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Adrenal cholesterol uptake from plasma lipoproteins: Regulation by corticotropin

(cholesterol metabolism/high density lipoprotein/low density lipoprotein/apolipoprotein A-I)

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ABSTRACT **The transfer of lipoprotein-bound cholesterol into adrenal cells was examined. Adrenal glands from unstimulated or corticotropin stimulated hypophysectomized rats were incubated with high density lipoprotein (HDL) or low density lipoprotein LDL containing radiolabeled cholesterol. The rate of transfer of labeled cholesterol from HDL into the glands was two to three times greater than from LDL. Corticotropin stimulation increased the transfer of cholesterol from HDL but not LDL. The effects of corticotropin were not dependent on subsequent cholesterol utilization for steroidogenesis. The process of cholesterol transfer from HDL was linear with time over 2 hr at 37° and greatly reduced at 4°. In addition, the transfer process became saturated above an HDL cholesterol concentration of 900 μg/ml. About 25% of the labeled adrenal cholesterol arising from HDL was recovered within the mitochondria. The labeled cholesterol within isolated mitochondria could undergo mitochondrial conversion to pregnenolone. Finally, the delipidated HDL apolipoproteins, apoA-I and apoA-II, when added to incubations containing less than saturating concentrations of HDL, stimulated transfer of labeled cholesterol from HDL to adrenal cells. These studies suggest that rat adrenal tissue possesses an HDL specific hormonally-responsive mechanism for accumulating extracellular cholesterol and that apoA-I and apoA-II have a significant function in the uptake process.**

The mechanisms responsible for transferring cholesterol from plasma into cells are of particular interest because of their possible role in atherosclerotic disease. Adrenal tissue may serve as a useful model for studying these mechanisms because studies in man (1, 2) and the rat (3, 4) showed that 80% or more of the cholesterol substrate for adrenal steroidogenesis may come from plasma. However, little is known about the process by which extracellular cholesterol enters the adrenal cells. Dexter *et al.* (4) have observed that the uptake process is stimulated by corticotropin (adrenocorticotrophic hormone, ACTH), and furthermore that the stimulatory effect of ACTH persists even when utilization of accumulating cholesterol is blocked by specific inhibitors.

Serum cholesterol is lipoprotein bound, predominantly by the high density lipoprotein (HDL) and low density lipoprotein (LDL) fractions. In the rat (5), 60% of the circulating cholesterol is found in HDL and 30% in LDL, while in man 30% and 60% are found in HDL and LDL, respectively (6). In order to better understand the uptake of cholesterol by adrenal cells as well as ACTH regulation of the uptake process, we have examined the ability of HDL and LDL to serve as substrate for transfer of cholesterol to the adrenal. The observations reported here suggest that HDL is the preferred substrate for adrenal cholesterol uptake, and that uptake from HDL is regulated by ACTH. Furthermore, these studies suggest that the HDL apoproteins, apoA-I and apoA-II, play a role in the cholesterol uptake process.

Abbreviations: ACTH, corticotropin; HDL, high density lipoprotein; LDL, low density lipoprotein; AG, aminoglutethimide.

MATERIALS AND METHODS

Adrenal cholesterol uptake was determined by incubating quartered rat adrenal glands with purified human lipoproteins containing [4-^{14}C]- or [1,2-^{3}H]cholesterol. Because the level of LDL in rat serum is very low, we have employed LDL and also HDL isolated from human serum. Studies comparing homologous lipoprotein classes remain to be performed.

Treatment of Animals. Two groups of Sprague-Dawley rats weighing 180–250 g were transaurally hypophysectomized 24 hr prior to sacrifice. Our group received 20 units of ACTH (Acthar Gel, 45 United States Pharmacopoeia units/ml, Armour Pharmaceutical Co.) subcutaneously every 6–8 hr while a second group received no treatment. In some cases, a third group received aminoglutethimide (AG) (30 mg in 1 ml of 0.9% NaCl) intraperitoneally 30 min prior to each ACTH treatment. Adrenal glands from ether anesthetized animals were then excised, trimmed of fat, weighed, quartered, and placed into standard buffer (Krebs-Ringer bicarbonate with glucose, 200 mg/100 ml at pH 7.4).

Adrenal Incubations. Individual or paired quartered adrenal glands were incubated in standard buffer containing [1,2-^{3}H]- or [4-^{14}C] cholesterol-labeled lipoproteins at the concentration and specific activity indicated in the figure and Table legends. Incubations were performed for up to 2 hr at 37° or 4° in an Eberbach shaker bath in a 95% O_2-5% CO_2 atmosphere. The incubation media for all experiments using quartered adrenal glands contained AG (0.25 mg/ml) to prevent conversion of cholesterol to pregnenolone (7). At the completion of the incubation, medium was removed and the glands washed three times with 3–5 ml of standard buffer at 4°. The final wash contained less than 2% of the counts retained within the glands.

Incubated washed glands were then homogenized in 2 ml of standard buffer and total protein, cholesterol, and radioactivity measured. Greater than 80% of the radioactivity associated with incubated glands could be sedimented by centrifugation at 100,000 × *g* for 1 hr. In initial experiments, the identity of the radiolabeled species accumulated in the incubated glands was determined by thin-layer chromatography of chloroform/methanol (3/1) tissue extracts performed as described below. About 95% of the extracted counts comigrated with the cholesterol standard while another 2–5% of the counts comigrated with cholesterol oleate. This indicates that the basic cholesterol structure remained intact throughout the experiment. In later experiments, extraction and chromatography were omitted.

Mitochondrial Incubation. Conversion of cholesterol to pregnenolone, the rate limiting step in adrenal steroidogenesis, occurs in the mitochondria (8). It was therefore of interest to determine the amount of labeled cholestesterol within mitochondria from incubated adrenals and also to determine if it could be converted to pregnenolone.

The adrenal glands from either 10 ACTH-treated to 10 untreated hypophysectomized rats were pooled and each group incubated, as described above, for 45 min in the presence of [4-^{14}C]cholesterol-labeled HDL and AG to prevent conversion of accumulated cholesterol to pregnenolone. At the conclusion of the incubation, the pooled glands from each group were homogenized and the mitochondria isolated as previously described (9), a procedure which removes the inhibitor AG. The labeled cholesterol accumulated in the mitochondria was then measured. Duplicate aliquots of isolated mitochondria from each group were then incubated at 37° and 4° in the absence of AG to allow conversion of cholesterol to pregnenolone. The incubations were performed for 30 min in 30 mM Tris buffer containing 1% albumin, 0.05 M sucrose, 10 mM α-ketoglutarate (sodium salt), 1 mM disodium EDTA, 20 mM KCl, and 5 mM NaH_2PO_4 at pH 7.4 as previously described (9). At the completion of the incubation, the mitochondria, along with the incubation media, were extracted, and the labeled pregnenolone isolated by thin-layer chromatography as described below.

Analytical Methods. Cholesterol was extracted from tissue and lipoproteins by the method of Bligh and Dyer (10). Measurements of total, free, and esterified cholesterol were performed as described by Sperry and Webb (11).

Pregnenolone was extracted from mitochondrial homogenates by using dichloromethane as previously described (9). Pregnenolone, cholesterol, and cholesterol esters were separated by thin-layer chromatography with silica gel impregnated glass fiber (ITLC type SG., Gelman Instrument Co., Ann Arbor, Mich.) and by first developing the chromatogram in a solvent system consisting of benzene/ethyl acetate (3/1, vol/vol) to 2.5 cm from the origin. The sheets were then dried and placed in a second solvent system consisting of isooctane/ethyl acetate (100/2, vol/vol), and developed to 15 cm from the origin. Unlabeled pregnenolone, cholesterol, and cholesterol oleate were run on a separate track and located by exposure to I_2 vapor. Areas corresponding to the location of the standards were cut out and placed directly into counting vials containing 10 ml of the Permafluor/toluene scintillation mixture. Protein concentration was determined according to Lowry *et al.* (12), using bovine serum albumin as a standard. Radioactivity was determined by liquid scintillation counting using a Packard model 3220 Spectrometer. Unextracted tissue samples were counted in toluene/Permafluor (24/1, wt/vol) after treatment of the tissue with NCS tissue solubilizer (Amersham/Searle).

Preparation of Labeled Lipoproteins. Plasma, for isolation of human lipoproteins, was collected from normal donors by plasmaphoresis in acid citrate dextrose to which was added EDTA 1 mg/ml. LDL and HDL were isolated by ultracentrifugal flotation in KBr at a density of 1.006–1.063 and 1.063–1.21 g/ml, respectively (6). The lipoproteins were then dialyzed against three changes of at least 500 volumes of 0.1 M ammonium bicarbonate buffer at pH 7.4 at 4° for 48 hr. The purified lipoprotein gives a single band on disc gel electrophoresis with the system of Masket *et al.* (13). The isolation and purification of apoA-I (14) and apoA-II (15) were previously described. The individual purified lipoproteins were labeled with either [4-^{14}C]- or [1,2-^{3}H]cholesterol by the method of Avigan (16).

Materials. [4-^{14}C]cholesterol and [1,2-^{3}H]cholesterol were obtained from New England Nuclear and used without further treatment. All other chemicals were reagent grade or better.

RESULTS

Effects of ACTH on Adrenal Cholesterol Uptake. Shown in Table 1 are the results of experiments comparing the effects of ACTH on adrenal accumulation of labeled cholesterol from HDL to that from LDL. Substantially more labeled cholesterol was accumulated by adrenals from either ACTH treated or untreated rats when HDL rather than LDL, at equal cholesterol concentrations, was added to the incubation. This was true at all lipoprotein-cholesterol concentrations examined when the accumulated labeled cholesterol was related to the specific activity of the lipoprotein cholesterol in the media. Furthermore, ACTH caused an increase in accumulation of labeled cholesterol from HDL but not LDL. The actual amount of labeled cholesterol accumulated from HDL increased almost 2-fold ($P < 0.0005$) with ACTH treatment while that from LDL did not show a statistically significant increase. Because ACTH is known to cause adrenal hypertrophy (17), the accumulation of cholesterol was related to adrenal protein content. The conditions employed in this study caused an average increase in adrenal protein content from 3.5 to 4.3 mg/pair. This increase in adrenal protein fully accounted for the apparent increase in counts from LDL while the ACTH stimulated increase from HDL remained highly significant ($P < 0.0005$) even when related to the increase in adrenal protein.

Table 1. Effects of ACTH on rat adrenal [4-^{14}C] Cholesterol uptake from human HDL and LDL

	Untreated	ACTH treated	*P* value
	HDL		
cpm/pair	1159 ± 329 (30)	1987 ± 678 (33)	<0.0005
cpm/mg protein	314 ± 85 (22)	464 ± 153 (24)	<0.0005
	LDL		
cpm/pair	458 ± 125 (8)	558 ± 189 (8)	>0.1
cpm/mg protein	137 ± 53 (8)	136 ± 44 (8)	>0.4

Pairs of adrenal glands from hypophysectomized ACTH treated and untreated rats were incubated for 30 min in standard media containing AG and labeled lipoproteins as described. The HDL and LDL cholesterol concentrations in the incubation media varied from 90 to 880 μg/ml and 290 to 770 μg/ml, respectively. The ^{14}C activity in each case varied from 1 to 8 × 10^5 cpm/ml. To compare results among experiments employing varying amounts of label in the media, we expressed the number of counts contained within each pair of washed glands relative to each 1000 counts/ml of incubation media. The data shown are the means ± standard deviation. The number of observations is shown in parentheses. The level of confidence employing a two sided *t* test is also shown.

An additional effect of administering high doses of ACTH to rats is depletion of adrenal cholesterol stores (18). We, therefore, wished to determine if the effect of ACTH in enhancing transfer to labeled cholesterol from HDL to the adrenal was secondary to its depleting effect on adrenal cholesterol content. The transfer of labeled cholesterol from HDL to three groups of pooled adrenal glands (8 pairs/group) containing widely varying amounts of cholesterol was determined. The glands were obtained from hypophysectomized rats receiving either no further treatment, or ACTH, or ACTH and AG as described above. Table 2 shows the results of these treatments on adrenal protein content, cholesterol content, and accumulation of labeled cholesterol. Both of the groups receiving ACTH showed an increase in adrenal protein content. The adrenals from the group receiving ACTH without AG were depleted of cholesterol. This reflects the stimulatory effect of ACTH on cholesterol utilization for steroidogenesis and suggests that under these circumstances, *in vivo*, the enhancement of ste-

Table 2. The effects of ACTH and aminoglutethimide (AG)/pretreatment on adrenal cholesterol uptake from human HDL

Sample	No treatment	ACTH treatment	ACTH plus AG
mg of protein/pair	2.2	3.5	3.5
μg of cholesterol/pair	860	265	1250
cpm/pair	2080	4330	4340

Excised, quartered adrenal glands (eight pairs in each group) from untreated hypophysectomized rats and hypophysectomized rats treated with either ACTH alone or ACTH and AG were pooled according to treatment group and incubated for 30 min in standard buffer containing [4-^{14}C]cholesterol labeled HDL (850 μg of cholesterol per ml, 214 cpm/μg of cholesterol) and AG (0.25 mg/ml). The glands were then washed and homogenized; the protein, cholesterol, and radioactivity were determined. The value shown is the mean of duplicate determinations of each parameter measured on duplicate aliquots of material obtained from homogenates of eight pooled pairs of adrenals.

roidogenesis is greater than the enhancement of cholesterol uptake from plasma (18). On the other hand, adrenals from rats treated with both ACTH and AG increased in cholesterol content. This reflects only the effect of ACTH in enhancing uptake of cholesterol from serum since AG blocked steroidogenesis (7). The total number of counts accumulated per pair of adrenal glands in each treatment group was unrelated to the cholesterol content of the glands. Despite their widely varying cholesterol content, glands from both groups of rats that received ACTH accumulated the same amount of labeled cholesterol from HDL. Thus, the effect of ACTH appears to be primary rather than secondary to adrenal cholesterol depletion.

Characteristics of Adrenal Cholesterol Uptake from HDL. Further studies were performed to characterize adrenal uptake of cholesterol from HDL. In glands from untreated rats, uptake was found to be linear with time at 37° and greatly inhibited at 4° (Fig. 1). Furthermore, while glands from ACTH pretreated rats showed an enhanced cholesterol uptake in comparison to glands from untreated rats when incubated at 37°

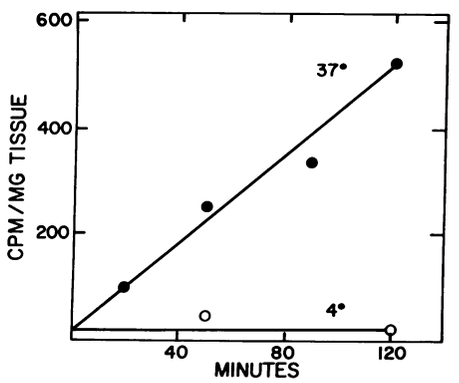

FIG. 1. Effects of time and temperature on *in vitro* accumulation of [4-^{14}C]cholesterol from human HDL by quartered rat adrenal glands. Pairs of quartered rat adrenal glands from intact untreated rats were incubated for the indicated length of time at either 37° or 4°. All incubation media contained 740 μg of HDL cholesterol per ml with a specific activity of 395 cpm/μg of cholesterol, and AG (0.25 mg/ml). At the end of each interval, two pairs of glands from each temperature group were washed and the amount of accumulated radiolabeled cholesterol determined in duplicate for each pair as described under *Materials and Methods*.

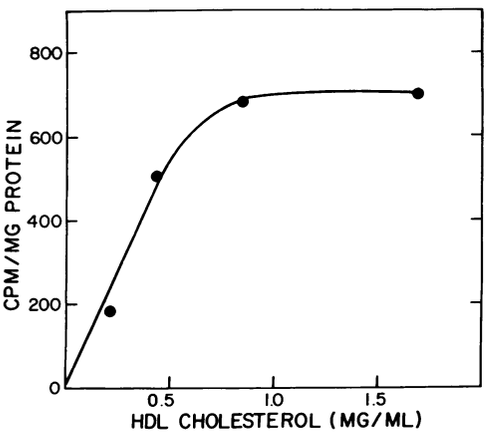

FIG. 2. Effects of HDL cholesterol concentration on uptake of [4-^{14}C]cholesterol from human HDL by quartered rat adrenal glands. Pairs of quartered rat adrenal glands, excised from hypophysectomized ACTH treated rats, were incubated for 30 min in 2 ml of standard buffer supplemented with varying concentrations of human HDL of constant cholesterol specific activity (340 cpm/μg of cholesterol). The glands were then washed, homogenized, and the protein and [^{14}C]cholesterol content determined. The points shown are the mean of duplicate determinations of duplicate incubations of adrenal pairs.

(Table 1), this difference was abolished and the uptake was low in both groups at an incubation temperature of 4°.

The dependence of uptake on HDL cholesterol concentration was also examined. The accumulation of labeled HDL cholesterol by glands from ACTH treated hypophysectomized rats was proportional to total HDL cholesterol concentration (of constant specific activity) up ot about 900 μg/ml. Above that concentration, very little further increase in accumulation occurred, which indicated that the uptake process from HDL is saturable (Fig. 2).

Effects of ACTH on Adrenal Mitochondrial Accumulation and Metabolism of HDL Cholesterol. Because the rate limiting step in adrenal steroidogenesis, conversion of cholesterol to pregnenolone, occurs in the mitochondria, uptake and metabolism of labeled cholesterol arising from extracellular HDL was examined in these organelles. Table 3 shows that more labeled cholesterol is transferred from extracellular HDL to in-

Table 3. Effect of ACTH pretreatment on mitochondria accumulation of [4-^{14}C]cholesterol from extracellular human HDL *in vitro*

	Untreated	ACTH treated
cpm/mg mitochondrial protein	400	1103
% of total accumulated counts found within the mitochondria	14%	22%

Two pools of 10 adrenal pairs each, one from treated hypophysectomized rats, and another from untreated hypophysectomized rats were each incubated for 30 min at 37° in standard buffer containing [4-^{14}C]cholesterol labeled HDL (850 μg of cholesterol/ml, 208 cpm/μg of cholesterol) and AG (0.25 mg/ml). The pooled glands in each group were then washed, homogenized, and the mitochondria recovered as previously described (19). Prior to isolation of the mitochondria, an aliquot of the total homogenate was used for determination of total gland radiolabeled cholesterol content. Protein and radiolabeled cholesterol content were determined on duplicate aliquots of isolated mitochondria from each group and the mean value is shown.

Table 4. Effects of ACTH on mitochondrial pregnenolone formation from [4-^{14}C] cholesterol accumulated from human HDL

Temperature (°C)	% of initial mitochondrial [4-^{14}C] cholesterol converted to pregnenolone	
	Untreated	ACTH treated
4	5.7	12.9
37	8.5	19.7

Two groups of 10 adrenal pairs each (one group from ACTH treated and one from untreated hypophysectomized rats) were incubated for 1 hr in standard buffer containing AG (0.25 mg/ml) and [4-^{14}C]cholesterol labeled HDL (850 μg of cholesterol/ml, 208 cpm/μg of cholesterol). Mitochondria from each pooled group of adrenal glands were isolated as previously described (19). Duplicate aliquots from each group were incubated, not in the absence of AG, at both 37° and 4° for 30 min as previously described (19). Each incubation mix containing mitochondria was extracted and pregnenolone separated by thin layer chromatography and counted as described in *Materials and Methods*.

tracellular mitochondria by glands from ACTH treated hypophysectomized rats than by glands from untreated hypophysectomized rats. Furthermore, the percentage of the total labeled cholesterol accumulated by the gland which is transferred to the mitochondria is increased by ACTH treatment. Whereas ACTH treatment increases total adrenal gland uptake of labeled cholesterol from HDL 1.5 times (Table 1), it increases mitochondrial uptake 2.75 times (Table 3). Thus, ACTH treatment appears not only to increase adrenal cholesterol uptake but also promotes delivery of accumulating cholesterol to the mitochondria.

It was of particular importance to determine if the labeled cholesterol accumulating within the mitochondria of adrenal glands incubated with cholesterol-labeled HDL could be used as substrate for conversion to pregnenolone. Mitochondria were therefore isolated and the inhibitor of pregnenolone formation, AG, removed as described earlier. Table 4 shows that labeled cholesterol transferred to the mitochondria could serve as substrate for conversion to pregnenolone. A greater amount of labeled pregnenolone was recovered from mitochondria incubated at 37° than at 4° for both the ACTH treated and untreated groups. This suggests that labeled cholesterol entering the adrenal from extracellular HDL can serve the physiologic function of providing steroidogenic substrate.

Effects of Added ApoA-I and ApoA-II on Adrenal Uptake of HDL Cholesterol. After we determined that ACTH stimulated adrenal transfer of labeled cholesterol from HDL, it was of interest to examine the action of the HDL apoproteins, apoA-I and apoA-II, on this process. The adrenal glands from intact untreated rats were individually incubated in media containing [4-^{14}C]cholesterol labeled HDL at a concentration of 480 μ/ml. The indicated protein (Fig. 3), at a final concentration of 0.5 mg/ml, was added to one of each pair of glands while the remaining gland incubated in the absence of added protein served as the control. Both apoA-I and apoA-II markedly enhanced the adrenal accumulation of [4-^{14}C]cholesterol from HDL during the 30 min incubation period. Albumin and γ-globulin were without effect (Fig. 3).

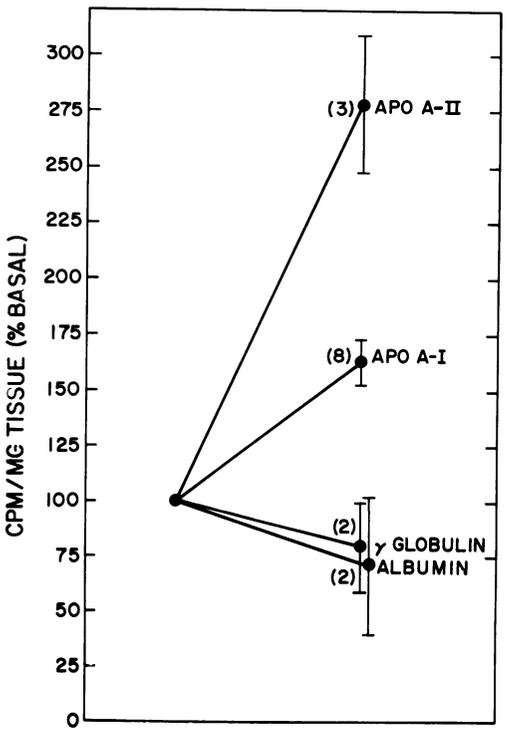

FIG. 3. Effects of human apoA-I and apoA-II on rat adrenal uptake of [4-^{14}C]cholesterol from human HDL. Pairs of excised, quartered adrenal glands from untreated rats were incubated in standard buffer containing [^{14}C]cholesterol-labeled human HDL (480 μg of cholesterol per ml, 218 cpm/μg of cholesterol) and AG (0.25 ng/ml) at 37°. To one of each pair of glands, we added the indicated apoprotein or protein at a final concentration of 0.5 mg/ml. The remaining gland incubated in the absence of added protein served as the control. The glands were then thoroughly washed, homogenized, and the protein and [^{14}C]cholesterol content determined. The number in parentheses is the number of adrenal gland pairs examined. The bars indicate the range for each analysis.

DISCUSSION

Previous studies (1–4) showed that a major portion of the cholesterol substrate used by the adrenal cortex for production of steroid hormones may originate from plasma. The *in vivo* studies of Dexter *et al.* (17) demonstrated that adrenal accumulation of extracellular cholesterol increased with ACTH stimulation and did not depend on subsequent cholesterol utilization.

The current studies suggest that adrenal cholesterol uptake involves specific participation by the HDL class of serum lipoproteins. Transfer of cholesterol from human HDL to rat adrenals is two to three times greater than from human LDL and more importantly responds to ACTH stimulation.

The response to ACTH stimulation is consistent with the observed stimulatory effect of ACTH on adrenal cholesterol uptake *in vivo*. Similarly, transfer of cholesterol from HDL to the adrenal does not depend on adrenal cholesterol content or utilization, another property shared by the uptake process *in vivo*. These two facts strongly support the relevance of the current observations to the *in vivo* situation.

Several important properties of adrenal transfer of HDL cholesterol were observed. Transfer was linear with time up to 2 hr and greatly retarded at lower temperatures (Fig. 1). The process was also found to saturate at an HDL cholesterol concentration of about 900 μg/ml (Fig. 2). Although this is well above the circulating levels of HDL cholesterol in normal rats, levels of greater than 200 μg/ml were reported in genetically obese rats (5). Thus, the levels under investigation are consistent with physiologically and pathologically circulating levels *in vivo*. In addition, the use of human rather than rat lipoproteins

may account, in part, for the high HDL cholesterol concentration required to saturate the uptake process in rat adrenal glands. Stein and Stein (20) have found, by using iodinated lipoproteins, that the apoprotein constituents of either human HDL or LDL are neither accumulated nor catabolized as readily by rat aortic smooth muscle cells as are the apoprotein constituents of rat HDL and LDL.

In our studies, it was important to determine if the labeled cholesterol transferred from HDL to the adrenal gland had entered normal physiologic pathways. The fact that the cholesterol could not be removed by washing and remained associated with sedimentable fractions after homogenization of the tissue indicates that it had become incorporated into or become bound to the tissue. Furthermore, the labeled cholesterol associated with mitochondria from glands previously incubated with HDL could be converted to pregnenolone. Thus, the labeled cholesterol arising from HDL appeared to have become incorporated into the gland and to serve normal physiologic functions.

The mechanism by which labeled cholesterol from HDL enters the adrenal cell is not yet known. The fact that the process is lipoprotein specific, is markedly influenced by HDL apoproteins, responds to ACTH stimulation, and is unaffected by endogenous cholesterol pool size makes isotopic exchange alone a highly unlikely explanation. Although isotopic exchange may account for some movement of labeled HDL cholesterol into the adrenal, preliminary studies employing adrenal cortical tumor cells in culture indicate that net movement of cholesterol from HDL into adrenal cells does occur (unpublished results). Another possible mechanism is bulk phase or nonselective endocytosis, a nonsaturable process (21, 22). The fact that adrenal uptake of HDL cholesterol is saturable and enhanced by ACTH while uptake from LDL is not makes this an unlikely explanation for the current observations.

An additional mechanism by which cells accumulate extracellular macromolecules proceeds by saturable cell membrane binding, endocytosis, and lysosomal fusion (22). Such a mechanism may account for the uptake of LDL by normal cultured human fibroblasts (23, 24). Further studies are underway to determine if this accounts for adrenal uptake of HDL cholesterol. In any event, the process of cholesterol uptake in the adrenal shows very different specificity from that in cultured fibroblasts. HDL instead of LDL appears to be the preferred substrate in the adrenal. Because the HDL apoproteins, apoA-I and apoA-II, are water soluble in the delipidated state, we were able to examine their effects on adrenal cholesterol uptake from HDL. At less than saturating HDL concentrations, both apoproteins stimulate cholesterol transfer. The mechanism by which they produce this effect is not yet known but studies employing ^{125}I-labeled-apoA-I showed a reversible hormonally sensitive binding site to be present in adrenal tissue (unpublished results).

Although the current studies show that HDL cholesterol enters the adrenal cell, they do not allow us to say if the whole HDL particle also enters. Bierman *et al.* (19) used lipoproteins labeled in both the apoprotein and lipid moieties and observed a disproportionate uptake of lipid relative to protein. Lipoprotein specific receptor mediated transmembrane movement specific for small-molecular-weight constituents of the serum lipoproteins, therefore, offers another alternative explanation for our observations. Further studies are underway to gain greater insight into the mechanism by which HDL cholesterol enters the adrenal cell.

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1. Borkowski, A. J., Levin, S., Delcroix, C., Mahler, A. & Verhas, V. 1967) *J. Clin, Invest.* **46,** 797–811.
2. Borkowski, A. J., Levin, S., Delcroix, D. & Klastersky, J. (1970) *J. Appl. Physiol.* **28,** 42–49.
3. Morris, M. D. & Chaikoff, I. L. (1959) *J. Biol. Chem.* **234,** 1095–1097.
4. Dexter, R. N., Fishman, L. M. & Ney, R. L. (1970) *Endocrinology* **87,** 836–846.
5. Schonfeld, G., Felski, C. & Howald, M. A. (1974) *J. Lipid Res.* **15,** 457–464.
6. Havel, R. J., Eder, H. A. & Bragdon, J. H. (1958) *J. Clin. Invest.* **34,** 1345–1353.
7. Dexter, R. N., Fishman, L. M., Ney, R. L & Liddle, G. W. (1967) *J. Clin. Endocrinol. Metab.* **27,** 473–480.
8. Garren, L. D., Gill, G. N., Masui, H. & Walton, G. M. (1971) *Recent Prog. Horm. Res.* **27,** 433–478.
9. Mahafee, D., Reitz, R. C. & Ney, R. L. (1974) *J. Biol. Chem.* **249,** 227–233.
10. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37,** 911–917.
11. Sperry, W. M. & Webb, M. (1950) *J. Biol. Chem.* **187,** 97–106.
12. Lowry, O. H., Rosebrough, N. J., Farr, D. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
13. Masket, B. H., Levy, R. I. & Fredrickson, D. S. (1973) *J. Lab. Clin. Med.* **81,** 794–802.
14. Gwynne, J., Brewer, H. B., Jr. & Edelhock, H. (1974) *J. Biol. Chem.* **249,** 2411–2416.
15. Lux, S. E., John, K. M. & Brewer, H. B., Jr. (1972) *J. Biol. Chem.* **247,** 7510–7518.
16. Avigan, J. (1959) *J. Biol. Chem.* **234,** 787–790.
17. Farese, R. V. & Reddy, W. J. (1963) *Biochim. Biophys. Acta* **76,** 145–148.
18. Sayers, G., Sayers, M. A., Fry, E. G., White, A. & Long, C. N. H. (1944) *Yale J. Biol. Med.* **16,** 361–392.
19. Bierman, E. L., Stein, O. & Stein, Y. (1974) *Circ. Res.* **35,** 136–150.
20. Stein, O. & Stein, Y. (1975) *Circ. Res.* **36,** 436–442.
21. Steinman, R. M. & Cohn, Z. A. (1972) *J. Cell Biol.* **55,** 186–204.
22. deDuve, C., deBarsy, T., Poole, B., Trouet, A., Tulkins, P. & VanHoof, F. (1974) *Biochem. Pharmacol.* **23,** 2495–2531.
23. Brown, M.S. & Goldstein, J. L. (1976) *Science* **191,** 150–153.
24. Anderson, R. G. W., Goldstein, J. L. & Brown, M. S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2434–2438.