carried out earlier, using mice as the test system, indicated that virus persisted in the body of the roach for at least 2 weeks; but by utilizing the more sensitive tissue culture techniques in this study it was demonstrated that survival was of longer duration in the roach: *viz.*, up to 51 days.

The results obtained also indicate that although virus was recovered for as long as 51 days, limited investigations did not disclose evidence of its multiplication. Rather, the pattern seemed to be one of simple mechanical carriage with a gradual decline in virus titre. The peculiar anatomy of the roach probably is a factor favoring sequestration and persistence of virus. Thus, at the junction of the stomach and the intestine, there are present in the roach tubular appendages known as gastric caecae. In these hollow structures it is quite possible that the ingested virus becomes trapped and is protected from the flushing action of the intestinal tract. The significance of the persistence of virus is apparent in that roaches, because of their demonstrated habits and anatomy, may be carriers of poliovirus for weeks and may serve as potential vectors for dissemination in certain communities.

Summary. Sabin's attenuated strain of poliovirus type 3 persisted in cockroaches (*Blaberus discoidalis*) after experimental infection for as long as 51 days. No evidence was obtained indicating multiplication of the virus. Rather, the pattern was one of mechanical carriage of the virus with a gradual decline in titre. It was possible to recover virus only from the gastrointestinal tract of roaches dissected 27 days after infection but not from nerve trunk or other parts of the body of the roaches.

The authors wish to thank Dr. John R. Paul, Dr. Dorothy M. Horstmann, and Mr. John T. Riordan for valuable advice and assistance in this study. The authors also are indebted to Prof. Charles R. Remington, Dept. of Biology, Yale University, for supplying the culture of *B. discoidalis* used in this work.

1. Carp, R., Koprowski, H., Virology, 1962, v16, 71.

2. Hsiang, C. M., Pollard, M., Micks, D. W., Texas Rep. Biol. Med., 1952, v10, 329.

3. Hurlbut, H. S., J. Infect. Dis., 1950, v86, 103.

4. Rehn, J. A. G., Sci. Month., 1945, v61, 265.

5. Rehn, J. W. H., Classification of the Blattaria as indicated by their wings (orthoptera). Mem. Am. Ent. Soc., No. 14, 1951.

6. Sailer, R. I., Common names of insects approved by Entomological Society of America. Bull. Ent. Soc. Am., 1955, v1 (4), 1.

7. Syverton, J. T., Fischer, R. G., Smith, S. A., Dow, R. P., Schoof, H. F., Fed. Proc., 1952, v11, 483.

Received January 26, 1965. P.S.E.B.M., 1965, v119.

Uptake, Hydrolysis and Synthesis of Cholesterol Esters by a Transplantable Adrenal Cortical Tumor.* (30116)

W. J. LOSSOW, G. SHYAMALA, S. SHAH† AND I. L. CHAIKOFF
Department of Physiology, University of California, Berkeley

Cholesterol is stored principally as esters in the mammalian adrenal gland(1-3). The uptake of these esters from plasma by the gland and their hydrolysis and formation in the gland have been under investigation in this laboratory. The adrenal gland of the rat derives cholesterol, in part at least, from cholesterol esters of the very low-density chyle lipoproteins(4). Several tissues, including the adrenal gland, were shown to be capable of hydrolyzing chyle cholesterol esters(5). But the manner in which the rat adrenal gland metabolized cholesterol esters is unique(4). Twenty-four hours after an injection of C^{14}-cholesterol-labeled chyle lipoproteins in which about 75% of the sterol C^{14} was in the esterified form, approximately

* This investigation was supported by USPHS Research Grant CA-00879-16 from Nat. Cancer Inst.

† Postdoctoral Research Fellow of Alameda County Heart Assn. Present address: Dept. of Biochemistry, M. S. University of Baroda, Baroda, India.

90% of the sterol C^{14} was in the free form in liver, adipose tissue, small intestine, muscle, bone, kidney and spleen. In the adrenal gland the proportion of sterol C^{14} recovered in the free form also increased during the first few hours after the injection, but it soon decreased, and by 24 hours about 90% of it was esterified. This suggests that the gland first hydrolyzes the chyle cholesterol esters it removes from the circulation, as do the other tissues, but subsequently resynthesizes new cholesterol esters to the extent that the latter becomes the predominant form in which the sterol is stored in the gland.

The Snell transplantable adrenal cortical tumor 494H is capable of producing certain of the hormones secreted by the normal adrenal gland(6). Since cholesterol is a precursor of corticosteroids(7-11), it became of interest to determine whether this tumor would show the same pattern for its *in vivo* metabolism of chyle cholesterol that we observed in the normal gland. To compare further the normal with the tumor tissue, we also studied esterification of free cholesterol and hydrolysis of cholesterol esters by homogenates prepared from them.

Experimental. Materials. Cholesterol-4-C^{14} (specific activity 13.7 mc/mmole) and cholesterol-7-α-H^3 were purchased from the New England Nuclear Corp., Boston, Mass. Cholesterol-7-α-H^3-oleate (specific activity 0.34 mc/mmole) was synthesized by the procedure of Page and Rudy(12). The labeled compounds were purified on silicic acid columns(13). Adenosine triphosphate (ATP) and coenzyme A (CoA) were purchased from Pabst Laboratories, Milwaukee Wis., and reduced glutathione (GSH) from Calbiochem, Los Angeles, Calif.

Tumor-bearing rats. Two groups, one male and the other female, of Osborne-Mendel rats bearing adrenal cortical tumor 494H(6) were supplied by Dr. Katherine Snell of the National Cancer Institute, Bethesda, Md. The first group had been inoculated intraperitoneally with the 30th generation transplants of the adrenal carcinoma, and the second group with the 33rd. The rats were used 6-7 weeks after inoculation of the transplants, at which time they weighed 230-260 g and their tumor masses 5-15 g. Their adrenal glands were atrophied. All rats, including the normal controls (Osborne-Mendel), were maintained on Purina Laboratory Chow.

In vivo *procedures.* The cholesterol-labeled, very low-density chyle lipoproteins ($S_f > 20$) were prepared, as previously described(4), from chyle collected from the thoracic ducts of normal, Long-Evans female rats that had been fed cholesterol-4-C^{14}. While under light anesthesia, each tumor-bearing and control rat received intravenously (leg or tail vein) 1.0 ml of the labeled chyle preparation. Animals were killed 0.5, 2, 4, 12 or 24 hours after the injection. All of the tumor tissue was removed and necrotic areas were discarded. The tumor tissue was then blotted, weighed and homogenized in a Waring blendor with a 3:1 (v/v) ethanol-ethyl ether solution. Adrenal glands removed from the normal rats were weighed, placed in Erlenmeyer flasks containing the same ethanol-ethyl ether solution, and disintegrated by grinding with a stirring rod.

In vitro *procedures.* Adrenal glands from the control rats and the tumor tissue from the tumor-bearing ones were removed after the animals had been exsanguinated by heart puncture while lightly anesthetized with ether. Normal adrenal glands were excised from both Osborne-Mendel and Long-Evans female rats weighing 180-300 g.‡ Non-necrotic tumor tissues were removed and blotted. Each tissue was homogenized in 50 volumes of cold 0.25 M sucrose in a Potter-Elvehjem type homogenizer provided with a motor-driven, tight-fitting teflon pestle. Two ml of each homogenate (40 mg of tissue) were incubated with a mixture consisting of (a) 3.0 ml of either 0.1 M K phosphate buffer (pH 6.0 or higher) or 0.1 M Na acetate buffer (pH 5.0 or lower) and (b) the labeled substrate (10,000 cpm dissolved in 0.1 ml of acetone). When used, cofactors (20 μmoles of ATP, 0.5 μmoles of CoA, 20 μmoles of $MgCl_2$ and 20 μmoles of GSH) were first dissolved in 0.25 M sucrose and added in a volume of 0.2 ml. A final adjust-

‡ The extent of esterification of labeled cholesterol and of hydrolysis of labeled cholesterol esters by the glands of the 2 strains were the same.

ment was made for the pH of each buffer before the labeled substrate was added. Incubations were carried out at 37°C for 3 hours with continuous shaking. The reactions were terminated by addition of 15 ml of ethanol-ethyl ether, 3:1 (v/v) to each incubation flask.

Analytical procedures. In both *in vivo* and *in vitro* experiments, tissue lipids were extracted as previously described(4), and esterified and free sterol fractions were separated from the extracts on silicic acid columns (13). The sterol fractions eluted from the columns were dried, dissolved in 15 ml of toluene containing 45 mg of 2,5-diphenyloxazole and 1.5 mg of 1,4-bis-2(5-phenyloxazolyl)-benzene, and assayed for C^{14} or H^3 with the Packard Tri-Carb liquid scintillation spectrometer.

The sterol contents of the esterified fraction (after hydrolysis) and free cholesterol fraction were determined by the $FeCl_3$ reaction(14) after the sterols had been precipitated with digitonin according to the procedure of Sperry and Webb(15).

Results and discussion. The C^{14} recovered as total lipids, expressed as percentage of the injected C^{14} per 100 mg of tissue, in the *in vivo* experiments with the normal adrenal glands and tumor tissues in shown in Table I. In earlier studies(16) in which normal rats were injected with cholesterol-labeled chyle lipoprotein prepared as in this investigation, it was revealed that most of the C^{14} is removed from the circulation earlier than 30 minutes (the first interval studied in the present experiments). The values for tumor tissues are about one-fortieth those for normal adrenals. However, because of the large mass of tumor tissues, the total amount of lipid C^{14} recovered in the entire mass of tumor (approximately 1 to 5% of the injected C^{14}) was about 10 times that recovered in both adrenals of each normal rat. In our earlier study(4) with injected cholesterol-labeled chyle lipoproteins, C^{14} uptake was not recorded as per unit weight of adrenal, and values for the C^{14} contents of the whole glands were too variable to indicate a change with time. In the present study, no consistent difference in the lipid C^{14} recovered at the various time intervals was observed in the tumor, but in the normal gland the recoveries were higher at 12 and 24 hours than at the earlier intervals. Since, a few hours after injection of cholesterol chyle lipoproteins, the C^{14} in the bloodstream is largely C^{14} recirculated from the liver following uptake of the chyle lipoproteins by tissues(16), it is likely that the isotope accumulated by the normal adrenal gland at the later intervals was recirculated labeled cholesterol.

TABLE I. Per Cent of C^{14} of Intravenously Injected Cholesterol-4-C^{14}-Labeled, Very Low-Density Chyle Lipoproteins Recovered as Lipids in Transplanted Adrenal Tumor and Adrenal Glands Excised from Normal Rats.

Hr after injection	% of injected C^{14} recovered as total lipids: Tumor (per 100 mg)	Normal adrenal (per 100 mg)
.5	.008	.60
.5	.021	
2	.020	.64
4	.021	.37
4	.020	
6	.028	.69
12	.002	1.63
24	.033	1.18
24	.020	1.35

The two preparations of labeled chyle lipoproteins contained 73.0 and 71.3% of the sterol C^{14} in the esterified form. Fig. 1 shows the percentages of the recovered sterol C^{14} present in the esterified form in tumor and normal gland at 6 different intervals after the injection. The changes observed with time in the proportions of C^{14} in the esterified form in the normal adrenal gland were almost identical with those observed in our earlier study(4). In both normal adrenal and tumor there occurred a decline followed by a rise in the proportions of esterified sterol C^{14}. In the normal gland the decrease in the proportion of sterol C^{14} in the esterified form was evident at the very first interval (30 minutes) but it was not detected until the second interval (2 hours) in the tumor. Furthermore, the extent of the increase in the proportion of esterified sterol C^{14} was considerably smaller in the tumor than in the normal tissue. If we assume that the proportions of labeled esterified and free cholesterol taken up by the normal gland

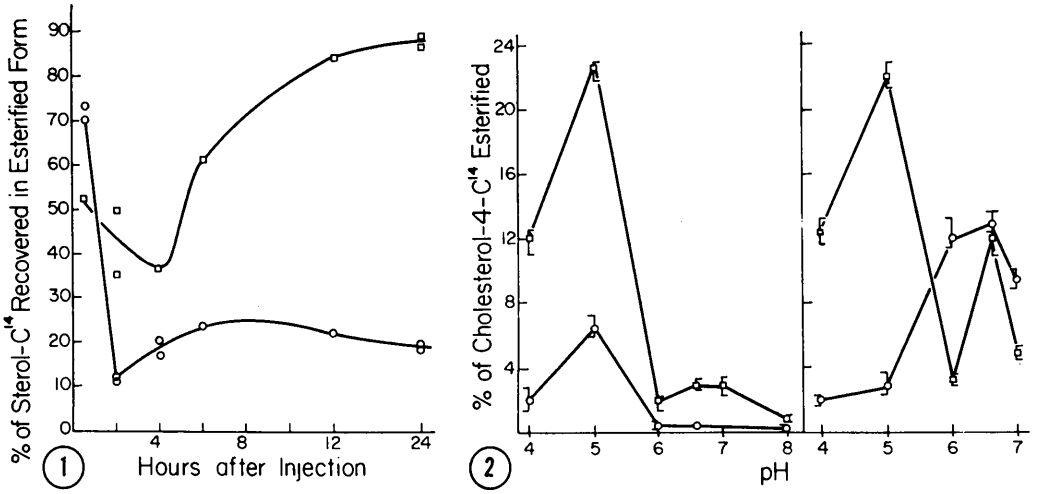

FIG. 1. Proportion of the recovered sterol C^{14} present in esterified form in adrenal tumors (○) and normal adrenal glands (□) at various intervals after injection of cholesterol-labeled, very low-density ($S_f>20$) chyle lipoproteins. The 2 preparations contained about 70% of the sterol C^{14} in esterified form.

FIG. 2. Effect of pH and cofactors on esterification of C^{14}-cholesterol by homogenates of transplanted adrenal tumor (○) and normal adrenal gland (□). Left: No cofactors added to incubation mixtures. Right: ATP, CoA, $MgCl_2$ and GSH added to each flask. Acetate buffers were used at pH 5.0 and lower, and phosphate buffers at pH 6.0 and higher. Each point is the average of 4 determinations. Range of values shown by vertical lines.

and tumor were the same, these observations suggest (a) that in both tumor and normal gland the chyle cholesterol esters removed from the circulation were first hydrolyzed and the free cholesterol released then re-esterified, and (b) that hydrolysis was less rapid and reesterification less extensive in the tumor than in the normal tissue. Since, in the rat, the fatty acid composition of sterol esters in the adrenal gland differs from that in the plasma(17,18), it would seem that, in the normal gland at least, hydrolysis of the esters is followed by synthesis of new esters characteristic of the gland. The percentages of sterol C^{14} recovered in the esterified form at the end of the experiment—about 88% in the normal gland and 19% in the tumor—correlate well with the percentages of endogenous sterol present as the ester—about 89% and 16% in the normal tissue and tumor, respectively (calculated from the values in Table II).

The effect of pH and cofactors on the esterification of cholesterol (Fig. 2) show the following for both tumor and the normal adrenal: (a) pH optimum of 5.0 in the absence of cofactors; (b) no increase in cholesterol esterification at that pH upon the addition of ATP, CoA and Mg^{++} and GSH, but a pronounced increase upon their addition at pH 6.6. These curves also show that the maximum values for esterification were considerably higher in the normal adrenal than in the tumor. The lower esterification in the tumor cannot be accounted for by a greater dilution of the added labeled cholesterol by endogenous free cholesterol because the concentration of free cholesterol in the tumor was less than half that in the normal adrenal (Table II).

TABLE II. Endogenous Sterol Concentrations.

Tissue	Mg free sterol per g tissue*	Mg esterified sterol per g tissue*
Normal adrenal	4.40	26.8
Tumor	1.93	.37

* Average values for 4 normal and 4 tumor-bearing rats.

In both the tumor and normal adrenal the highest values for hydrolysis of cholesterol oleate were observed when the pH of the medium was 7.0 or higher (Fig. 3). The maximum values for hydrolysis were considerably higher in the normal adrenal than

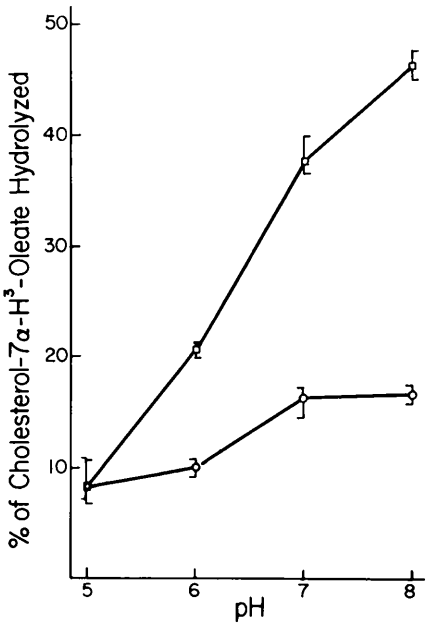

FIG. 3. Effect of pH on hydrolysis of cholesterol-7α-H³-oleate by homogenates of transplanted adrenal tumors (O) and normal adrenal glands (□). Acetate buffer was used at pH 5.0 and lower, and phosphate buffer at pH 6.0 and higher. Each point is average of 4 determinations. Range of values shown by vertical lines.

in the tumor. Since the cholesterol ester content of the normal adrenal is about 80 times that of the tumor (Table II), it is apparent that the lower values for the tumor cannot be ascribed to a greater dilution of the added cholesterol oleate by endogenous cholesterol oleate in the tumor than in the normal adrenal.

This study shows that, qualitatively, the normal and tumor tissues have similar patterns for the *in vivo* hydrolysis of chyle cholesterol esters and subsequent reesterification of the free cholesterol as well as similar characteristics for *in vitro* esterification and hydrolysis. But there are quantitative differences between the two tissues. The quantitative differences observed *in vivo* reflected those observed *in vitro*. Thus, *in vivo*, the reduction in the proportion of labeled sterol in the esterified form occurred later in the tumor than in the normal gland, a finding in accord with the tumor's lowered *in vitro* capacity for hydrolyzing cholesterol oleate; also, the lesser extent of the reesterification *in vitro* capacity to esterify free cholesterol. Since in the tumor, in contrast to the normal gland, the endogenous sterol is present predominantly in the free rather than esterified form, it would appear that the more pronounced of the above two cited defects in cholesterol metabolism in the tumor is the reduced capacity to esterify free cholesterol.

Summary. A very low-density, cholesterol-labeled lipoprotein fraction of rat chyle, in which about 70% of the sterol C^{14} was in the esterified form, was injected intravenously into normal rats and into rats bearing the transplanted Snell adrenal cortical tumor 494H. The esterified and free sterol C^{14} contents of the tumor and of the normal adrenal glands were measured at several intervals after the injection. The same general pattern—an initial decrease followed by an increase in the proportion of sterol C^{14} esterified—was observed for tumor and normal gland. This suggests that in both cases the uptake and hydrolysis of labeled chyle cholesterol esters were followed by cholesterol reesterification. In the tumor, however, the hydrolysis occurred later and the subsequent reesterification was much less pronounced. A comparison of esterification of free cholesterol and hydrolysis of cholesterol oleate by homogenates prepared from tumors and normal glands revealed the following for both tissues: The pH optimum, in the absence of cofactors, was 5.0. Addition of CoA, ATP, Mg^{++} and GSH at that pH did not increase the esterification, whereas their addition at pH 6.6 did. Hydrolysis of cholesterol oleate was highest at pH 7.0 or above. Quantitative differences between the tumor and the normal adrenal *in vitro* reflected differences observed *in vivo*. Homogenates prepared from the tumor had a lower capacity for hydrolyzing cholesterol oleate and for esterifying free cholesterol than did homogenates of the normal gland. In the tumor, in contrast to the normal adrenal, the predominant form of the endogenous sterol was the free rather than the esterified. This finding, as well as those cited above, suggests that the major of the two described defects in cholesterol metabolism in the tumor is the impairment in capacity to esterify free cholesterol.

We are indebted to Dr. Katherine Snell, Nat. Cancer Inst., N.I.H., Bethesda, Md., for providing us with the tumor-bearing rats. Our thanks are due to Miss Christine Giotas for technical assistance.

1. Cook, R. P., in Chloresterol, R. P., Cook, Ed., Academic Press, New York, 1958, p166.

2. Deuel, H. J., Jr., The Lipids, Interscience Publishers, Inc., New York, 1955, v2, p766.

3. Deane, H. W., Handbuch der Experimentellen Pharmakologie, H. W. Deane, Ed., Springer-Verlag, Berlin, 1962, v14, p51.

4. Lossow, W. J., Brot, N., Chaikoff, I. L., J. Lipid Res., 1962, v3, 207.

5. Brot, N., Lossow, W. J., Chaikoff, I. L., ibid., 1962, v5, 63.

6. Snell, K. C., Stewart, H. L., J. Nat. Cancer Inst., 1959, v22, 1119.

7. Zaffaroni, A., Hechter, O., Pincus, G., J. Am. Chem. Soc., 1951, v73, 1390.

8. Hechter, O., Solomon, M. M., Zaffaroni, A., Pincus, G., Arch. Biochem. Biophys., 1953, v46, 201.

9. Werbin, H., Bergenstal, D. M., Gould, R. G., Le Roy, G. V., J. Clin. Endocrinol. and Metab., 1957, v17, 337.

10. Werbin, H., Chaikoff, I. L., Arch Biochem. Biophys., 1961, v93, 476.

11. Peron, G., J. Biol. Chem., 1961, v236, 1764.

12. Page, I. H., Rudy, H., Biochem. Z., 1930, v220, 304.

13. Creech, B. G., Sewell, B. W., Anal. Biochem., 1962, v3, 119.

14. Zlatkis, A., Zak, B., Boyle, A. J., J. Lab. Clin. Med., 1953, v41, 486.

15. Sperry, W. M., Webb, M., J. Biol. Chem., 1950, v187, 97.

16. Naidoo, S. S., Lossow, W. J., Chaikoff, I. L., J. Lipid Res., 1962, v3, 309.

17. Daily, R. E., Swell, L., Field, H., Jr., Treadwell, C. R., Proc. Soc. Exp. Biol. and Med., 1960, v105, 4.

18. Swell, L., Field, H., Jr., Treadwell, C. R., ibid., 1960, v104, 325.

Received January 28, 1965. P.S.E.B.M. 1965, v119.

Transfer of Gamma Globulin from Mother to Offspring in Mink.*† (30117)

DAVID D. PORTER (Introduced by Frank J. Dixon)

Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

The transfer of immunoglobulins from mother to offspring occurs by different mechanisms in various species, and can be roughly correlated with the type of placentation. For example, immunoglobulins in man are transferred *in utero*, in the cow post partum *via* the colostrum, and in mice by both methods. During the study of Aleutian disease of mink (*Mustela vison*), we have made observations on the mechanism of transfer of immunoglobulin from mother to offspring. It is known that young mink have maternal antibody(1), but no study of the mechanism of transfer has been reported.

Materials and methods. Ranch raised pregnant female mink were obtained from the Utah Mink Growers Cooperative and were judged to be free from Aleutian disease on the basis of serum protein electrophoresis(2).

Mink 6.4S gamma globulin and albumin were prepared by ion exchange cellulose chromatography and were homogeneous by electrophoretic, ultracentrifugal and immunologic tests. The proteins were trace labelled with I^{131}. Six female mink were given 165 to 500 μc I^{131} gamma globulin and 2 were given 500 μc I^{131} albumin by intracardiac injection ½ to 10 days before delivery. The animals received potassium iodide in their drinking water throughout the experiment. At time of delivery, some kits were immediately removed, and others were allowed to nurse. Colostrum was removed from no more than 2 of the mothers eight breasts. Maternal and kit serum, colostrum, and milk were examined for trichloroacetic acid precipitable radioactivity using a well type scintillation counter.

* Publication No. 103 from Division of Exp. Pathol., Scripps Clinic and Research Foundation, La Jolla, Calif.

† Supported by Grant AM-06577 from Nat. Inst. of Metab. Dis., N.I.H., U.S.P.H.S.