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In Vitro Labeling of Hamster and Gerbil Adrenal Cholesterol: Effect of Triamcinolone Administration[1] (38431)

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Investigations into the regulation of adrenal cholesterol metabolism in mammals are complicated by the presence of two distinct types of adrenal cortices based on cholesterol concentrations. Most mammalian species have adrenal cortices with typically high cholesterol concentrations (1–4), but in other mammals such as the cow (5, 6) and the sheep (7) cholesterol concentrations in the adrenal cortex are relatively low. Additionally, the sterol of the cholesterol-rich adrenal cortices is largely esterified (1–4) while that of the cholesterol-poor adrenal cortices, for example, the sheep, is primarily unesterified (8, 9).

These differences in sterol composition are reflected in differences in *in vitro* formation of ^{14}C-cholesterol from labeled precursors. Cholesterol-rich adrenal glands from species such as the dog, guinea pig, or man either do not form ^{14}C-labeled cholesterol *in vitro* from ^{14}C-acetate or require long periods of incubation with high specific radioactivity of the labeled precursor in order to provide significant ^{14}C incorporation into adrenal cholesterol (10–12). Slices of sheep adrenal cortex, however, incorporate significant amounts of ^{14}C into cholesterol from tracer amounts of [3-^{14}C]pyruvate added to a large pool of pyruvate, and when subjected to a pulse of [2-^{14}C]acetate sheep adrenal cortical slices form ^{14}C-cholesterol of high specific radioactivity (7, 9).

The adrenal glands of the golden (Syrian) hamster are cholesterol-poor glands (8, 13), while those of the Mongolian gerbil contain the high cholesterol concentrations (see below) characteristic of most mammalian adrenal glands. The primary objective of these experiments was to determine the extent to which adrenal cholesterol synthesis, as estimated by the formation of ^{14}C-cholesterol from labeled sterol precursors, is carried out by each type of adrenal gland. A second objective was an estimation of the extent to which adrenal cholesterol synthesis would be modified by the administration of the potent antiinflammatory steroid triamcinolone (14), an experimental procedure known to suppress ACTH secretion and adrenal steroid synthesis (15, 16).

Methods. Male and female golden hamsters were grouped according to sex. One-half of the hamsters in each group were given daily subcutaneous injections of 0.154 *M* NaCl solution for 6 days, and the remainder of the hamsters in each group were given daily subcutaneous injections of triamcinolone acetonide 0.25 mg/100 g/day for 6 days. Hamsters were exsanguinated by decapitation and the adrenal glands were removed, bisected, and placed on an iced Petri dish. Pooled adrenal halves, taken from 6–8 hamsters evenly divided between males and females to provide approximately 100 mg of adrenal tissue, were placed into 50-ml Erlenmeyer flasks containing 6 ml of Krebs–Henseleit solution. The flasks were capped with rubber sleeves and incubated for 3 hr following equilibration with 95% O_2–5% CO_2. The substrate was sodium pyruvate 2.5 m*M* to which was added sodium [3-^{14}C]pyruvate to give an initial specific activity of 14,000 cpm/μmole pyruvate. The incorporation of ^{14}C into adrenal cholesterol and into medium bicarbonate was determined in these experiments.

In a second series of experiments, adrenal halves from four or five hamsters were incubated as described above, the substrate being sodium pyruvate 2.5 m*M* to which sodium [1-^{14}C]pyru-

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vate was added to give an initial specific activity of 9500 cpm/μmole pyruvate. ^{14}C incorporation into medium bicarbonate was determined in these experiments.

Mongolian gerbils were treated with triamcinolone acetonide or with physiological saline solution as described for the hamster experiments. Pooled adrenal halves from two gerbils were incubated with pyruvate as described for hamster adrenal tissue to determine the incorporation of ^{14}C into medium bicarbonate and cholesterol.

A final series of experiments utilizing gerbil adrenal glands from triamcinolone-treated and control gerbils was performed to label the adrenal cholesterol fractions. The method used was that described by Biliar, *et al.* (11), for the *in vitro* labeling of sterols of guinea pigs adrenal glands, with the following modifications: Pooled adrenal halves (total weight approximately 50 mg) from two gerbils were incubated for one hour following equilibration with 95% O_2–5% CO_2 in 25-ml Erlenmeyer flasks with 3 ml of Krebs–Henseleit solution containing glucose 5 m*M*. The adrenal tissue was then incubated for three successive 2-hr periods in 3-ml Krebs–Henseleit solution containing sodium acetate 1.5 m*M* to which was added sodium [2-^{14}C]acetate 5.0 μCi/3 ml to provide an initial specific radioactivity of 3.5×10^6 cpm/μmole acetate. At the end of each 2-hr period, the incubation medium was discarded and 3 ml of fresh medium containing the labeled acetate was added to each flask.

Total lipid, extracted from gerbil adrenal glands with a modification (18) of the method of Folch *et al.* (19), was used to obtain both total and unesterified cholesterol. Tissue cholesterol determinations and ^{14}C-incorporation into cholesterol and bicarbonate were performed with methods described by Gans and Cater (17) and Shaefer and Gans (9).

The spleen from each hamster and gerbil was removed, weighed and placed into 10% buffered formaldehyde solution. Paraffin sections were cut at 6 μm and stained with hematoxylin-eosin.

Results. The adrenal gland of the hamster in terms of body weight weighed less than did that of the gerbil (Table I) and the total cholesterol concentration of the hamster adrenal gland, while comparable to the concentration of the unesterified cholesterol of the gerbil adrenal gland, was hardly 10% that of the total cholesterol concentration of the gerbil adrenal gland (Tables II and III). Hamster adrenal glands effectively incorporated ^{14}C from tracer amounts of [3-^{14}C]pyruvate into cholesterol *in vitro* (Table II). When gerbil adrenal glands were incubated *in vitro* with [3-^{14}C]pyruvate in tracer quantities, no ^{14}C-labeled cholesterol was recovered (Table IV).

The unesterified fraction of gerbil adrenal cholesterol was effectively labeled with ^{14}C when the glands were incubated for 6 hr with three changes of incubation solution containing high specific radioactivity [2-^{14}C]acetate (Table III). Esterified cholesterol comprises about 90% of the total gerbil adrenal cholesterol concentration and the dilution of the label in the total cholesterol fraction suggests that the *in vitro*

TABLE I. Effects of Triamcinolone on Body Weight, Splenic Weight, and Adrenal Weight in Hamsters and Gerbils.[a]

Group	N^b	Body wt (g)			Splenic wt mg/100 g body wt	Adrenal wt mg/100 g body wt
		Start	End	Mean change		
			Hamsters			
Control	92	114 ± 4.1	118 ± 3.5	+ 4 ± 2.0	110 ± 10.7	7.3 ± 0.35
Triamcinolone	90	121 ± 4.2	101 ± 3.0	−20 ± 1.9	41 ± 2.5	8.6 ± 0.37
	p $(t)^c$			< 0.001	< 0.001	< 0.02
			Gerbils			
Control	38	66 ± 2.8	66 ± 2.8		115 ± 11.0	24.9 ± 1.2
Triamcinolone	36	66 ± 5.7	64 ± 6.2		50 ± 6.7	22.9 ± 0.8
	p $(t)^c$				< 0.001	

[a] All data in this and subsequent tables are expressed as the average ± 1 SE of the mean.

[b] N = number of hamsters. Adrenal glands from six to eight hamsters were pooled to provide approximately 100 mg wet wt.

[c] Significance of difference between groups.

TABLE II. Cholesterol Concentrations and Incorporation of ^{14}C-Pyruvate into Bicarbonate and Cholesterol by Hamster Adrenal Glands *in Vitro*.[a]

Group	N[b]	Adrenal cholesterol % wet wt	% Incorporation added ^{14}C/100 mg in 3 hr into Bicarbonate	% Incorporation added ^{14}C/100 mg in 3 hr into Cholesterol	Cholesterol specific radioactivity (cpm/mg)
			[b] from [3-^{14}C]pyruvate		
Control	10	0.32 ± 0.0054	9.6 ± 0.71	0.54 ± 0.049	3,220 ± 758
Triamcinolone	10	0.40 ± 0.0066	6.8 ± 0.55	0.30 ± 0.037	1,181 ± 177
		p(t)[c] < 0.01	< 0.001	< 0.001	< 0.001
			from [1-^{14}C]pyruvate		
Control	5		32.0 ± 2.8		
Triamcinolone	5		25.4 ± 2.4		
		p(t)[c]	< 0.001		

[a] Adrenal cholesterol concentrations were determined on the basis of initial wet wt.
[b] N = number of flasks, each flask containing pooled adrenal glands from six to eight hamsters.
[c] Significance of difference between groups.

procedures did not label the esterified cholesterol (Table III).

Triamcinolone administration to hamsters and gerbils was accompanied by significant decreases in splenic weight (Table I). Striking changes were observed in splenic morphology, the spleens from triamcinolone treated animals showing a virtual disappearance of lymphatic nodules and marked reduction in sinusoidal blood volume. The changes in splenic weight and morphology were so large and consistent that they were taken as evidence of a significant biological effect of triamcinolone, since these changes are compatible with the depressant effects of anti-inflammatory steroids on lymphoid tissue (20).

This regimen of triamcinolone administration resulted in severe systemic consequences in both species, but particularly in hamsters. Varying degrees of sedation were evident by the third day of treatment and ten hamsters and one gerbil, moribund at the end of the experimental period, were discarded. Body weight was significantly decreased in triamcinolone treated hamsters, but not in the steroid treated gerbils (Table I). Food intake was not measured directly, but the presence (or absence) of food in the stomach or cheek-pouches at the end of the experiment indicated diminished food intake in one-third of the steroid treated hamsters. By contrast, the stomachs of all gerbils treated with triamcinolone were filled with food.

The incorporation of ^{14}C from [3-^{14}C]pyruvate into bicarbonate and cholesterol and from [1-^{14}C]pyruvate into bicarbonate was significantly decreased by adrenal glands taken from triamcinolone treated hamsters (Table II). There were significant increases in adrenal weight and in the cholesterol concentration of the adrenal glands from steroid treated hamsters (Table II).

Wet weight and cholesterol concentrations of

TABLE III. Cholesterol Concentrations and Specific Radioactivity Following *in Vitro* Incubation of Gerbil Adrenal Glands with [2-^{14}C]Acetate.

Group	N[a]	Adrenal cholesterol % wet wt: Unesterified[b]	Adrenal cholesterol % wet wt: Total	Specific radioactivity of adrenal cholesterol (cpm/mg): Unesterified[b]	Specific radioactivity of adrenal cholesterol (cpm/mg): Total
Control	6	0.341	4.2 ± 0.28	7,350 ± 874	668 ± 157
Triamcinolone	6	0.382	4.0 ± 0.74	3,520 ± 927	247 ± 37
	p(t)[c]			<0.02	<0.01

[a] N = Number of flasks, each flask containing adrenal halves from two gerbils.
[b] Accurate determinations of unesterified adrenal cholesterol concentrations were obtained in only three experiments.
[c] Significance of differences between groups.

TABLE IV. Incorporation of ^{14}C From [3-^{14}C]Pyruvate into Bicarbonate by Gerbil Adrenal Glands *in Vitro*.

Group	N^a	% Incorporation added ^{14}C/50 mg in 3 hr into Bicarbonate	Cholesterol
Control	10	7.5 ± 0.30	None
Triamcinolone	10	4.9 ± 0.26	None
	$p(t)^b$	<0.001	

[a] N = number of paired flasks, each flask containing adrenal glands from two gerbils.

[b] Significance of difference between groups.

gerbil adrenal glands were not changed by treatment with triamcinolone (Tables I and II). Adrenal glands from steroid treated gerbils incorporated a significantly smaller percentage of ^{14}C from [3-^{14}C]pyruvate into bicarbonate than did the adrenal glands from control animals (Table IV). There was a 50% decrease in the specific radioactivity of both adrenal unesterified and total cholesterol following the incubation of adrenal glands from triamcinolone treated gerbils with [2-^{14}C]acetate (Table III).

Discussion. The results of these experiments on hamster adrenal cholesterol concentrations confirm the data from other investigations (8, 13) classifying the hamster adrenal gland as a cholesterol-poor adrenal gland. The gerbil, by contrast, possesses an adrenal gland which is cholesterol-rich and, therefore, similar to most mammalian adrenal glands. Hamster adrenal glands also carry out a relatively high level of *in vitro* cholesterol synthesis comparable to that observed in sheep adrenal cortical slices (7, 9). *In vitro* cholesterol synthesis in gerbil adrenal glands could only be demonstrated following the incubation of the adrenal glands with high specific radioactivity ^{14}C-acetate. Thus, the gerbil adrenal gland both morphologically and metabolically resembles the adrenal glands of man (12) and possibly the guinea pig as well (11). However, both guinea pig adrenal slices and human adrenal tissue *in vitro* incorporate some ^{14}C from ^{14}C-acetate into esterified cholesterol (11, 12), while our data suggest that gerbil adrenal glands do not esterify the ^{14}C-cholesterol formed *in vitro*.

Cholesterol synthesis in the liver is regulated by a negative feedback control system responsive to the amount of cholesterol in the enterohepatic circulation (21). The marked differences in the *in vitro* formation of cholesterol by cholesterol-poor and cholesterol-rich adrenal glands suggests that in the latter cholesterol synthesis is depressed by the large concentrations of esterified sterol. Intestinal cholesterol synthesis, by contrast, is modulated by the presence of the hepatic steroids, the bile acids, in the intestinal lumen (22). Steroids may represent an additional regulatory component of adrenal cholesterol synthesis (7) thereby providing the adrenal cortex with dual modes for the control of cholesterol formation.

Cholesterol synthesis *in vitro* is undectable in human adrenal cortical tissue from patients treated with dexamethasone (12) and is decreased by 90% in adrenal fasciculate-reticular slices from triamcinolone treated sheep (7). Despite the large doses of triamcinolone and their consequences, both systemically and on adrenal oxidative metabolism, a considerable degree of cholesterol synthesis was observed in adrenal tissue from steroid treated hamsters and gerbils. The adrenal glands of these laboratory rodents appear to be more resistant to the suppressive effects of steroid administration on adrenal cholesterol synthesis than is the human or sheep adrenal cortex.

Summary. The cholesterol concentrations of hamster adrenal glands are 0.32 ± 0.005% wet wt confirming their classification as cholesterol-poor adrenal glands. Hamster adrenal glands incubated *in vitro* with [3-^{14}C]pyruvate in tracer quantities, incorporate significant amounts of ^{14}C into adrenal cholesterol. Gerbil adrenal gland cholesterol concentrations are 4.2 ± 0.28% wet wt, less than 10% of which is unesterified. The typically cholesterol-rich gerbil adrenal glands do not form ^{14}C-cholesterol when incubated *in vitro* with tracer quantities of [3-^{14}C]pyruvate. When incubated for 6 hr with three changes of fresh solution containing high specific radioactivity [2-^{14}C]acetate, gerbil adrenal glands form ^{14}C-labeled unesterified cholesterol, but little or none of the labeled cholesterol is esterified. The administration of triamcinalone acetonide, 0.25 mg/100 g body wt/day for 6 days to both hamsters and gerbils, results in decreased *in vitro* oxidative metabolism and ^{14}C-cholesterol formation by the adrenal glands of both laboratory animals.

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