moidal dose-response curve would be considered linear if only 1- to 6-unit doses were employed. Perhaps the linear relationship observed by others using urine concentrates(13) and anemic plasma extracts(11,14) falls within that dose range.

Although a single dose of 6 units of erythropoietin yielded a maximum reticulocytosis in the plethoric mouse(6), our results suggest that increased erythropoiesis is produced by doses greater than 6 units. Larger groups of animals may be required to lend some degree of statistical significance to this assertion.

Proof of the concept that erythropoietin is the sole regulator of physiological erythropoiesis(3) (and not merely a "panic mechanism"(5)) requires a sensitive assay to demonstrate its presence in normal animal's plasma. The transfused plethoric mouse described here is to our knowledge the most sensitive assay animal available, and in our experience is 10 to 20 times more sensitive than the commonly employed starved rat when one is testing serum with slight erythropoietic potency. This enhanced discrimination doubtlessly will aid in demonstration of activity in human plasma in concentrations well below those detected using the starved rat. Currently, we are investigating the presence of erythropoietic factor in the plasma of normal and mildly anemic patients.

Summary. The transfused plethoric mouse is sensitive to quantities of erythropoietin as small as 0.05 unit as measured by radioiron administered 2½ days after challenge with the hormone. A sigmoidal curve describes the relationship between incremental log-doses of purified erythropoietin and incorporation of radioiron into circulating red blood cells.

1. Carnot, P., Deflandre, C., *Compt. Rend. Acad. Sci.,* 1906, v143, 384.

2. Goldwasser, E., White, W. F., *Fed. Proc.,* 1959, v18, 236.

3. Jacobson, L. O., Goldwasser, E., *Brookhaven Symposia in Biology,* 1958, v10, 110.

4. Gurney, C. W., Goldwasser, E., Pan, C., *J. Lab. Clin. Med.,* 1957, v50, 534.

5. Stohlman, F., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 885.

6. Filmanowicz, E., Gurney, C. W., *J. Lab. Clin. Med.,* 1961, v57, 65.

7. Jacobson, L. O., Goldwasser, E., Gurney, C. W., *Haemopoiesis, Ciba Symposium,* London, 1960, p423.

8. Fried, W., Plzak, L. F., Jacobson, L. O., Goldwasser, E., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 237.

9. Schleuter, R. J., Norgello, H., White, W. F., *ibid.,* 1960, v103, 43.

10. Gurney, C. W., Pan, C., *ibid.,* 1958, v98, 789.

11. Reichlin, M., Harrington, W. J., *Blood,* 1960, v16, 1298.

12. Dahl, J. D., Blaisdell, R. K., Beutler, E., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 622.

13. Hodgson, G., Perrata, M., Yudlevich, D., Eskuche, I., *ibid.,* 1958, v99, 137.

14. Gallagher, N. I., Hagan, D. Q., McCarthy, J. M., Lange, R. D., *ibid.,* 1961, v106, 127.

Received February 26, 1962. P.S.E.B.M., 1962, v110.

Cholesterol Metabolism in the Gerbil. (27419)

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Clarkson *et al.*(1) showed that the blood sterol level of the Mongolian gerbil increases after cholesterol feeding. It was subsequently shown that the increase in plasma sterol is roughly proportional to the amount of cholesterol fed(2). In spite of this, Gordon and Cekleniak were unable to produce atheromatous lesions in these animals(3). These results are in contrast to those in other animals, *i.e.,* the chicken and rabbit, in which cholesterol feeding leads to both an elevated blood sterol and atheromatous lesions(4,5). These data indicate that cholesterol metabolism in the gerbil may differ from that of other ani-

mals. Therefore, it was of interest to study the metabolism of cholesterol in this animal.

Materials and methods. 4-C^{14}-cholesterol. 4-C^{14}-cholesterol* was diluted with an appropriate amount of unlabeled cholesterol, previously purified through its dibromide(6) to give a specific activity of 1.7 μc/mg. The radioactive cholesterol was then recrystallized twice from methanol and stored at −18° until used.

Cholesterol emulsion. Ten mg of 4-C^{14}-cholesterol (1.7 μc/mg) dissolved in diethyl ether was placed in a Potter-Elvehjem homogenizer. To the ether solution was added 0.9 ml of Tween 80.† The ether was evaporated by placing the homogenizer under hot tap water, after which 9.1 ml of distilled water was added and the mixture was homogenized for 3 minutes. This procedure produced an emulsion containing 1.0 mg of cholesterol/ml which was stable for several days.

Administration of labeled cholesterol and collection of samples. Each of 3 male gerbils,‡ weighing 60 to 80 g, received 1.0 ml of the C^{14}-cholesterol emulsion (1.7 μc) by intraperitoneal injection. During the experiment the animals were fed ground Wayne Rabbit pellets and water *ad libitum*. Feces were collected daily and were stored at −18° until used. Urine was collected in alcohol every 2 days and stored at 4°. Fourteen days following administration of radioactive cholesterol, the animals were sacrificed by cervical dislocation. Organs were carefully removed, trimmed of fat and connective tissue, washed and placed in flasks containing 9N KOH.

Preparation of samples for counting. Feces. Two consecutive daily fecal samples were combined and homogenized in glass centrifuge tubes with distilled water by use of a glass stirring rod. Homogenized feces were then extracted for 48 hours with 90% ethanol in a Soxhlet extractor. The alcoholic extract was then made to a convenient volume and an aliquot was removed for counting.

Urine was centrifuged to remove solid matter, and an aliquot of supernatant liquid was counted.

Organs and carcass. To the organs in 9N KOH was added an equal volume of absolute ethanol, and the mixture was saponified for 2 hours under gentle reflux. The carcass was treated similarly except that it was allowed to dissolve in the KOH solution at room temperature prior to addition of alcohol and heating. The saponification mixture was reduced to one-half its original volume, acidified with HCl to pH 1.8 and extracted 3 times with 1 volume of chloroform, followed by a single extraction with 1 volume of diethyl ether. The extracts were combined and brought to dryness under a gentle stream of air at room temperature. The residue was dissolved in 50 ml of absolute ethanol, and an aliquot was removed for counting.

Counting procedure. Aliquots of the above samples were pipetted into scintillation vials and 15 ml of DAM phosphor(7) were added. Samples were counted in a Tracerlab Liquid Scintillation Spectrophotometer. Counts per minute were converted into disintegrations per minute by use of an internal standard.

Results. Distribution of radioactivity in the gerbil following administration of 4-C^{14}-cholesterol. At the end of 2 weeks following injection of labeled cholesterol (Table I) 60% of the C^{14} had been excreted. Of the 40% of radioactivity remaining in the animal, a little more than half was found in the carcass. The remainder was distributed in the organs, predominantly in the gut, plus its contents, and liver.

Fig. 1 shows excretion of C^{14} in urine and feces. The feces is the main excretory route for cholesterol and its metabolic products in the gerbil. Of total radioactivity excreted, 90% appeared in feces and 10% in urine. The finding that 10% of radioactivity excreted after injection of labeled cholesterol was present in urine is in contrast to only 1% excreted in urine by the rat. Since the steroids appearing in urine represent excretion products of the steroid hormones, we were prompted to examine the adrenal glands in these animals. Adrenal weights and corre-

* Volk Radiochemical Co., Chicago, Ill.

† Polyoxyethylene Sorbitan Monooleate, Atlas Powder Co., Wilmington, Del.

‡ These animals were bred in our own colony. The original breeding stock was obtained from Tumblebrook Farm, Brant Lake, N. Y.

TABLE I. Distribution of C^{14} in the Gerbil 2 Weeks after Injection of 4-C^{14}-Cholesterol.

Organ	No. of animals	Total radioactivity, D.P.M. $\times 10^{-3}$/animal	% of administered dose
Feces	3	2044	53.7
Urine	3	234	6.2
Heart	3	3	<.01
Lungs	3	14	.4
Adrenals	3	8	.2
Kidneys	3	16	.4
Spleen	2*	4	<.01
Gut and contents	3	249	6.6
Liver	3	81	2.1
Testis	3	17	.5
Ventral prostate & sem. vesicles	2*	12	.3
Brain	3	3	<.01
Carcass	3	844	22.2
Total		3529	92.7
Total D.P.M. administered		3805	

* One sample lost.

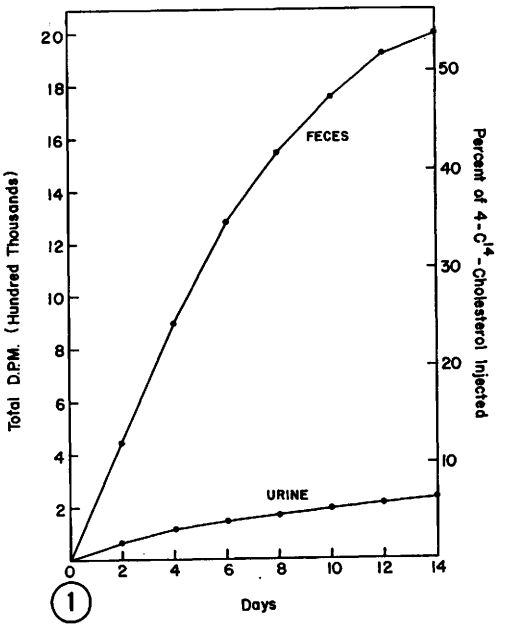
sponding adrenal to body weight ratios were determined on normal adult male gerbils taken at random from our colony. These were found to be in good agreement with values previously reported(8). Table II shows average values found for gerbil adrenals compared with those of adult male rats.§ The average wet weight of adrenal glands of the gerbil is slightly greater than that of the rat. However, when compared on a body weight basis, the weight of adrenal glands in the gerbil is 4 times that of the rat.

Absorption of 4-C^{14}-cholesterol injected intraperitoneally. A possible objection to the excretion data presented here is that the labeled cholesterol was injected intraperitoneally. Therefore, it is possible that not all of the injected cholesterol was absorbed. Hence, calculations of percent radioactivity originally injected, which appeared in the excreta, would be in error. Thus, it was important to measure absorption of 4-C^{14}-cholesterol injected

TABLE II. Comparison of Adrenal Weights of Adult Male Gerbil and Rat.

Animal	No. animals	Avg adrenal wt, mg	Avg body wt, g	Adrenal wt / Body wt
Gerbil	6	43.9	71.0	.62
Rat	8	35.5	232.0	.15

§ CFE Strain, Carworth Farms, New City, N. Y.



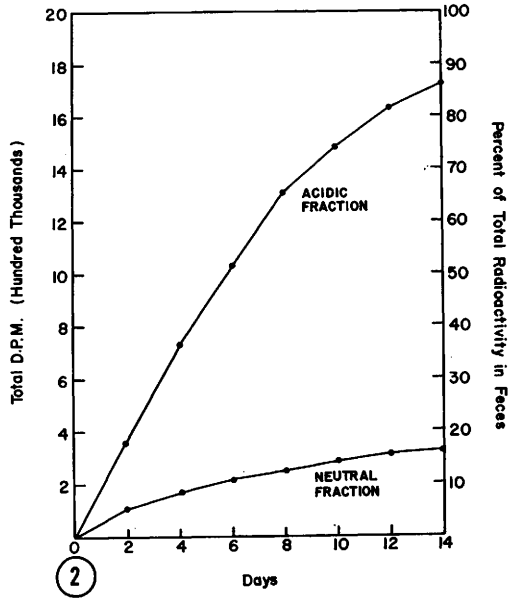


FIG. 1. Appearance of C^{14} in urine and feces after inj. of 4-C^{14}-cholesterol (cumulative).
FIG. 2. Distribution of C^{14} in feces after inj. of 4-C^{14}-cholesterol (cumulative).

intraperitoneally. 2.0 μc of C^{14}-cholesterol, prepared as described above, was injected intraperitoneally into each of 5 male gerbils weighing 50 to 60 g. At various intervals following injection of labeled cholesterol, the animals were sacrificed by ether inhalation. The abdominal cavity was opened and washed

with 5.0 ml of isotonic saline, followed by two 5.0 ml portions of petroleum ether. The washings were combined and brought to dryness under a stream of air at room temperature. The residue was extracted with four 5.0 ml portions of boiling ethanol. The ethanolic extracts were combined and an aliquot was removed and counted as described above.

The experimental results are summarized in Table III. Within 4 hours after administration of labeled cholesterol, 92% of the radioactivity had been absorbed. The value of 8% remaining does not accurately represent unabsorbed cholesterol since we have found that the washing procedure employed also removes sterol from the tissues. Thus, in a parallel experiment 0.59, 0.94, 0.87, 0.64 mg sterol, respectively, were found in the washings from the abdominal cavities of 4 gerbils weighing 60 to 80 g, which received no cholesterol. Thus, essentially all the cholesterol injected intraperitoneally is absorbed within 4 hours.

Distribution of radioactivity in feces of the gerbil following administration of 4-C^{14}-cholesterol. Many workers have shown that bile acids are quantitatively the most important catabolic products of cholesterol metabolism(9,10,11). It was of interest, therefore, to determine the distribution of radioactivity between bile acids and neutral steroids in feces. To accomplish this, the anion exchange resin, Dowex-1, was employed to replace the tedious classical extraction methods. Several authors have reported use of ion-exchange methods for determination of bile acids in feces and serum(12), and for separation of bile acids from bile(13). The procedure which in our hands proved satisfactory follows: Columns were conveniently prepared from 10 ml graduated pipettes by cutting off the tip and clamping the pipette with the mouthpiece down. A small plug of glass wool was inserted and a slurry of Dowex-1 X4, 200-400 mesh, OH form, in 90% aqueous ethanol, was added. The resin was allowed to settle by gravity until it reached a volume of 2.0 ml from the top of the glasswool plug. The 90% ethanol was allowed to drain until it reached the top of the resin, at which time a 2.0 ml aliquot of alcoholic extract of feces was added. At the time of addition of the sample, a scintillation vial was placed beneath the column. When the meniscus of the sample reached the top of the resin, 10 ml of 90% equeous ethanol was added and the entire eluate was allowed to flow through the resin. The scintillation vials, which now contained the neutral steroid fraction, were allowed to come to dryness overnight under a gentle stream of air at room temperature. 15.0 ml of phosphor was added to the residue and samples were counted as described above. The acidic fraction was determined by difference. Fig. 2 shows the distribution of fecal radioactivity between the neutral steroids, which include both free and esterified steroids, and acidic steroids, *i.e.*, bile acids. That the acidic fraction actually represented bile acids was confirmed by classical extraction procedures. No radioactivity was extractable from an acidified fecal extract with n-hexane subsequent to removal of neutral steroids. However, the radioactivity was easily extractable with n-butanol. Of total radioactivity found in feces, 84% appeared in the bile acids and 16% were present as neutral steroids. These results correspond closely to values found for the rat by Siperstein and Chaikoff(14).

Discussion. The data presented indicate that 50% of the radioactivity originally administered as C^{14}-cholesterol was excreted by the 8th to 9th day. This is in contrast to the rat in which half the original radioactivity appeared in the excreta 4 to 5 days after injection of labeled cholesterol(11). The apparent lower rate of turnover of cholesterol found in the gerbil may be a reflection of its enhanced gastrointestinal absorption of cholesterol(2) compared with the rat.

The amount of radioactivity found in urine of the gerbil represents 10% of total C^{14} excreted, the remaining 90% being excreted in

TABLE III. Radioactivity Remaining in Abdominal Cavity Following Intraperitoneal Injection of 4-C^{14}-Cholesterol.

Hr after inj.	No. of animals	% radioactivity remaining
4	2	8.1
7	2	7.5
24	1	4.6

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.

We are indebted to Mr. Theodore Van Trabert for his technical assistance in fecal fractionations.

1. Clarkson, T. B., King, J. S., Jr., Warnock, N. H., *Proc. Animal Care Panel,* 1957, v7, 220.

2. Gordon, S., Stolzenberg, S. J., Cekleniak, W. P., *Am. J. Physiol.,* 1959, v197, 671.

3. Gordon, S., Cekleniak, W. P., *ibid.,* 1961, v201, 27.

4. Dauber, D. V., *Arch. Path.,* 1944, v38, 46.

5. Anitschkow, N., in *Arteriosclerosis* ed. by Cowdry, E. V., Macmillan, New York, 1933, pp. 271-322.

6. Fieser, L. F., *J. Am. Chem. Soc.,* 1953, v75, 5421.

7. Davidson, J. D., Feigelson, P., *Inter. J. Applied Rad. Isotopes,* 1957 v2, 1.

8. Gordon, S., Cekleniak, W. P., Stolzenberg, S. J., Benitz, K. F., Moraski, R. M., *Toxicol. Appl. Pharmacol.,* 1961, v3, 315.

9. Ekdahl, P. H., Sjovall, J., *Acta Physiol. Scand.,* 1955, v34, 329.

10. Bergstrom, S., *Proc. Roy. Physiographic Soc. Lund.,* 1952, v22, Nr. 16.

11. Siperstein, M. D., Jayko, M. E., Chaikoff, I. L., Dauben, W. G., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 720.

12. Kuron, G. W., Tennent, D. M., *Fed. Proc.,* 1961, v20, 268.

13. Gordon, B. A., Kuksis, A., Beveridge, J. M. R., *ibid.,* 1961, v20, 248.

14. Siperstein, M. D., Chaikoff, I. L., *ibid.,* 1955, v14, 767.

Received February 26, 1962. P.S.E.B.M., 1962, v110.

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)

* This work was supported in part by USPHS grant.