sharing of common antigens by the cells tested.

Although it was shown that lymphoid cells are susceptible to serum factors of a skin-grafted rabbit, it remains to be seen whether other types of cells are also affected. Preliminary tests have shown that polymorphonuclear leucocytes of the skin donor are agglutinated by active sera and that epidermal cells are killed by such sera.

Summary. The sera of rabbits which were homografted with skin are shown to be toxic to lymph node cells of the skin donor rabbit. Cytotoxicity was demonstrated upon incubation *in vitro* for 2 hours at 37°C. Sera of grafted rabbits became toxic after graft breakdown had commenced (11 days), and reached a peak after the rejection (20-30 days). The sera were in all instances more toxic to lymph node cells of the skin donor than to cells of other rabbits.

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Negative Feedback Mechanism of Cholesterol Synthesis in Experimental Nephrosis.* (26262)

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To date the mechanism for production of hypercholesterolemia in the nephrotic syndrome is unknown. Various postulates have been offered including hypothyroidism(1), increased intestinal cholesterol absorption (2), defective lipid transport(3), etc. Recently the existence of a negative feedback mechanism for hepatic cholesterol synthesis has been demonstrated in normal animals (4,5). It seemed possible that an absence of, or a defect in this mechanism might explain, at least in part, the increase in serum cholesterol in nephrosis. To test this possibility, the effects of cholesterol feeding upon normal and nephrotic animals were studied. Observations were made of C^{14}-acetate incorpora-

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TABLE I. Effect of Cholesterol Feeding on Liver Slice Incorporation of C^{14} Acetate into Cholesterol.

	# rats	Proteinuria, mg/24 hr	Diet	C^{14}, cpm/mg liver Cholesterol-digitonide	P value (# test)	
Pair-fed normal	6	0	Normal	994.9 ± 324.2*	<.025	<.01
	6	0	2% cholesterol†	81.1 ± 14.5		
Pair-fed nephrotic	9	214.0‡	Normal	535.9 ± 141.8	<.001	
	10	214.5	2% cholesterol	45.7 ± 38.4		

* Mean ± stand. error of mean. † 5 day diet. ‡ Mean.

tion in cholesterol in hepatic tissue slices and of the disposition of cholesterol in various tissues.

The results indicate that in experimental nephrosis, the hepatic feedback mechanism is operational and normally responsive to exogenous cholesterol. Certain abnormalities of cholesterol disposition were noted.

Material and methods. Sixty-four male rats of the Sprague-Dawley strain with starting weights of 100-120 g were studied. Animals were housed individually and pair-fed cholesterol-containing and control diets for periods of 5 to 15 days prior to sacrifice. The cholesterol diet was prepared by addition of 2% cholesterol U.S.P. to ground Purina chow, after solution of cholesterol in ether, while the control diet was prepared accordingly with the solvent alone.

The nephrotic syndrome was produced in 35 animals by 11 daily subcutaneous injections of the aminonucleoside of puromycin as previously described(6,7). The classical picture of nephrosis with proteinuria, ascites and hypercholesterolemia was evident by the tenth day. Urinary protein was estimated by the Esbach method(8).

The experiments were divided into 2 types. In the first, studies were designed to measure the effect of cholesterol feeding on C^{14}-acetate incorporation into liver slice cholesterol. Normal rats fed a 2% cholesterol diet were pair-fed with normal animals on the control diet. Nephrotic rats were similarly pair-fed against each other.

Modifications were made of the Baruch and Chaikoff(9) method for assaying incorporation of acetate 2-C^{14} into cholesterol in hepatic tissue slices. Slices weighing 500 ± 5 mg with a thickness of about .5 mm were cut with the aid of a plastic hand-microtome. Incubations in duplicate for each animal were carried out in flasks containing 4.5 ml of Krebs-Ringer phosphate buffer, 0.5 ml of cold sodium acetate (2.49 mg) made isotonic with NaCl and tracer amounts of sodium 2-C^{14} acetate (specific activity 9075 cpm/μM). Flasks were gassed with 95% oxygen and 5% CO_2 for 3 hours at 37°C in a Dubnoff shaker. After hydrolysis, saponification and extraction of the non-saponifiable lipids, cholesterol was precipitated in chloroform with a .5% solution of digitonin (Sperry method); the precipitate was filtered (Whatman #50 paper cut to fit the planchettes) and after washing and drying, counted in a proportional gas flow counter at infinite thinness.

The second type of experiment was designed to study the distribution of cholesterol in tissues of normal and nephrotic rats before and after cholesterol feeding. In these experiments, normal rats were pair-fed against nephrotic rats both on control diets and cholesterol-containing diets. Cholesterol determinations in serum and tissue homogenates were carried out by the method of Saifer and Kammerer(10). All animals were sacrificed by decapitation after an overnight fast except for animals used for the C^{14}-acetate experiments, which were fed until time of sacrifice, to prevent the starvation effect upon acetate incorporation.

Results. Hepatic cholesterol feed-back: In Table I it may be seen that C^{14}-acetate incorporation into cholesterol by liver slices of nephrotic animals on a normal diet is considerably less than that in normal animals (54% decrease). It is likely that this difference reflects the fact that food intake of nephrotic animals compared with normals is

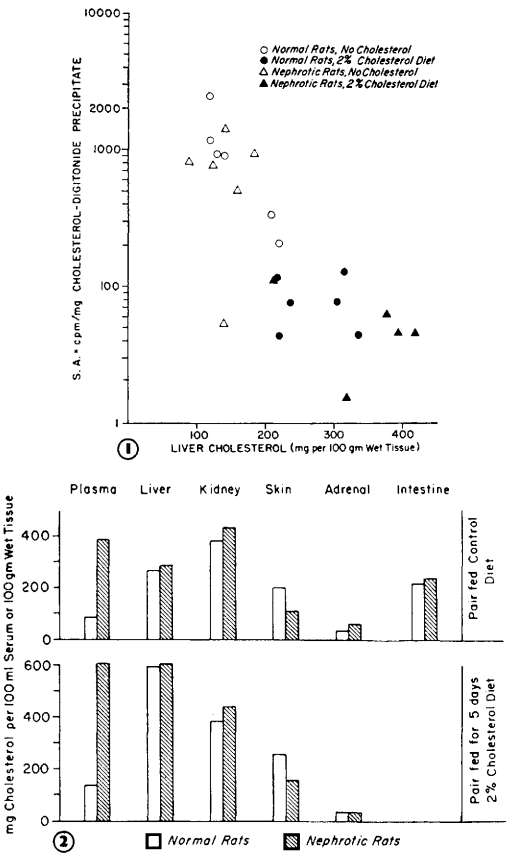

FIG. 1. Relationship between S.A. liver cholesterol and concentration of liver cholesterol in normal and nephrotic animals before and after cholesterol feeding. Of the 16 nephrotic rats noted in Table I, in only 11 animals were studies made of liver cholesterol concentration as well as S.A. of liver cholesterol.

FIG. 2. Effect of cholesterol feeding on distribution of cholesterol in tissues. Sixteen rats were used in each group of pair-feeding experiments.

decreased. After 5 days of cholesterol feeding, both groups of animals show almost complete inhibition of hepatic cholesterol synthesis (91.9% reduction in controls and 91.5% in nephrotics). Since the level of hepatic cholesterol doubled (Fig. 1) in both groups of animals after cholesterol feeding, it seems reasonable that a simple dilution effect was not completely responsible for the striking depression in specific activity of liver cholesterol. Fig. 1 also shows the relationship between specific activity of the cholesterol-digitonide precipitate and cholesterol content of liver. Despite considerable scatter, it may be noted that an inverse relationship exists between the two. These findings suggest that a negative feed-back mechanism for hepatic cholesterol synthesis obtained in the nephrotic animal, is dependent upon liver cholesterol concentration, and does not differ from the normal.

Tissue distribution of cholesterol. Distribution of tissue cholesterol may be seen to be quite similar in liver, kidney, adrenal, and intestine (Fig. 2) of pair-fed nephrotic and normal rats maintained on a normal diet. However, skin and plasma levels differ. Feeding of a 2% cholesterol diet results in a profound increase in hepatic cholesterol in both groups. The magnitude of this change appears similar, suggesting that nephrotic liver is capable of trapping cholesterol. Other tissues behave similarly in both groups of rats with kidney and adrenal showing no change, and skin increasing somewhat. Plasma response differs, in that the absolute rise in nephrotics from their control levels is considerably greater (200 mg%) than the rise in plasma levels in normal rats (30 mg%).

These observations indicate that nephrotic tissues, excluding plasma, behave as do normal tissues in response to exogenous loads of cholesterol.

Discussion. Gould and Taylor(11,12) and Frantz, *et al.*(13), working with hepatic tissue slices of normal animals, have shown that addition of cholesterol to the diet causes a depression in rate of synthesis of cholesterol in the liver. Studies on incorporation of C^{14}-acetate into cholesterol in liver slices obtained from rats made nephrotic with anti-kidney serum have been reported by Heyman (14) and Marsh and Drabkin(3). The former investigator found no change in cholesterol synthesis in 9 animals sacrificed within 2 days after injection of the nephrotoxic serum. In contrast, the latter authors reported(15) an increased *in vitro* and *in vivo* hepatic cholesterol synthesis 48 hours after nephrosis was established. Byers, *et al.*(16) found no change or only a slight decrease of hepatic cholesterol formation in nephrotoxic-nephrotic rats. Marsh and Drabkin(3) obtained decreased hepatic cholesterol formation and unchanged renal cholesterol synthe-

sis in rats 5 to 7 days after induction of the nephrotic state.

Our data obtained in severely and acutely nephrotic rats, in agreement with the work of Marsh and Drabkin, demonstrate a statistically significant decrease in hepatic cholesterol synthesis ($P<.01$) in nephrotic animals, although these animals were not pair-fed with normals.

It may be possible to resolve some of these differences in terms of the nutritional state of the animals studied. Since nephrotic animals may be partially starved as the disease progresses, it is likely that decreased synthesis may be noted in later stages of the disease. In general, it would appear from our observations that hepatic cholesterol synthesis is certainly not increased.

Cholesterol added to the diet for 5 days decreased the rate of hepatic cholesterol synthesis to very low levels in both normal animals ($P<.025$), and nephrotics ($P<.001$). Feeding this diet for 15 days in 6 nephrotic animals did not further decrease hepatic cholesterol synthesis. It does not seem, therefore, that a faulty feedback mechanism can be invoked to explain nephrotic hypercholesterolemia.

The response of nephrotic animals to exogenous cholesterol appeared to be similar to normal except as to retention of cholesterol in plasma. Assuming a plasma volume of 6 ml per rat, normal rat plasma retained 1.8 mg of cholesterol while nephrotics retained 12 mg of cholesterol (Fig. 2). This type of difference could be explained by alteration in plasma binding of cholesterol in nephrosis. It seems unlikely that the problem is a tissue (hepatic) inability to take up cholesterol, since nephrotic liver and other tissues responded as did normal tissue to exogenous cholesterol. On the other hand, since normal liver cholesterol doubled with only a small increase in plasma cholesterol level, one might have anticipated a considerably greater increase in nephrotic liver cholesterol relative to the plasma increase.

These findings do not permit differentiation between a hepatic defect or a plasma defect but do suggest an abnormality of the interrelationship.

Summary. Feeding of cholesterol resulted in marked inhibition of hepatic cholesterol synthesis in nephrotic and normal animals. A faulty hepatic feedback mechanism cannot be invoked to explain nephrotic hypercholesterolemia.

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