the feces. This enhanced urinary excretion of radioactivity compared with that of the rat suggests that formation of steroid hormones is a more important pathway of cholesterol metabolism in the gerbil. This conclusion is supported by the finding that the gerbil has 4 times the adrenal weight/body weight of the rat. The greater size of adrenals in the gerbil may be a reflection of its need to retain salts and water under arid conditions, since the gerbil is normally a desert inhabitant.

The data also show that in the gerbil, as in all higher animals thus far studied, bile acids constitute the major pathway of cholesterol catabolism.

Summary. The metabolism of C^{14}-cholesterol has been studied in the gerbil. At the end of 14 days, 60% of the radioactivity, originally administered as cholesterol, was excreted. Of this, 90% appeared in feces and 10% in urine. The radioactivity in feces was found predominantly in the bile acid fraction. The enhanced urinary radioactivity found in the gerbil suggests that conversion into steroid hormones is a more important pathway of cholesterol metabolism in this animal than in the rat. The distribution of radioactivity remaining in organs and carcass following administration of C^{14}-cholesterol was also studied.

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Sterol Ester Formation by Rat Liver Cell Fractions.* (27420)

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It has been shown(1) that an enzyme system(s) is present in rat liver homogenate capable of forming C^{14}-sterol ester from acetate-1-C^{14}, mevalonic acid-2-C^{14}, and cholesterol-4-C^{14}. Esterification of added cholesterol-4-C^{14} was found to occur in the absence of added cofactors and degree of esterification was increased by adding ATP, CoA, DPN and TPN in various combinations; the reaction appeared to be inhibited by oleic acid. The most active systems were those to which DPN and/or TPN had been added. The purpose of the present study was to obtain additional information on the occurrence and distribution of cholesterol esterifying systems in different cellular fractions of rat liver.

Methods and materials. The livers of 2 rats weighing 175-200 g were homogenized in a medium (1 part liver to 2.5 parts medium)

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composed of 0.004 M magnesium chloride, 0.03 M nicotinamide, 0.01 M glutathione, 0.125 M sucrose, and 0.1 M potassium phosphate buffer, pH 7.2, as described by Frantz and Bucher(2). The homogenate was centrifuged first at 500 × *g* for 10 minutes to remove nuclei and then fractionated by differential centrifugation into mitochondria (5,000 × *g* for 10 minutes), microsomes (105,000 × *g* for 60 minutes), and soluble fraction(3). The mitochondria were washed twice with buffer medium and the microsomes once. Individual cellular fractions or combinations of several cellular fractions were then incubated with cholesterol-4-C^{14} in the absence and presence of DPN as described in Table I. Incubation was carried out in 95% O_2 - 5% CO_2 for 2 hours in a Dubnoff Incu-Shaker at 37°C. The digests were inactivated with 50 ml 2:1 chloroform-methanol and the extract washed with 25 ml of water. The chloroform layer was separated, taken to dryness carefully under nitrogen; the lipids were dissolved in petroleum ether and the extract dried over sodium sulfate. The esterified sterol fraction was separated from the other lipids by silicic acid chromatography (4). The esterified sterol fraction was hydrolyzed and the sterols precipitated with digitonin(5). The sterol digitonides were dissolved in 2:1 chloroform-methanol, plated directly on copper planchets, and C^{14}-activity determined in a gas flow counter; the samples were counted at infinite thinness.

TABLE I. Esterification of Cholesterol-4-C^{14} by Rat Liver Cell Fractions.

Additions to digests†	CPM recovered as C^{14}-sterol ester* Exp. 11	Exp. 12	Exp. 13
Whole homogenate			
0	9280		
DPN	15250	12090	9000
Mitochondria			
DPN		470	275
Soluble fraction + DPN		5855	6343
Heated‡ sol. frac. + DPN			231
Microsomes			
DPN		405	121
Soluble fraction			5502
Soluble fraction + DPN		13625	10985
Heated sol. frac. + DPN			198
Soluble fraction			
DPN		3083	4010

* Determined as sterol digitonide after hydrolysis of ester.

† Incubation system contained 1 ml each of homogenate or cell fraction(s), 5 μmoles DPN when present, 2 × 10^5 cpm cholesterol-4-C^{14}; made to a volume of 2.5 ml with phosphate buffer medium; incubation time 2 hr; duplicate incubation flasks.

‡ Heated at 90°C for 10 min.

Results. Several typical experiments are shown in Table I. Esterification occurred in whole homogenates in the absence of added cofactors. Addition of DPN to the homogenate markedly stimulated esterification. Esterifying activity was present in systems containing microsomes plus soluble fraction, mitochondria plus soluble fraction, and soluble fraction alone. The most active sytem was microsomes plus soluble fraction; DPN stimulated esterification in that system and gave a level of activity equal to the original homogenate. Virtually no activity could be demonstrated in systems containing mitochondria or microsomes plus DPN, but without soluble fraction. When heated soluble fraction was added to mitochondria or microsomes, virtually no esterification occurred. Also, no activity was noted in sytems with heated whole homogenate, mitochondria, microsomes, or heated soluble fraction alone.

Discussion. The present data are in agreement with previous findings(1) and also indicate that there may be one or more cholesterol esterifying systems in rat liver. The systems present in mitochondria and microsomes appear to be similar in that they require soluble fraction for activity. The data also suggest that the soluble fraction contains one or more heat labile cofactors or a soluble enzyme necessary for the esterification reaction to occur in mitochondria and microsomes. The occurrence of cholesterol esterifying system in different parts of the cell may be related to the finding that there are a number of different cholesterol esters in rat liver(6). Those cholesterol esters may be synthesized in different parts of the cell and have different functions.

Fredrickson(7) noted cholesterol esterifying activity in mouse liver mitochondria in the presence of heat-stable soluble fraction of whole liver homogenate. It was suggested that esterification might serve to protect the

hydroxyl group at position 3 of the sterol during oxidation of cholesterol to bile acid. Mukherjee *et al.*(8) reported on the occurrence of an enzyme sytem in whole rat liver homogenate which esterified cholesterol only in the presence of ATP, CoA, and palmitate or palmityl CoA. This system differs in certain respects from that reported here and in the previous study(1). Activity was obtained in the absence of those cofactors and esterification was markedly stimulated by the presence of DPN. In the light of present information concerning the mechanism of formation of ester bonds in the synthesis of phosphatides and triglycerides, the effect of DPN in the present sytem is not clear. Further studies are underway more fully to characterize the microsomal cholesterol esterifying system.

Summary. Rat liver homogenates were fractionated into mitochondria, microsomes and soluble fraction. Cholesterol-4-C^{14} was found to be esterified in sytems containing whole homogenate, mitochondria plus soluble fraction, microsomes plus soluble fraction and soluble fraction alone. Virtually no esterifying activity was obtained in the presence of heated soluble fraction. A stimulatory effect of DPN on sterol ester formation was noted. The findings suggest that different parts of the liver cell possess the capability of forming sterol esters.

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Iodinated Protein Patterns in Thyroids from Normal and Iodine Deficient Hamsters. (27421)

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When hamsters have subsisted on iodine-deficient diets for some months, many of the hyperplastic follicles of the thyroid gland, after fixation and staining for microscopic examination, are found to contain a poorly coagulable material. This contrasts with the dense "colloid" observed in the follicles of normal animals or of hamsters which have chronic colloid goiters(1). It seemed of interest to characterize the protein patterns of glands from normal and iodine deficient animals to determine differences, if any, which might explain the histological appearances. This communication describes certain dissimilarities which have been observed.

Materials and methods. Adult male hamsters were fed either a stock diet or an iodine-deficient ration previously described(2). After varying periods on the latter diet (Table II) these animals, as well as normal controls, were sacrificed, each having received intraperitoneally 24 hours before 20-25 μc I^{131} without carrier. The thyroids of all animals were perfused with .9% sodium chloride through the ascending aorta after clamping the descending portion. Glands were dissected under low magnification, cleaned of fat and muscle and weighed. Since the entire gland of a normal animal weighs only 4 mg, two to three were pooled. The glands from iodine deficient animals ranged from 7 to 73 mg, depending on the duration of the deficiency. Small portions of the iodine deficient glands were taken for microscopic ex-