suggest that there may be some relative difficulty in the catabolism of the monosialoganglioside which may then persist in the blood stream as a glycolipid hapten.

Summary. Five human patients' sera containing antiganglioside activity were examined with various ganglioside preparations utilizing hemagglutination and hemagglutination inhibition techniques. The results indicate that the activity in these cases is specific for monosialoganglioside.

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Effect of Cholestyramine,* A Bile Acid Binding Polymer on Plasma Cholesterol and Fecal Bile Acid Excretion in the Rat. (28674)

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The hypercholesteremic state has received considerable clinical and laboratory attention in recent years because of its possible importance in atherogenesis. Siperstein *et al.*(1) have suggested that lowering plasma cholesterol through the binding of bile acids in the intestine may serve as a means of controlling arteriosclerosis. These authors found that feeding ferric chloride to cholesterol-fed cockerels inhibited the rise in plasma cholesterol and retarded the accompanied atheromata. They associated this effect with decreased cholesterol absorption due to precipitation of the bile acids in the intestinal tract.

More recently(2) it has been shown that feeding of cholestyramine, a bile acid binding polymeric organic base, inhibited the plasma cholesterol rise and aortic plaque formation in cholesterol-fed cockerels and depressed plasma cholesterol levels in normocholesteremic cockerels and dogs. Bergen *et al.*(3) have described the lowering of serum cholesterol levels by cholestyramine in patients suffering from coronary artery disease. The present report describes the effect of cholestyramine on plasma cholesterol level, fecal bile acid excretion and cholesterol synthesis rate in the rat.

Methods and materials. In vivo *experiments.* Holtzman male albino rats were obtained at 130-140 g in weight and maintained on Purina Laboratory Chow for one week. The rats were then randomized according to weight into groups of 10 rats each, with 20 rats in the control group, and placed on the experimental diets. The normocholesteremic diet consisted of Purina Laboratory Chow to

* Generic name for a quaternary ammonium anion exchange resin in which the basic groups are attached to a styrene-divinyl benzene copolymer skeleton by carbon-to-carbon bonds. Its equivalent weight is about 230. The material used in these experiments is in the chloride form. It is essentially dry as prepared, but picks up moisture upon exposure to air. The dietary con'ents reported in the tables represent the dry weight of the resin incorporated.

TABLE I. Effect of Cholestyramine on Plasma Cholesterol Level and Fecal Bile Acid Excretion in the Rat on a Normal Diet.

% cholestyramine in diet	Plasma cholesterol, mg %	Wt gain, g	Cholic acid	Dihydroxy-cholanic acid	Total bile acids
			mg/rat/day		
		10-day Exp			
0	70.6	54	.9	3.9	4.8
.5	69.7	60	.9	5.4	6.3
1.0	74.8	61	.9	9.7	10.6
2.0	68.0	59	.9	13.6	14.5
		9-wk Exp			
0	64.7	262	1.0	5.9	6.9
.5	65.1	270	1.3	10.8	12.1
2.0	62.3	260	1.3	21.9	23.2

which 10% lard had been added. For the hypercholesteremic diet, 0.5% cholic acid and 0.5% cholesterol were incorporated by dissolving them in the 10% lard prior to addition of the lard to the diet. Cholestyramine* was administered by incorporation into the diet. After 10 days, or after 9 weeks for the long term experiment, 2 ml blood samples were taken by cardiac puncture under light nembutal anesthesia and placed in tubes containing 0.2 ml of 0.4 M sodium citrate. The plasma was analyzed for total cholesterol by the method of Abell *et al.*(4). Reported plasma cholesterol concentrations have been corrected for the citrate dilution. Feces were collected during the last 4 days of the experiment and analyzed for cholic and dihydroxycholanic acids by an ion-exchange procedure(5). For liver cholesterol determinations, the whole liver was weighed, homogenized and diluted 10-fold with H_2O. A 1 ml aliquot was hydrolyzed for 1 hour at 40°C with 5 ml of 80% alcohol containing 3 N KOH. Five ml of H_2O was added and the mixture extracted with 10 ml of petroleum ether. Cholesterol was determined on the petroleum ether extract according to the method of Abell *et al.*(4) for plasma.

In vitro *experiments*. A cell-free rat liver homogenate system which synthesizes cholesterol was prepared employing the technique described by Bucher(6), as modified by Rabinowitz and Gurin(7). Livers were taken from rats receiving the normocholesteremic diet and the same diet plus cholestyramine for 10 days and for 9 weeks. The substrates employed were 2-C^{14}-DL-mevalonic acid (2-C^{14}-MVA), and 1-C^{14}-acetic acid (1-C^{14}-HAc) having specific activities of 0.0125 mc/mM and 2 mc/mM, respectively, as measured by liquid scintillation counting. Each tube† contained either 2.7 μM of 2-C^{14}-MVA or 1 μM of 1-C^{14}-HAc, 1 ml of rat liver homogenate and 0.2 mg each of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD). The volume was made to 2.2 ml with pH 6.9 buffer which has been previously described(7). The gas phase was 95% O_2 and 5% CO_2. Incubation was carried out at 37° for 30, 45 and 60 minutes with agitation. Two mg of carrier cholesterol were added after incubation and the cholesterol was isolated and counted as the digitonide according to a previously described procedure(7).

Results. Table I shows results obtained when cholestyramine was fed to rats on the normocholesteremic diet (Purina Laboratory Chow containing 10% lard) for periods of 10 days and 9 weeks. At levels up to 2.0% in the diet, this substance did not influence the plasma cholesterol in either experiment. However, cholestyramine did produce a rise in total fecal bile acid excretion in both the short and long term experiments. The extra fecal bile acids resulted chiefly from an increase in the dihydroxycholanic fraction with no significant contribution from the cholic acid portion. The liver cholesterol content of the rats on the 9-week experiment was unchanged by administration of cholestyramine, with values of 9.1, 9.0 and 9.1 mg/g

† Screw cap culture tubes (20 x 125 mm).

TABLE II. *In Vitro* Cholesterol Biosynthesis by Liver Homogenates Prepared from Rats Fed Cholestyramine.

% cholestyramine in diet	Plasma cholesterol, mg %	Substrate	Recovered cholesterol, cpm/mg carbon Incubation time, min 30	45	60
		10-day Exp			
0	70.6	Acetate	188	274	353
1.0	74.8	"	1329	1877	2186
2.0	68.0	"	2784	4036	4280
0	70.6	Mevalonate	2241	2274	2883
1.0	74.8	"	4213	4428	6137
2.0	68.0	"	3924	6865	7009
		9-wk Exp			
0	64.7	Acetate	34	37	38
2.0	62.3	"	260	174	186
0	64.7	Mevalonate	691	895	899
2.0	62.3	"	1561	1989	2588

dry liver for the 3 groups respectively.

At the end of the 10-day and 9-week experiments reported in Table I, the livers were removed from the rats and the *in vitro* ability of homogenates to incorporate C^{14}-acetate and C^{14} mevalonate into cholesterol was determined. The results are shown in Table II. In the 10-day experiment the incorporation of acetate into cholesterol by the controls was low, but the feeding of 2% cholestyramine caused an approximately 12-fold increase in conversion. The incorporation of mevalonate was also elevated by cholestyramine feeding but to a lesser extent, with a 2- to 3-fold rise. In the 9-week experiment, the acetate incorporation into cholesterol by these older rats was very low, but was elevated about 5-fold by cholestyramine treatment. The mevalonate conversion was also stimulated but to a slightly smaller degree.

Table III shows the effect of feeding cholestyramine for 9 days, at varying levels, to rats rendered hypercholesteremic by incorporation of cholesterol and cholic acid in the diet. The expected increase in plasma cholesterol was prevented with increasing intake of cholestyramine and at the 4% level it completely prevented the hypercholesteremia. Reduction in food intake of animals rendered hypercholesteremic by dietary cholesterol and cholic acid will result in lowered plasma cholesterol values. Food consumptions determined during the experiment indicated no decrease in intake by the cholestyramine treated animals.

Total bile acid excretion of rats receiving the cholesterol-cholic acid diet was elevated over the normocholesteremic controls with a rise in both the cholic and dihydroxycholanic fractions. Administration of increasing amounts of cholestyramine in the hypercholesteremic diet brought about a small elevation in the total bile acid excretion. The rise in the dihydroxycholanic fraction was more marked than the total, but was accompanied by a depression in the cholic portion of the total bile acid excretion. Liver cholesterol concentrations were markedly elevated by the hypercholesteremic diet and were not appreciably reduced by resin feeding. Values for the first 4 groups shown in Table III were: 90, 92, 89 and 70 mg cholesterol/g of dry liver. A value was not obtained at the 4% resin level. Livers of rats receiving the normocholesteremic diet contained 9.1 mg/g dry tissue.

TABLE III. Effect of Cholestyramine on Plasma Cholesterol Level and Fecal Bile Acid Excretion in the Rat on a Hypercholesteremic Diet.

% cholestyramine in diet	Plasma cholesterol, mg %	Wt gain, g	Cholic acid	Dihydroxy-cholanic acid	Total bile acids
			mg/rat/day		
0	176	58	15	27	42
.5	156	62	12	35	47
1.0	139	59	5	45	50
2.0	96	55	2.4	50	52
4.0	74	59	2.8	59	62
Normocholest. diet*	70	54	.9	3.9	4.8

* Normocholesteremic diet.

Discussion. Administration of cholestyramine to rats receiving a normocholesteremic diet had no effect on their plasma or liver cholesterol concentrations. However, it increased the excretion of fecal bile acids, the increase being confined to the dihydroxycholanic portion. Cholestyramine is an anion exchange polymeric organic base known to bind bile acids *in vitro*(2) and one might have predicted an increase in fecal bile acids in these experiments. The loss of steroid nucleus in the feces was not reflected by a drop in plasma cholesterol, suggesting that increased *de novo* synthesis may have counteracted the anticipated depression of plasma cholesterol. That the latter may indeed be the case is suggested by the marked increase in rate of *in vitro* cholesterol synthesis from acetate and mevalonate by liver homogenates from the rats receiving cholestyramine.

When the rats were rendered hypercholesteremic by inclusion of cholesterol and cholic acid in the diet, it was not unexpected to find that this bile acid binding polymer should prevent the elevation in plasma cholesterol. Under the conditions of the experiment, administration of either cholesterol or cholic acid alone has no appreciable hypercholesteremic effect. The hepatic *de novo* cholesterol synthesis would be markedly reduced due to the dietary sterol(8,9) thus inhibiting compensation, therefore the removal of cholic acid by the polymer would prevent the development of hypercholesteremia.

On the hypercholesteremic diet the reduction of plasma cholesterol to normal by cholestyramine was accompanied by only a slight elevation in total fecal bile acid excretion. However, on the hypercholesteremic diet the ratio dihydroxycholanic:cholic acid in the feces was 1.8 which rose with increasing intake of cholestyramine to a value of 21 at the high dose. This suggests a depressed enterohepatic circulation of the administered bile acid due to the resin, with greater metabolism by the intestinal micro-flora.

Summary. Administration of cholestyramine, a bile acid binding polymer to rats on a normal diet was without effect on plasma or liver cholesterol concentration, but produced a rise in fecal bile acid excretion. These effects were accompanied by an increase in hepatic *de novo* cholesterol synthesis. Cholestyramine prevented the rise in plasma cholesterol resulting from administration of cholesterol and cholic acid. The fecal bile acids were increased slightly with a marked elevation in the dihydroxycholanic to cholic acid ratio.

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