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Received September 20, 1965. P.S.E.B.M., 1966, v121.

Cholesterol Flux into Aorta in Experimental Nephrosis.* (30798)

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Hyperlipemia and hypercholesterolemia are characteristic features of the nephrotic syndrome. In nephrosis in man, the lipemia is due largely to an increase in the s_f 10-20 lipoproteins, the class associated with atherogenesis(1). Though there are few studies of the incidence and severity of atherosclerosis in nephrosis, the available evidence suggests that the prolonged hyperlipemia of this disease is associated with increased atherosclerosis(1,2). Experimental nephrosis in the rat, however, was not found to be associated with increased atherosclerosis(3,4), perhaps because of the relatively short duration of nephrosis in the animals studied. The purpose of the present work was to determine whether the hypercholesterolemia of experimental nephrosis is associated with increased deposition of cholesterol in the aorta and with an increased flux of cholesterol from plasma into aortic wall, a situation which might be expected to lead over a long period to increased atherosclerosis.

Methods. Male Sprague-Dawley rats weighing 200-225 g were divided into 4 groups: (i) Normal (non-nephrotic) rats fed the standard laboratory diet (Purina laboratory chow). (ii) Normal rats fed a diet based on that described by Hartroft *et al* (6). It contained 1% cholesterol. Initially the content of butter fat was 3%, but this was increased to 5% after 2 weeks and to 6% after 4 weeks, in order to maintain a relatively constant level of plasma cholesterol. This diet will be referred to as "medium fat." (iii) Normal rats fed a diet similar to that of Group (ii), but containing 5% cholesterol and 20% butter (referred to as "high fat" diet). Both medium and high fat diets contained 0.1% thiouracil rather than 0.3% as originally used(6). (iv) Nephrotic rats fed the standard laboratory diet. Nephrosis was induced by injection of rabbit anti-rat kidney serum(5); animals were considered to be nephrotic if urinary protein excretion was 300 mg or more per day, 12-14 days after injection.

Plasma cholesterol concentrations were measured at weekly intervals. After 8 weeks, the animals were sacrificed. Flux rate of cholesterol from plasma to aorta was calculated by the method of Newman and Zilversmit(7) as previously described(8). Plasma lipoproteins were separated in the ultracentrifuge(9). The adventitia was stripped from the aorta. Cholesterol(10) and phospholipid (11) were measured in chloroform:methanol (2:1) extracts of the residual media and intima, in whole plasma and in the plasma lipoprotein fractions.

Results. The results are shown in Tables I and II. It is evident that the increase in plasma cholesterol in nephrosis was due primarily to an increase in the "chylomicron" fraction (density <1.006). With a moderate

* Supported by grants from Medical Research Council, Ottawa, Canada, and Quebec Heart Foundation.

increase in fat content of the diet, the increase in plasma concentration in non-nephrotic rats was due almost entirely to the low density fraction (density 1.006-1.063). With a further increase in dietary fat, there was a further increase in the low density cholesterol together with less marked increases in the other fractions.

The C/P ratio was slightly elevated in the chylomicron and low density fractions in nephrosis, but these changes were not apparent in whole plasma, in which the C/P ratio was not significantly altered from the normal. An increase in dietary fat, however, produced marked elevations of the ratio in both these fractions, which were reflected in a distinct increase in the C/P ratio of whole plasma.

Of the cholesterol concentrations in the various plasma lipoproteins, only that in the low density fraction showed a significant relationship to the influx rate (Fig. 1). In the non-nephrotic group, there was an apparent tendency for the aortic cholesterol to rise as the influx rate increased ($P<.01$); this relationship was not present in the nephrotic animals which had, for the same influx rates, higher aortic cholesterol concentrations than animals with dietary hypercholesterolemia (Fig. 2).

Discussion. Previous evidence has shown the dependence of the influx rate of choles-

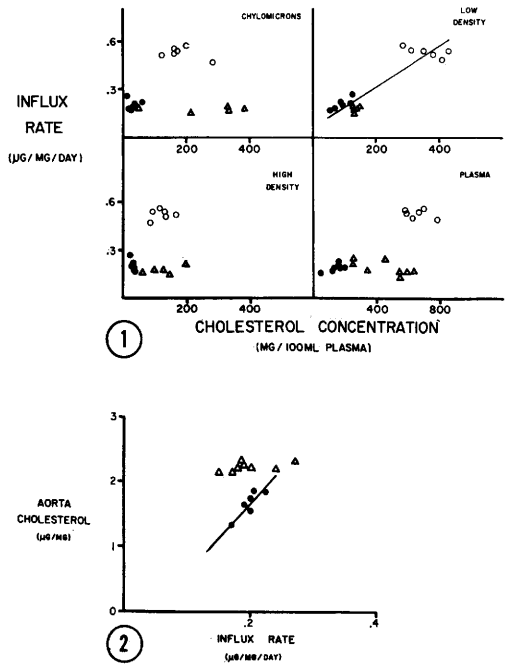

FIG. 1. Relationship of cholesterol concentration in plasma lipoproteins to rate of influx of cholesterol into aorta. The correlation for low-density lipoproteins was significant at the 1% level. ○ Non-nephrotic, high-fat diet. ● Non-nephrotic, medium-fat diet. △ Nephrotic, standard diet.

FIG. 2. Relationship of cholesterol influx rate to aortic cholesterol content. ● Non-nephrotic, medium-fat diet. △Nephrotic, standard diet. Correlation for non-nephrotic animals is significant at the 1% level.

TABLE I. Plasma and Aortic Lipids in Hypercholesterolemia.

	Diet		Plasma			Aorta		Cholesterol influx, μg/g/day
			Cholesterol, mg/100 ml	Phospholipid, mg/100 ml	C/P	Cholesterol, mg/g	Phospholipid, mg/g	
Normal	Standard	(6)	71 ± 8	118 ± 8	.60 ± .03	1.43 ± .10		163 ± 14
	Medium fat*	(6)	150 ± 28	130 ± 12	1.15 ± .07	1.66 ± .17	5.5 ± .9	201 ± 19
	High fat†	(6)	658 ± 69			2.39 ± .21		519 ± 74
Nephrotic	Standard	(8)	467 ±118	695 ±157	.66 ± .12	2.22 ± .08	6.88 ± .25	199 ± 33

Figures shown are mean ± S.E.M.
Figures in parentheses represent number of animals.

* Cholesterol 1%; butter 6%.
† Cholesterol 5%; butter 20%.

TABLE II. Cholesterol and Phospholipid Content of Lipoprotein Fractions.

	Diet		Lipoprotein class*	Cholesterol mg/100 ml	Cholesterol % of total	P lipid, mg/100 ml	C/P
Normal	Standard	(6)	Chylo	14	19	24	.58
			LD	19	27	20	.94
			HD	38	54	74	.52
	Medium fat	(6)	Chylo	28	19	16	1.70
			LD	94	61	43	2.30
			HD	29	20	67	.50
	High fat	(6)	Chylo	182	27		
			LD	357	54		
			HD	119	18		
Nephrotic	Standard	(5)	Chylo	263	47	341	.80
			LD	134	26	116	1.20
			HD	128	27	275	.50

* Chylomicrons: D<1.006 LD: D = 1.006–1.063 HD: D>1.063
Figures in parentheses represent number of animals.

terol into the aorta on the plasma cholesterol concentration(7,8). This is again evident for animals with dietary hypercholesterolemia. However, in the nephrotics, the influx rate was independent of the plasma concentration in whole plasma. Only when the influx rate was related to the cholesterol concentration in low density lipoproteins did the nephrotics approach the relationship found in animals with dietary hypercholesterolemia. It is therefore probable that cholesterol entering the aorta is that of the low density lipoproteins. Such a conclusion agrees with that based on other indirect evidence implicating this particular plasma fraction in atherogenesis(12, 13).

An apparent protective action of serum phospholipids against dietary atherosclerosis was demonstrated by Ladd, Kellner, and Correll(14) and attributed(15) to a stabilizing influence on colloidal lipid. Subsequently, it was shown that administration of surface-active agents also inhibited lipid deposition in the aortic wall of rabbits with dietary hypercholesterolemia(16,17). It is of interest therefore that the influx rate of cholesterol was not affected by the C/P ratio of the low density lipoproteins. Thus the nephrotic animals, having virtually the same low density cholesterol concentrations and influx rates as the animals given the medium-fat diet, had much lower C/P ratios. This finding may indicate that the C/P ratio is in fact not of significance in controlling lipid deposition, or that other factors overcame the protective action of the lower C/P ratio. Published values for the C/P ratios of the lipoproteins of normal rat serum show considerable variation(9,18); the present results fall within the range covered by the earlier reports. The differences may be due in part to the use of different strains of rat and in part to differences in the techniques of separation of the lipoproteins. Though our results differ from those of Borden *et al*(18) with regard to site of the changes in the serum lipoproteins (cholesterol distribution and C/P ratio) induced by a high-fat diet, comparison is difficult because of differences in diet and in mode of separation of the lipoprotein fractions, but primarily because these authors used serum from animals fasted for 12 hours.

Summary. Concentrations of cholesterol and phospholipid in plasma lipoproteins, rates of cholesterol influx into the aortic wall, and concentration of cholesterol in the aorta have been measured in rats with hypercholesterolemia induced by diet or by experimental nephrosis. Nephrotic hypercholesterolemia was due primarily to an increase in the lipoproteins of density <1.006; the cholesterol/phospholipid ratio was moderately increased in this fraction and in the fraction of density 1.006-1.063. Mild dietary hypercholesterolemia was associated with an increase in the latter fraction and with marked increases in the C/P ratio of the low and very low density fractions. Severe dietary hypercholesterole-

mia was due to increases in all fractions though the major increase was still in the fraction of density 1.006-1.063. The rate of cholesterol influx was most closely related to the concentration of cholesterol in this fraction. Among animals with dietary hypercholesterolemia, there was a significant relation between influx rate and aortic cholesterol concentration; in nephrosis, aortic cholesterol was higher than in animals with comparable influx rates produced by dietary means.

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Received September 20, 1965. P.S.E.B.M., 1966, v121.

Placental Barrier to the Fetal Transfer of Maternal Chloroleukemia In Rats.* (30799)

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At least two types of virus transmissible rodent leukemia are capable of transplacental passage with resultant vertical transmission patterns(1,2). The object of this study was to determine whether or not the direct transplacental transmission of Shay chloroleukemia in the rat could be demonstrated. Injection of cellular suspensions from Shay chloroleukemic rats to normal newborn recipients less than 7 days old results in virtually 100% transmission of the leukemia(3). If maternal leukemic factors enter the fetal circulation the offspring of the pregnant leukemic rat either should have congenital leukemia or should develop the disease during the usual incubation period of 4 to 12 weeks. The results of this study indicate that either the placenta or some other factors associated with pregnancy are inhibitory to the transmission of this type of rodent leukemia.

* This project was supported by USPHS Grant HE 08493-02, Nat. Heart Inst.

Experimental method. The initial experimental subjects for these studies were adult, albino, female Sprague-Dawley rats. Each was caged separately and placed on a diet of Purina Chow biscuits and water. Several groups of rats were identified as follows:

Group I was composed of 5 rats with the Shay chloroleukemia which had been kept in remission with thiotepa (N,N′,N″-triethylenethiophosphoramide) in order to reach maturity. Leukemia had been transferred initially to these rats during the first few days of life. At 4-5 months of age each was mated with a normal male Sprague-Dawley rat. The