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Cholesterol Esterification by Adrenal Homogenates.* (28481)

CHRISTOPHER LONGCOPE AND ROBERT H. WILLIAMS
Department of Medicine, University of Washington School of Medicine, Seattle

In certain animal species the cholesterol in the adrenal gland is present largely in the ester form(1,2), and it has been postulated that these cholesterol esters are derived from the lymph esterified cholesterol(3). It has recently been shown, however, that homogenates of rat adrenal tissue are able to esterify cholesterol(4), suggesting that the rat adrenals may form their own cholesterol esters. We now wish to report the extension of these studies with homogenates of adrenal tissue from other species.

Materials and methods.† The animals were killed and the adrenal glands rapidly removed and placed in ice-cold saline. After carefully removing the adipose tissue from the glands, they were homogenized, using 1 ml 0.25 M sucrose per 20 mg adrenal tissue. The homogenates were centrifuged at 1,000 $\times$ *g* for 10 minutes and the supernatant A collected. This fraction, unless otherwise noted, was centrifuged at 20,000 $\times$ *g* for 20 minutes, and the supernatant from the resulting sample centrifuged at 85,000 $\times$ *g* for 20 minutes. The remaining pellet, numbered 3, was then resuspended in the original volume of 0.25 M sucrose and incubated in 2 ml homogenate and 1 ml KH_2PO_4 buffer containing the cofactors and either 1-C^{14}-palmitic acid or 4-C^{14}-cholesterol. The labeled lipids were dissolved in propylene glycol to a final volume of less than 0.25% per flask. All incubations were done at 37°C in a Dubnoff metabolic shaker in room air.

At the end of the incubation period, 2 ml of medium was removed and extracted twice with 5 ml portions of chloroform:methanol (2:1 v/v) and once with 5 ml petroleum ether. The extracts were pooled, dried under nitrogen, and the residue dissolved in hexane and placed on a silicic acid column(5). The cholesterol esters were eluted with 35 to 50 ml of 15% benzene in hexane and free cholesterol was eluted with 10 ml ether and then 10 ml methanol. Aliquots of each fraction were dried under air, dissolved in toluene phosphor(6) and the radioactivity was determined in a liquid scintillation counter. Cholesterol, free and esterified, was determined on an aliquot of each fraction by the method of Courchaine, Miller and Stein(7). Proteins were determined using a modification of the Lowry method(8).

Results. As seen in Table I, supernatant A of rat and guinea pig adrenal was quite active in esterifying cholesterol while rabbit adrenal was slightly less active. Supernatant A of bovine adrenal, however, was relatively deficient in cholesterol-esterifying activity. With added 1-C^{14}-palmitic acid and unlabeled cholesterol, pellet 3 of both rat and guinea pig adrenal was very active (Table II) while bovine adrenal displayed only a fraction of this cholesterol-esterifying ability. With 4-C^{14}-cholesterol, pellet 3 of rat and rabbit adrenal was able to esterify cholesterol to a significant amount (Table III), while pellet 3 of bovine adrenal was relatively deficient in this regard.

Discussion. Using a system which is optimal for esterification of cholesterol by homogenates of rat adrenal tissue(4), similar

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† Described more fully in Longcope, C., Williams, R. H., *Endocrinology*, in press.

TABLE I. Esterification by Supernatant A.

Species	No. of animals	Ester cholesterol (μg per flask)	Free cholesterol (μg per flask)	CPM* recovered as ester cholesterol (mean ± SEM)
Rat adrenal	4	213.2	115.2	6,800 ± 50
Guinea pig adrenal	3	46.1	84.7	4,100 ± 100
Rabbit adrenal	3	287.4	151.3	2,700 ± 600
Calf adrenal	1	11.5	80.8	650

* Based on 50,000 CPM of 4-C^{14}-cholesterol added to each incubation flask.

Two ml supernatant A was incubated in 1 ml KH_2PO_4 buffer containing 40 μM ATP, 5 μM $MgCl_2$, 0.2 μM CoA, 20 μM GSH (reduced glutathione) and 4-C^{14}-cholesterol.

TABLE II. Esterification Using Palmitic-1-C^{14} Acid.

Species	No. of animals	CPM* recovered as cholesterol ester (mean ± SEM)	Free cholesterol/flask (μg)	Protein/flask (mg)
Rat adrenal	4	26,350 ± 345	35.9	.66
Guinea pig adrenal	3	26,325 ± 1,125	34.7	1.02
Bovine adrenal	3	3,565 ± 240	22.9	.54

* Based on 50,000 CPM 1-C^{14}-palmitic acid added to each incubation flask.

Two ml of pellet 3 suspension was incubated in 0.25 M sucrose and 1 ml of KH_2PO_4 buffer containing 40 μM ATP, 5 μM $MgCl_2$, 0.2 μM CoA, 20 μM GSH, 200 μM NaF, 5 γ unlabeled cholesterol and 1-C^{14}-palmitic acid.

TABLE III. Esterification by Pellet 3.

Species	No. of animals	CPM* recovered as cholesterol ester (mean ± SEM)	Free cholesterol/flask (μg)	Protein/flask (mg)
Exp 1				
Rat adrenal	4	10,698 ± 100	27.0	.66
Bovine adrenal	3	503 ± 33	25.1	.54
Exp 2				
Rat adrenal	6	17,371 ± 287	26.8	—
Rabbit adrenal	3	11,090 ± 312	41.5	

* Based on 50,000 CPM of 4-C^{14}-cholesterol added to each incubation flask.

Two ml of pellet 3 suspension was incubated in 0.25 M sucrose and 1 ml of KH_2PO_4 buffer containing 40 μM ATP, 5 μM $MgCl_2$, 0.2 μM CoA, 20 μM GSH and 4-C^{14}-cholesterol.

levels of activity have been demonstrated by homogenates of adrenal glands from guinea pigs and rabbits. This esterifying activity was present in supernatant A as well as the more purified pellet 3, and could be demonstrated using either 1-C^{14} palmitic acid or 4-C^{14}-cholesterol.

Under similar conditions, however, only minimal esterification occurred in homogenates of bovine adrenal tissue. Both supernatant A and pellet 3 of bovine adrenal esterified only 5 to 10% of the cholesterol esterified by the other adrenal preparations, although their protein contents did not differ markedly. That this is not due to a dilutional phenomenon is shown by the levels of unlabeled cholesterol found in the homogenates. The conditions used may not have been optimal for bovine tissue, but it was noted(4) that homogenates of rat adrenals caused significant esterification at a higher and lower pH, and the cofactor requirements probably do not differ markedly. Therefore, the low levels of cholesterol esterification found in bovine preparations are thought to be due to low levels of the cholesterol esterifying enzyme(s).

While bovine adrenal glands contain only small amounts of cholesterol ester, in contrast to the adrenal glands of rats, rabbits

and guinea pigs, blood levels of cholesterol and per cent of cholesterol esters are similar in all 4 species(9). This suggests that significant cholesterol-esterifying activity is needed to maintain high levels of cholesterol esters in the adrenal gland and that the blood level *per se* is not the determining factor.

These findings correlate well with those of Lossow, Brot and Chaikoff(10). These workers injected 24-C^{14}-cholesterol intravenously into rats and noted that initially the adrenal contained large amounts of labeled free cholesterol, but subsequently the label was found largely in the ester fraction. They suggested that cholesterol esters of the blood were hydrolyzed prior to entry into the adrenal cells and were subsequently esterified *in situ*. Our finding cholesterol-esterifying activity in adrenals that normally contain large amounts of cholesterol in the ester form, and our failure to demonstrate this activity in adrenals that do not contain significant amounts of cholesterol ester, would seem to support this thesis.

Summary. Cholesterol-esterifying activity was demonstrated in homogenates of rat, rabbit and guinea pig adrenals. Cholesterol-esterifying activity was found to be deficient in homogenates of bovine adrenal tissue. This suggests that the amount of cholesterol esters in the adrenal gland is dependent on the level of cholesterol-esterifying activity in the gland, rather than on blood levels of cholesterol, free or esterified.

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Transferrins of Cattle Twins.* (28482)

S. P. DATTA† AND W. H. STONE (Introduced by M. R. Irwin)
Division of Genetics, University of Wisconsin, Madison

About 90% of dizygotic (DZ) cattle twins are chimeras and have a mixture (mosaicism) of 2 antigenically distinct kinds of erythrocytes(1). Erythrocyte mosaicism or chimerism is believed to arise from *in utero* vascular anastomosis between dizygotic twins, permitting an interchange of primordial erythropoietic cells and the persistence of mixed blood types in each twin(2), consequently members of a twin pair show identical blood types. Anastomosis of the vessels of unlike-sex twins presumably allows for the exchange of hormones resulting in the freemartin condition(3). Tissues that give rise to histocompatibility antigens are likewise exchanged, since dizygotic twins with erythrocyte mosaicism (DZM) usually accept each other's skin grafts(4,5). In contrast, the tissues that produce the J factor of cattle blood are presumably not exchanged, since DZM twins may be phenotypically different in J even if they had communal circulation as

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† Present address: Dept. of Microbiology and Immunology, Albert Einstein College of Medicine, New York.