ganisms(6,7,9,10). Further studies should be conducted on the gerbil as well as on other animals to establish age-related modifications in glycoprotein values both in pregnant and non-pregnant females, as well as in males. Since plasma proteins high in carbohydrate content have been found in the embryos of various animals(11), it may be that such proteins are produced by the female for subsequent transfer to developing embryos.

Summary. Age-related changes in the plasma glycoproteins of the Mongolian gerbil *(Meriones unguiculatus)* were studied in animals ranging in age from 1-3 days to 28 months. Analyses were performed using paper electrophoresis and periodic acid-Schiff staining, and glycoprotein percent composition values were calculated for albumin, $alpha_1$-, $alpha_2$-, beta- and gamma-globulin fractions. $Alpha_1$-/beta-globulin glycoprotein ratios decreased with increasing age. Least affected by animal aging were the $alpha_2$- and beta-globulin fractions, whereas the greatest percent changes were shown by $alpha_1$-globulin, which steadily decreased with increasing age. All five plasma fractions contained significant quantities of glycoprotein material.

The authors express their gratitude to The West Foundation, Brant Lake, N. Y., for supplying the gerbils used in this study.

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. Ringle, D. A., Dellenback, R. J., *ibid.,* 1963, v204, 275.

5. Winzler, R. J. In: *The Plasma Proteins,* F. W. Putnam, ed., New York, Academic Press, 1960, v1, 309.

6. Payne, R. W., *Ann. N. Y. Acad. Sci.,* 1961, v94, 284.

7. Wakamatsu, H., *Keio J. Med.,* 1961, v10, 225.

8. Peterson, R. P., Turbyfill, C. L., Soderwall, A. L., *Fert. Steril.,* 1962, v13, 486.

9. Hill, N. C., Goldstein, N. P., McKenzie, B. F., McGuckin, W. F., Svien, H. J., *A.M.A. Arch. Neur.,* 1960, v2, 341.

10. Cooper, G. R. In: *The Plasma Proteins,* F. W. Putnam, ed., New York, Academic Press, 1960, v1, 51.

11. Moore, D. H., Shen, S. C., Alexander, C. S., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 307.

Received September 26, 1963. P.S.E.B.M., 1963, v114.

Esterification of Free Cholesterol and Hydrolysis of Cholesterol Oleate by Acetone Powder Extracts of Hog Adrenals.* (28798)

NATHAN BROT, W. J. LOSSOW AND I. L. CHAIKOFF
Department of Physiology, University of California, Berkeley

Dailey *et al*(1) failed to observe either esterification of free cholesterol-4-C^{14} or hydrolysis of cholesterol-4-C^{14} esters when they added either one to a hog adrenal gland homogenate preparation in which they were able to show conversion of cholesterol-4-C^{14} to steroids. In this system the endogenous esterified cholesterol remained constant during the steroid synthesis. One explanation offered for these findings was that hog adrenals lacked a cholesterol esterase system. In subsequent studies(2,3) these workers found that homogenates of dog adrenals converted free cholesterol-C^{14} to cholesterol esters and labeled cholesterol esters to free cholesterol under conditions in which these substrates were incorporated into steroids. Dailey *et al* (2,3) stated that the hog adrenal has a much lower ratio of esterified to free cholesterol than does the dog adrenal, and suggested that a relationship might exist between that ratio and the capacity of the adrenal gland of a particular species to interconvert free and esterified cholesterol.

* Aided by grants from U. S. Public Health Service and Life Insurance Medical Research Fund.

We have observed that homogenates of rat, guinea pig and beef adrenal glands, as well as extracts of acetone powders prepared from these glands, readily esterify labeled free cholesterol, and that the optimum pH for this reaction in adrenal preparations of all 3 species lies between 4 and 6, with little or no esterification taking place above pH 7.† Since the experiments of Dailey *et al*(1) with hog adrenal homogenates were performed at pH 7.4 (the pH favorable for steroid synthesis), it seemed desirable to explore the possibility that, in the adrenal gland of the hog, esterification of free cholesterol and/or hydrolysis of cholesterol esters could be observed at a pH other than 7.4.

Experimental. Preparation of radioactive β-lipoprotein substrates. 0.4 ml of a 5% dextran sulfate solution and 0.1 ml of a 11% $CaCl_2$ solution were added to each 1 ml of rat serum. After standing for 2 hours at 4°, the mixture was centrifuged at about 1,450 × *g* for 10 minutes. The supernatant fraction was decanted, and the β-lipoprotein precipitate was dissolved in a volume of 0.9% NaCl equal to the original volume of serum. The β-lipoproteins were again precipitated and redissolved as described above, and then incubated for 20 hours with either free cholesterol-4-C^{14} or cholesterol-7α-H^3 oleate dispersed on celite, as described by Avigan(4). At termination of the incubation the celite was removed by centrifugation and the β-lipoproteins were again precipitated and dissolved in 0.9% NaCl. About 50% of the incubated C^{14}-labeled cholesterol and about 20% of the incubated H^3-labeled esters had been incorporated into the β-lipoproteins.

The labeled cholesterols were purchased from the New England Nuclear Corp., Boston, Mass. Cholesterol oleate was synthesized by the procedure of Page and Rudy(5). Both labeled compounds were purified on silicic acid columns before use(6).

Incubation procedure. Acetone powders of hog adrenals were prepared as described by Morton(7). The powders were extracted with 15 volumes of 0.05 M potassium phosphate buffer of pH 6.1 at 4°C for 1 hour and the mixtures were then centrifuged at 16,000 × *g* for 1 hour. 1.0-ml aliquots (containing about 21 mg of protein) of the clear supernatant fraction were incubated for 3 hours at 37°C in flasks containing 3.5 ml of 0.1 M citrate-phosphate buffer of various pH's and 0.5 ml of a radioactive β-lipoprotein substrate.

† Unpublished observations.

TABLE I. Esterification of Free Cholesterol- and Hydrolysis of Esterified Cholesterol-Labeled β-Lipoproteins by Acetone Powder Extracts of Hog Adrenal Glands.

Flask	β-Lipoprotein substrate labeled with	% of cholesterol-4-C^{14} esterified (flasks 1-4)* or % of cholesterol-7-H^3-oleate hydrolyzed (flasks 5-8)† pH 5.7	pH 7.4
1	Free cholesterol-4-C^{14}	7.6	
2	"	7.5	
3	"		.4
4	"		.4
5	Cholesterol-H^3-oleate	6.1	
6	"	6.1	
7	"		12.4
8	"		9.6

* The avg % sterol-C^{14} in esterified form in control flasks containing the free cholesterol-4-C^{14} substrate and no acetone powder extract was 0.4% at both pH's.

† The avg % sterol-H^3 in free form in control flasks containing the cholesterol-7-H^3-oleate substrate and no acetone powder extract was 1.6% at pH 5.7 and 1.4% at pH 7.4.

Analytic procedures. The incubation mixtures were extracted as previously described (8), and the esterified and free sterol fractions were separated by silicic acid chromatography(6). The dried lipid samples were 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 either C^{14} or H^3 in a Packard Tri-Carb liquid scintillation spectrometer.

Results. It is apparent from the findings presented in Table I that esterification of labeled free cholesterol occurred at pH 5.7 and was virtually absent at pH 7.4. On the other hand, hydrolysis of the labeled ester was observed at both pH's, but was greater at the higher one. A more detailed pH study for the esterification reaction is shown in Fig. 1. Esterification of the cholesterol-4-C^{14}-labeled β-lipoprotein substrate by hog adrenal powder extracts was optimal in the

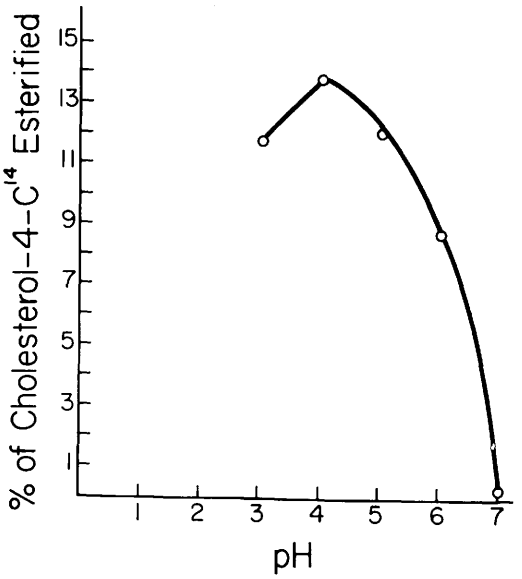

FIG. 1. Effect of pH on esterification of free cholesterol-4-C^{14} by acetone powder extracts of hog adrenal gland.

pH range between 3 and 5, with maximum activity observed at pH 4.0. No esterification was observed at pH 7.0.

Summary. It is demonstrated that extracts of acetone powders of hog adrenal glands do esterify free cholesterol and hydrolyze esterified cholesterol. The optimum pH range for esterification was between 3 and 5, and no esterification was observed at pH 7.4. Hydrolysis of the ester was observed at pH 7.4 and to a lesser extent below pH 6.

1. Dailey, R. E., Swell, L., Treadwell, C. R., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 571.
2. ———, *Arch. Biochem. Biophys.*, 1962, v99, 334.
3. ———, *ibid.*, 1963, v100, 360.
4. Avigan, J., *J. Biol. Chem.*, 1959, v234, 787.
5. Page, I. H., Rudy, H., *Biochem. Z.*, 1930, v220, 304.
6. Creech, B. G., Sewell, B., *Anal. Biochem.*, 1962, v3, 119.
7. Morton, R. K., in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Ed., Academic Press, New York, 1955, v1, 34.
8. Lossow, W. J., Brot, N., Chaikoff, I. L., *J. Lipid Res.*, 1962, v3, 207.

Received September 27, 1963. P.S.E.B.M., 1963, v114.

Renal Clearance of Glucosamine in the Rat.* (28799)

JACK D. CLAYTON AND M. KATHLEEN CARTER (Introduced by F. W. Schueler)
Department of Pharmacology, Tulane University School of Medicine, New Orleans, La.

Glucosamine (2-amino-2-deoxy-D-glucose) is an amino sugar widely distributed throughout all tissues of the body and exists almost entirely as a bound component of mucoproteins, nucleic acids, and mucopolysaccharides(1). Carter and Peters found that the renal clearance of exogenous glucosamine in dogs was equal to the glomerular filtration rate (GFR), measured by creatinine clearances, over a wide range of glucosamine plasma concentrations(2). Coulson and Hernandez, in testing the hyperglycemic effect of glucosamine in rats and alligators, found that the glucosamine excreted by the kidney was identical to that injected and was not a metabolic product(3). However, no information is available on renal clearance of glucosamine in rats. Glasser(4) has shown that creatinine clearances in rats are inaccurate as a measure of GFR if plasma levels are below 150 mg%, because of tubular secretion of creatinine in this species. Therefore, in the experiments reported here, glucosamine clearances in rats were compared with inulin clearances since it is difficult to maintain such a high plasma level of creatinine.

Materials and methods. Male Sprague-Dawley rats, weighing 400 to 500 g, were used. After being anesthetized with pentobarbital sodium, 35 mg/kg, i.p., the rats were prepared as follows: a tracheal cannula (PE 240-320) was inserted, the jugular vein was cannulated (PE 50) for infusion pur-

* This work was supported in part by training and research grants from USPHS.