were added. At the required times, 1 ml of each sample was centrifuged at 3000 rev/min for