# Abnormal cholesterol metabolism in renal clear cell carcinoma

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**Abstract** The clear cell form of renal cell carcinoma is known to derive its histologic appearance from accumulations of glycogen and lipid. We have found that the most consistently stored lipid form is cholesteryl ester. Clear cell cancer tissue contained 8-fold more total cholesterol and 35-fold more esterified cholesterol than found in normal kidney. Cholesteryl ester appeared to be formed intracellularly since it was not membrane-bound and since oleate was the predominant form, as opposed to linoleate in lipoprotein cholesteryl esters. The cholesterol in clear cell tumors did not appear to be a result of excessive synthesis from acetate since HMG-CoA reductase (EC 1.1.1.34) activity was lower in cancer tissue than in normal kidney ($2.9 \pm 0.8$ vs. $7.2 \pm 1.2$ pmol/mg of protein per min). In contrast, intracellular activity of fatty acyl-coenzyme A:cholesterol acyl transferase (ACAT, EC 2.3.1.26) was higher in tumor tissue than in normal kidney ($2405 \pm 546$ vs. $1326 \pm 301$ pmol/mg of protein per 20 min) while cytosolic cholesteryl ester hydrolase activity appeared normal. Cholesteryl ester storage in clear cell renal cancer may be a result of a primary abnormality in ACAT activity or it may be a result of reduced release of free cholesterol (relative to cell content) with a secondary elevation in ACAT activity.— **Gebhard, R. L., R. V. Clayman, W. F. Prigge, R. Figenshau, N. A. Staley, C. Reesey, and A. Bear.** Abnormal cholesterol metabolism in renal clear cell carcinoma. *J. Lipid Res.* 1987. **28:** 1177—1184.

**Supplementary key words** renal cell carcinoma • cholesterol metabolism • HMG-CoA reductase • fatty acyl-coenzyme A:cholesterol acyltransferase

The clear cell form of renal cell carcinoma, or hypernephroma, is the most common type of renal malignancy. The neoplastic cells are characterized histologically by a distinctive pale, glassy cytoplasm. It has been previously determined that the clear appearance of tumor cells results from cellular storage of lipid and glycogen (1). The lipid most consistently stored in these tumor cells is cholesterol, primarily in the ester form (2–4). It is apparent that renal cell carcinoma cells possess some abnormality in the metabolism of cholesterol or cholesteryl ester and that this abnormality results in sterol storage.

At least four metabolic abnormalities may be postulated to account for the excess of cholesteryl ester. Firstly, the tumor cells may incorporate lipoprotein cholesterol from serum in excessive amounts or to a greater degree than they are able to process, in a manner analogous to cholesteryl ester storage disease (5, 6). As a second possibility, the tumor cells may synthesize an excessive amount of cholesterol endogenously. A third possibility is that the cancer cells may possess exaggerated cholesterol esterification activity relative to esterase activity, possibly mediated by the microsomal enzyme fatty acyl-coenzyme A:cholesterol acyl transferase (ACAT). A final explanation would entail an abnormality in cholesterol (or cholesteryl ester) efflux from the cells; defective release of free cholesterol could be expected to produce a shunting of excess sterol into the ester pool.

The possibility that excessive uptake of lipoprotein cholesterol from serum might be the source of cholesteryl esters in renal cell carcinoma has been previously evaluated (4). We could find no increase in accumulation of an injected radioactive cholesterol analogue into tumor tissue as compared to normal renal parenchyma. In contrast, the analogue was actively accumulated by normal adrenal gland. These findings are in agreement with other in vivo and in vitro reports (7, 8) and are consistent with findings that a similar renal cancer of rats has no demonstrable low density lipoprotein receptors (9). Similarly, study of tissues preloaded with the cholesterol analogue in vivo has given rise to suggestive evidence that renal clear cell cancer cells might release cholesterol more slowly than normal kidney in vitro (10). The current studies utilize human renal cell carcinoma tissue to test hypotheses regarding excessive cholesterol synthesis or esterification by renal cell carcinoma and to further evaluate the release of free cholesterol from this tumor tissue.

Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; HMG, 3-hydroxy-3-methylglutaryl; HDL, high density lipoprotein.

## METHODS AND MATERIALS

### Tissue procurement

Twenty-seven patients with a clinical diagnosis of renal carcinoma underwent surgical resection of their tumors. All patients had a radical nephrectomy of the involved kidney. In a manner approved by the Human Subjects Institutional Review Board (investigational review board initial approval received December 1979), a small piece of grossly cancerous tissue and a small piece of grossly normal kidney were removed from the nephrectomy specimen for study immediately following resection. Tissue removal was done under the supervision of a pathologist so as not to compromise histologic diagnosis. Samples were kept in iced saline during transport to the laboratory.

### Microsomal preparation and lipid measurements

Weighed sections of paired cancer and normal kidney tissue were diced and homogenized in 0.1 M potassium phosphate buffer (pH 7.2) containing 0.2 M sucrose using a Polytron 10ST probe for 3–4 sec at setting 3. Aliquots of whole homogenate were taken to measure protein, free, and total cholesterol. The remainder was centrifuged at 12,000 *g* for 15 min. The supernate was centrifuged at 150,000 *g* for 40 min. The sedimented microsomes were resuspended in 0.1 M potassium phosphate buffer (pH 7.2) containing 0.2 M sucrose and 0.04 M EDTA. Protein was measured by the method of Lowry et al. (11).

Aliquots of whole tissue homogenates and of the subcellular microsomal fractions were extracted for total cholesterol determination by the method of Abell et al. (12). Free cholesterol was extracted from these same tissues using the same solvents at 60°C without KOH digestion or by using the method of Folch, Lees, and Sloane Stanley (13). Cholesterol was measured as the trimethylsilyl ether using a Hewlett-Packard 5830A gas chromatograph with a 6' × ¼" (2 mm ID) silane-treated glass column packed with 3% SP-2250 on 100/120 Supelcoport. Cholestane added during the extraction served as the internal standard. To check for authenticity of cholesterol, the steryl ester fraction of tumor lipid extract was separated by thin-layer chromatography with a solvent system of hexane–ethyl ether–acetic acid 85:15:1. Chloroform–methanol 2:1 eluates of the ester fraction were saponified and the trimethylsilyl ether and acetate ester of the sterol were formed. The unconjugated tumor sterol and the two derivatives chromatographed with identical retention times as native cholesterol.

Fatty acid components of tumor and normal kidney steryl ester were also identified. The steryl esters were subjected to saponification (12) and fatty acids were extracted with hexane after acidification to pH 2 with HCl. Hexane was removed under reduced pressure and fatty acids were methylated with diazomethane. Composition was determined by gas–liquid chromatography using a column packed with 10% SP2330 on 100/120 Chromosorb (Supelco, Inc.).

Tissue triglyceride content was measured by extraction of lipid from buffer-homogenized kidney and tumor with isopropanol at 60°C, and colorimetric quantitation of the glycerol moiety was obtained using the Sigma Diagnostic Kit number 405 (Sigma Chemical Co., St. Louis, MO).

### Microsomal enzyme activity

Activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase was measured in resuspended microsomal pellets by a method previously described (14–16). Basically, 0.1 mg of microsomal protein was incubated in a 0.17-ml volume of 0.1 M potassium phosphate buffer (pH 7.2) containing 0.2 M sucrose, 40 mM EDTA, 26 mM glucose-6-phosphate, 3 mM NADP, 15 mM dithiothreitol, 70 mM NaCl, and 1 unit/ml glucose-6-phosphate dehydrogenase. Following a 5-min preincubation, $^{14}$C-labeled HMG-CoA substrate was added at a concentration of 31 μM for a 15-min incubation at 37°C. The reaction was stopped with 0.025 ml of HCl and radioactive mevalonate (product) was separated from substrate by thin-layer chromatography as previously described. Addition of [$^{3}$H]mevalonate prior to separation was utilized to assess recovery of product, and HMG-CoA reductase activity was expressed as pmol of [$^{14}$C]mevalonate formed per mg of microsomal protein per minute. The conditions utilized are felt to measure total HMG-CoA reductase activity. In some instances, sufficient tissue was available to prepare microsomes and assay enzyme with 50 mM sodium fluoride present. These conditions are felt to allow measurement of the active fraction of enzyme.

Measurement of the microsomal enzyme acyl-CoA:cholesterol acyltransferase (ACAT) was performed by modification of the method of Lichtenstein and Brecher (17) and Rothblat, Naftulin, and Arbogast (18). ACAT activity was measured in a reaction mixture of 0.5 ml of 0.1 M potassium phosphate buffer (pH 7.4) containing 0.1 mg of microsomal protein, 1.25 mg of fatty acid-poor BSA, 1 μmol of dithiothreitol, and 20 nmol of [$^{14}$C]oleoyl-CoA (5 μCi/μmol). The BSA and $^{14}$C-labeled substrate were prepared as a mixture and added in 0.045 ml to start the reaction; endogenous microsomal free cholesterol was utilized as second substrate in the esterification reaction. After 20 min at 37°C, the reaction was stopped by addition of 10 ml of chloroform–methanol 2:1 containing 20 μg of cholesteryl oleate and 10 μg of methyl oleate as carriers. Lipids were extracted and washed by the method of Folch et al. (13) and the chloroform phase was taken to dryness under reduced pressure. The dried lipid extract was dissolved in 5% methanol in chloroform and spotted on Eastman Chromogram silica gel sheets and developed in hexane–diethyl ether–acetic acid 85:15:1

or hexane–ethyl acetate 9:1. Iodine vapor was used to stain the chromatogram prior to removing the cholesteryl ester band for scintillation counting. The $R_f$ values were: cholesteryl ester, 0.8–0.9; methylated fatty acids, 0.7; free fatty acids, 0.2; and any residual fatty acid acyl-CoA and phospholipids remained at the origin. ACAT activity was expressed as pmol of cholesteryl oleate formed per mg of microsomal protein per 20 min.

For some tissues, cytosolic cholesteryl ester hydrolase activity was measured by a modification of the method of Beaudet et al. (19). The 0.2-ml reaction mixture was composed of 50 mM Tris HCl (pH 7.4), 5 mM Mg $Cl_2$, 50 mM KCl, 0.2 mM EDTA, 1 mM $\beta$-mercaptoethanol, 0.4% BSA, 0.5% Triton 100-X, 30 $\mu$M [$^{14}$C]cholesteryl oleate (5.0 $\mu$Ci/$\mu$mol), and 0.1 mg of cytosolic protein from the 150,000 *g* supernate of the microsomal preparation. Incubation time was 1 hr at 37°C. Lipids were extracted with 2 ml of anhydrous ethanol and 4 ml of hexane at 60°C for 5 min. Two ml of 0.74% KCl was added to the mixture which was shaken and centrifuged to completely separate the layers. A portion of the hexane layers was removed and the free and esterified cholesterol were separated for scintillation counting as in the ACAT assay. Cholesteryl ester hydrolase activity was expressed as the quantity of ester converted to free cholesterol per mg of cytosolic protein per hr.

### Cholesterol efflux

Sufficient tissue was available from some patients to allow the study of cholesterol release from paired samples of renal cancer and normal kidney. A Stadie-Riggs microtome was used to prepare 0.5-mm-thick slices of cancer and normal renal tissues. Slices with a wet weight of 17–150 mg were incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) for 1–2 hr at 37°C. For most incubations, 1–3 mg of high density lipoprotein (HDL) protein was added to the incubation media either as delipidated HDL or as delipidated HDL reconstituted with phospholipid. Following incubation, medium was removed, filtered through 0.45-$\mu$m Millipore filters, and the cholesterol content of medium was measured. Cholesterol content of initial medium, a trace amount in most instances, was subtracted from the postincubation values. Cholesterol content of tumor and renal tissue slices was measured following incubation. For these studies, delipidated HDL was prepared by ultracentrifugation of normal human serum, isolating lipoproteins with density 1.063–1.20 g/ml, and delipidating by the method of Glickman and Green (20). HDL was reconstituted in 10 mM Tris buffer (pH 8.2) containing 1 mM EDTA. The delipidated HDL protein was added directly to incubation media for some studies and was sonicated with dimyristoyl phosphatidylcholine (0.9 mg/mg of HDL protein), to reconstitute a form of HDL/phospholipid prior to media addition, for other studies.

Radioactive chemicals (HMG-CoA, mevalonate, oleoyl-CoA, and cholesteryl oleate) were obtained from New England Nuclear (Boston, MA). Crystalline fatty acid-poor BSA and other chemicals and reagents were obtained from Sigma (St. Louis, MO). Results are expressed as mean ± standard error and statistical analyses utilized Student's two-tailed *t*-test, for paired values in most instances.

## RESULTS

### Surgical tissue and lipid analysis

All 27 patients studied had renal cell carcinomas. However, by histopathologic examination, three patients' tumors were of the granular type, three patients' tumors were categorized as mixed cell type, and one patient's tumor was categorized as an oncocytic carcinoma. The remaining 20 tumors were consistent with the clear cell type of renal cell carcinoma. Nonclear cell tumors were analyzed separately.

**Fig. 1** shows the cholesterol content of normal kidney and clear cell renal cancer tissue from 20 patients analyzed. Clear cell renal cancer showed a much higher content of total cholesterol than did normal kidney tissue (259 ± 58 vs. 33 ± 7 mg/g of protein). The increase was predominantly due to excessive cholesteryl ester storage in clear cell tumors (211 ± 49 vs. 6 ± 2 mg/g of protein), with only a modest increase in free cholesterol (48 ± 9 vs. 28 ± 5 mg/g of protein).

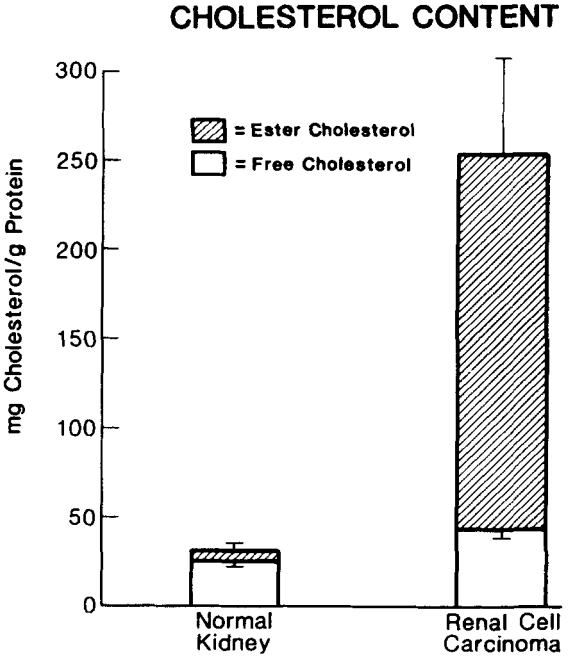


**Fig. 1.** Cholesterol content (total, free, and esterified) of clear cell renal carcinoma and adjacent normal kidney from 20 patients. Values are expressed as mg cholesterol per g of tissue protein, but similar relationships held when measured per g of tissue, wet weight.

In contrast to cholesteryl ester, the triglyceride content of renal clear cell carcinoma was highly variable with a range from 0 to 6380 mg/g of protein for the six tumors studied. This compares to the triglyceride content of normal kidney which was found to be 142 ± 65 mg/g of protein (mean ± SEM for six paired normal kidney samples from the same patients). The variability in tumor triglyceride content was further evidenced by the fact that two of the six tumors tested had a content less than or equal to their paired normal kidney counterparts. The difference in triglyceride content of the six tumor/normal kidney pairs did not reach statistical significance ($t = 1.375$) because of the extreme variability in triglyceride content of the tumors.

The fatty acid profiles from extracted cholesteryl esters of normal kidney and a representative clear cell tumor tissue are shown in **Table 1.** The finding of a predominance of oleate esters contrasts with the observation that serum lipoprotein cholesteryl esters are made up of nearly 50% linoleate and only 24% oleate, with a linoleate/oleate ratio of 1.9 (21). The linoleate/oleate ratio of 0.4 for tumor esters suggests that these esters have been formed intracellularly (22). **Fig. 2** shows an electron micrograph of renal cell cancer lipid inclusions. The electron-dense lipid material does not appear to be contained in lysosomes and does not appear to be membrane bound.

**Fig. 3** shows the measured values for total activity of microsomal HMG-CoA reductase in paired samples of normal kidney and clear cell carcinoma tissue from the 13 patients having this enzyme measured. Total expressed HMG-CoA reductase was significantly lower ($P < 0.001$) in cancer tissue (2.9 ± 0.8 pmol/mg per min) than in normal kidney (7.2 ± 1.2 pmol/mg per min). When HMG-CoA reductase was measured in the presence and absence of sodium fluoride, essentially all of the enzyme appeared to be active in normal kidney while 60% of total enzyme appeared to be active in tumor. Thus, both total enzyme activity and the active fraction of enzyme activity were low in tumor. The finding that the rate-limiting enzyme of cholesterol synthesis is substantially depressed in clear cell cancer suggests that excessive synthesis from acetate is not the origin of the cholesteryl ester stores. To confirm that HMG-CoA reductase activity indicated rate of cholesteryl synthesis, slices of normal kidney and histologically confirmed clear cell cancer tissue from four different patients were incubated with [$^3$H]water and incorporation of $^3$H into cholesterol was measured. Normal kidney tissue gave a value of 14.0 ± 5.2 nmol cholesterol formed/g of tissue per hr compared to a value of 7.3 ± 3.0 nmol cholesterol formed/g of tissue per hr for renal cell cancer.[1]

**Fig. 4** shows the microsomal ACAT activity measured in normal kidney and clear cell cancer tissue from the same 13 patients. Apparent ACAT activity is shown to be significantly higher ($P < 0.01$ by paired analysis) in clear cell tumor (2405 ± 546 pmol/mg per 20 min) than in normal kidney (1326 ± 301 pmol/mg per 20 min). Thus, in addition to having massive cholesteryl ester stores, these tumor cells persisted in having the enzyme capacity to synthesize more ester than nontumor renal cells. The 20-min incubation used in the ACAT assay appeared to be beyond the linear phase of the reaction. It is possible that the measured increase in ACAT activity was simply a reflection of increased free cholesterol content of clear cell tumor tissue. However, this seems unlikely since the free cholesterol content of the microsomal preparation was measured and found to be only modestly different (49 μg/free cholesterol per mg of protein for kidney vs. 61 μg free cholesterol per mg of protein for tumor). In one experiment, free cholesterol was added to a concentration of 100 μg/ml using 0.3% of the detergent WR 1339. Measured ACAT activity without exogenous cholesterol was 1322 pmol/mg per 20 min in tumor and 254 pmol/mg per 20 min in kidney; activity in the presence of additional free cholesterol was 1126 pmol/mg per min in the tumor and 328 pmol/mg per 20 min in the normal kidney. Therefore, it appears that these measurements of ACAT activity are representative of the actual tissue esterification rate.

**Table 2** shows data for total cholesterol content, esterified cholesterol content, HMG-CoA reductase activity, and ACAT activity for the nonclear cell tumor types. The cholesterol content of granular renal cell carcinoma was modestly elevated while that of the mixed cell type was nearly as high as that of the clear cell type. Reductase and ACAT activities of granular carcinoma paralleled those of clear cell cancer, consistent with a postulate that the two tumor types have a common origin. Enzyme activities for the mixed cellularity type tumor also suggest the same pattern, but the small number of samples does not allow statistical significance to be met. The oncocytic tumor type, a very low grade tumor, was quite dissimilar

TABLE 1. Fatty acid moiety of cholesteryl esters in normal kidney and clear cell renal carcinoma

| Fatty Acid | Normal Kidney | Renal Cell Cancer |
|---|---|---|
| | % | |
| Palmitate (16:0) | 23 | 11 |
| Stearate (18:0) | 16 | 8 |
| Oleate (18:1) | 30 | 59 |
| Linoleate (18:2) | 27 | 21 |

Cholesteryl esters were extracted and isolated from normal kidney and tumor of one patient. Esters were saponified and the fatty acid constituents were identified by gas-liquid chromatography.

[1]Studies done by R. V. Clayman in the laboratory of J. M. Dietschy, University of Texas Health Science Center at Dallas.

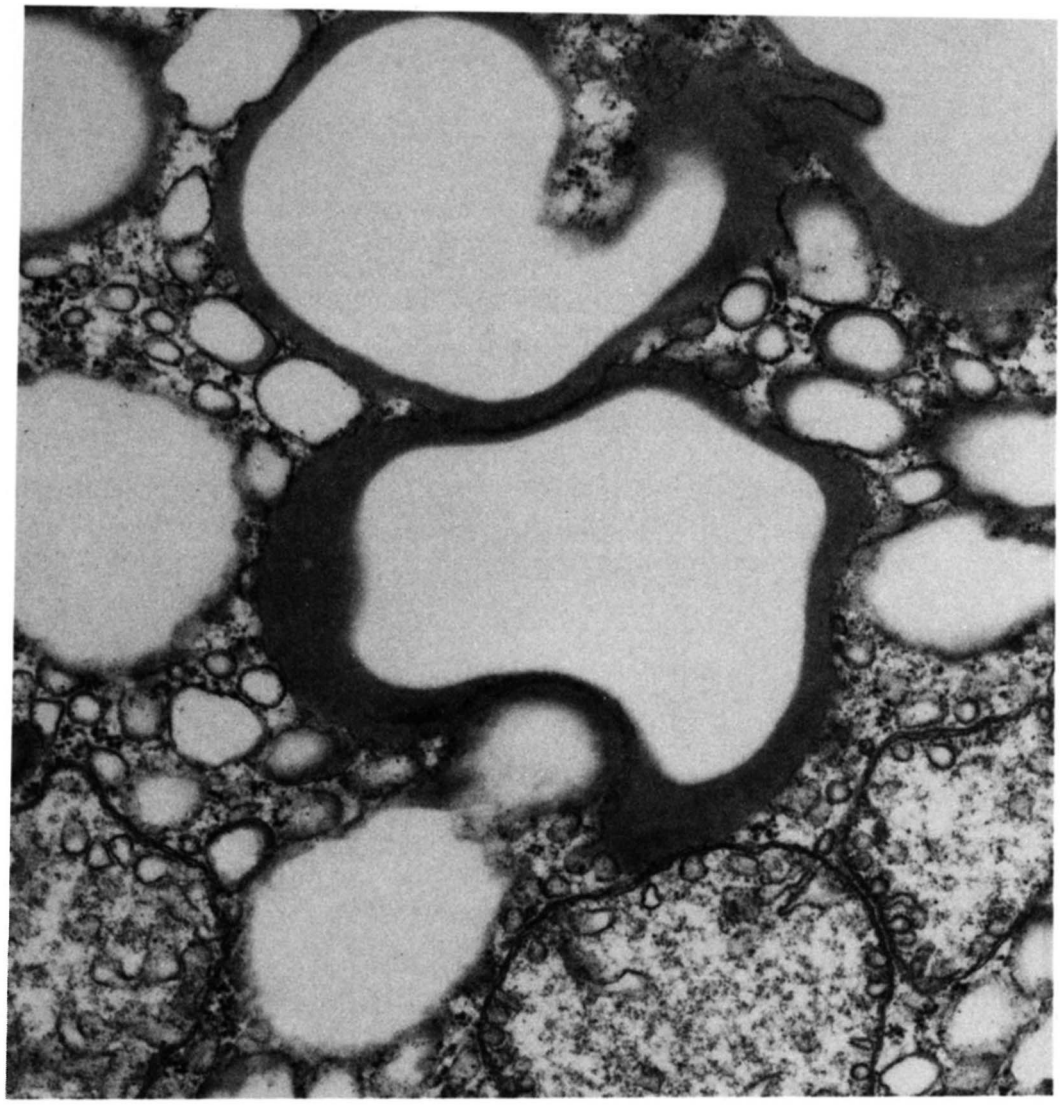

**Fig. 2.** Electron microscopic appearance of clear cell renal carcinoma. Tissue was fixed with glutaraldehyde. Magnification is 31,250 ×. Lipid inclusions do not appear to be contained within membranes.

and was more consistent with normal renal tissue in cholesterol content, but with much higher levels of HMG-CoA reductase for unclear reasons.

Cytosolic cholesterol esterase enzyme activity from paired tumor and normal tissue was measured in five clear cell cancer patients. Results are shown in **Table 3.** Ester hydrolytic activity was found to be variable from patient to patient, but tumor cell activity was basically the same as normal kidney cell activity for each patient. Thus, we were unable to discern a defect in hydrolysis of renal cell cancer cholesteryl esters.

### Cholesterol efflux

**Table 4** shows results of the measurement of cholesterol released from tissue slices to enter medium containing delipidated HDL, with or without phospholipid, during a 1–2 hr incubation of tissue from six patients. Final media cholesterol was expressed as percent of initial tissue free or total cholesterol for paired slices of normal kidney and clear cell renal cancer tissue from individual patients. It can be seen from the table that release of cholesterol was always greater to HDL protein which had been "reconstituted" with phospholipid. Although the free cholesterol content of cancer tissue was somewhat greater than normal kidney (as shown in Fig. 1), the percentage of free cholesterol release from the tumors was not greater (as a mean, the percent of free cholesterol released from tumor was 16% less than from kidney—not a significant difference). Furthermore, since the total cholesterol content of cancer tissue was much greater than normal kidney, the mean percentage of total cholesterol released from cancer (0.7%) was significantly less than from normal kidney (3.2%), $P < 0.02$. Thus, cancer cells did not appear to release a greater fraction of their cholesterol load to HDL;

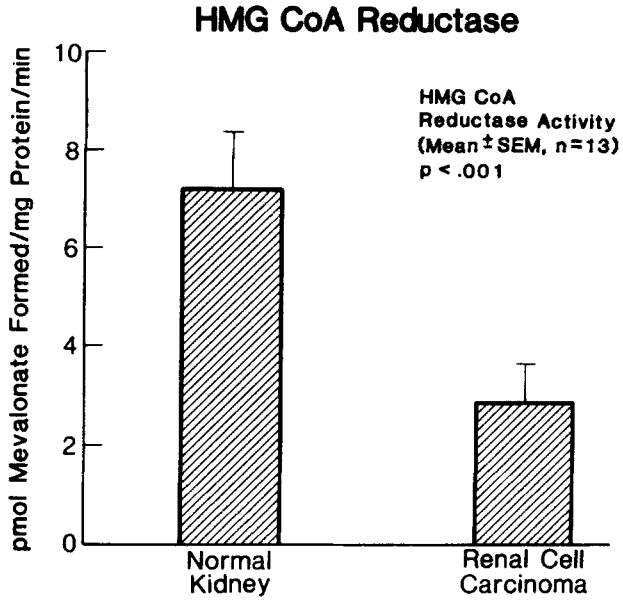


**Fig. 3.** Microsomal pellets were prepared from homogenized clear cell cancer tissue and adjacent normal kidney from 13 patients. Total activity of HMG-CoA reductase in resuspended microsomes was measured. Significantly lower activity was found in cancer tissue, $P < 0.001$ by paired analysis.

the total quantity of cholesterol released to HDL was nearly identical for both normal kidney slices and tumor slices ($28 \pm 5$ $\mu$g/g wet weight of normal tissue vs. $31 \pm 6$ $\mu$g/g wet weight of tumor tissue released to HDL, and

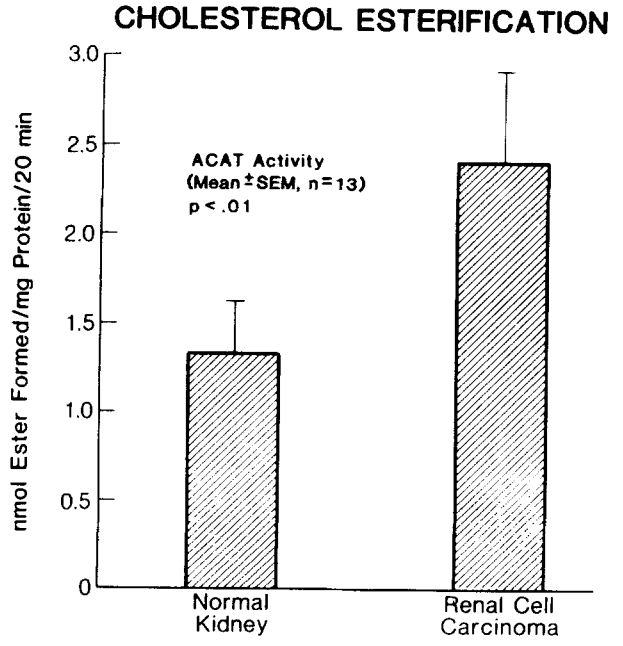


**Fig. 4.** Microsomal pellets were prepared from homogenized clear cell cancer tissue and adjacent normal kidneys from 13 patients. Apparent ACAT enzyme activity was measured in resuspended microsomes. ACAT activity was significantly higher in cancer tissue, $P < 0.01$ by paired analysis.

TABLE 2. Cholesterol content and enzyme activities in non-clear cell types of renal carcinoma

| | | Tumor Type | | | | | |
|---|---|---|---|---|---|---|---|
| | | Granular (3) | | Mixed (3) | | Oncocytic (1) | |
| | | Kidney | Tumor | Kidney[a] | Tumor | Kidney | Tumor |
| Cholesterol (mg/g protein) | Total | 23.5 (23–24) | 50.1 (28–122) | 48.1 (23–79) | 215.8 (120–380) | 25 | 24 |
| Cholesterol | Esterified | 2.4 (0–21) | 28.1 (4–95) | 20.7 (1–58) | 120.8 (41–338) | 3.5 | 0.0 |
| HMG-CoA reductase (pmol/mg per min) | | 8.5 (2–11) | 3.9 (0–7) | 8.1 (4–15) | 3.2 (2–5) | 8.4 | 27.7 |
| ACAT (pmol/mg per 20 min) | | 2690 (1150–3880) | 5903 (4420–7700) | 1073 (0–1870) | 2423 (710–4130) | 4780 | 11760 |

Seven non-clear cell renal carcinomas and adjacent normal kidneys were tested for cholesterol content, HMG-CoA reductase activity, and ACAT activity using the same methods as for clear cell tumor. Mean values are shown, with the range of values given in parentheses. A continuum of metabolic abnormalities appears to progress from granular tumors to mixed tumors to clear cell tumors. Oncocytic tumor was unique.

[a] "Normal" kidney from one of these patients may have contained tumor, since the cholesterol and ester content of one tissue sample was quite high.

TABLE 3. Cytosolic cholesteryl esterase activity

| Patient | Normal Kidney | Renal Cell Cancer |
|---|---|---|
| | *pmol of ester hydrolyzed/mg protein per hr* | |
| 1 | 114 | 93 |
| 2 | 96 | 110 |
| 3 | 35 | 38 |
| 4 | 46 | 36 |
| 5 | 122 | 110 |
| Mean | 82.6 | 77.4 |
| Mean % of normal | | 95% |

Tumor and adjacent normal kidneys from five clear cell renal carcinoma patients were tested, in duplicate, for cholesteryl ester hydrolase activity. Supernatant from the high speed centrifugation of homogenized tissue, primarily cytosol, was incubated with [$^{14}$C]cholesteryl oleate and release of [$^{14}$C]cholesterol was measured.

104 ± 16 μg/g vs. 116 ± 8 μg/g released to HDL/phospholipid).

## DISCUSSION

Studies of a large number of clear cell renal cancers confirm previous observations that tumor cells store large amounts of cholesterol, largely in the esterified form. The evidence presented here indicates that excessive accumulation of cholesteryl esters by these carcinoma cells involves the active intracellular processing of cholesterol. The prominance of cholesteryl oleate as the ester form indicates that these cells are not simply storing an ultrafiltrate of circulating lipoprotein lipids. Consistent with this thesis is the finding that the lipid is not specifically localized to lysosomes by electron microscopy, as might be seen in cholesteryl ester storage disease due to defective lysosomal hydrolases (23–25).

These results also suggest that excessive de novo synthesis of cholesterol from acetate is not the primary cause of ester accumulation in renal cancer, just as previous studies have suggested that excessive cholesterol uptake from lipoproteins is not the primary disorder (4, 7). Finding significantly lower levels of HMG-CoA reductase in nearly all renal cancers provides evidence that feedback inhibition of the enzyme by intracellular free cholesterol is present to at least some degree in this neoplasm. Malignant tissues might be expected to have increased HMG-CoA reductase activity to provide cholesterol for cell membranes or mevalonate as a precursor of transfer RNA (26), but this is not seen in clear cell cancers. These studies do not preclude the possibility that renal cancer cells exhibit increased synthesis of cholesterol from mevalonate or other precursors beyond the step involving HMG-CoA reductase. In fact, normal renal tubular cells show very active uptake of mevalonic acid from the circulation (27–29). Thus it remains possible that renal cancer tissue is also active in extracting mevalonic acid and synthesizing cholesterol from this point. Further investigation is necessary.

The evidence available from this study does indicate that free cholesterol within the tumor cells, whether arising from synthesis or uptake, is preferentially channeled into storage as cholesteryl ester via ACAT rather than being released from the cells to circulating HDL. The current data do not permit us to clearly determine whether

TABLE 4. Efflux of cholesterol from incubated tissue slices to Krebs-Ringer media containing HDL components

| Patient | Tissue | Medium Content | % Free Effluxed | % Total Effluxed |
|---|---|---|---|---|
| 1 | Kidney | 1 mg HDL[a] | 1.0 | 1.0 |
| | Cancer | | 1.7 | 0.6 |
| 2 | Kidney | 1 mg HDL | 1.5 | 1.3 |
| | Cancer | | 0.8 | 0.3 |
| 3 | Kidney | 3 mg HDL | 2.3 | 2.0 |
| | Cancer | | 1.2 | 0.4 |
| 4 | Kidney | 1 mg HDL + PL[b] | 4.3 | 3.9 |
| | Cancer | | 4.3 | 0.8 |
| 5 | Kidney | 1 mg HDL + PL | 8.3 | 5.8 |
| | Cancer | | 7.9 | 1.3 |
| 6 | Kidney | 1 mg HDL + PL | 5.6 | 5.4 |
| | Cancer | | 8.0 | 2.8 |

Slices of clear cell cancer tissue and normal kidney from six patients were incubated in Krebs-Ringer bicarbonate buffer with either HDL protein or HDL "reconstituted" with phospholipid. Cholesterol released to these acceptors in media and remaining tissue cholesterol components were then measured. Total amount of cholesterol effluxed was virtually identical for normal kidney and tumor (28 ± 5 μg/g tissue vs. 31 ± 6 μg/g released to HDL and 104 ± 16 μg/g vs. 116 ± 8 μg/g released to HDL + PL), in spite of the higher cholesterol content of tumor. Free cholesterol released to plain buffer without acceptor was also measured in six paired incubations from two patients. Normal kidney effluxed 12.8 μg/g tissue (0.8% of tissue free cholesterol) while tumor effluxed 11.2 μg/g (0.6%).

[a]HDL, Delipidated HDL protein.

[b]PL, Dimyristoyl phosphatidylcholine.

the underlying metabolic disorder is excessive ACAT activity or impaired free cholesterol release. On the one hand, apparent ACAT activity is clearly increased in the tumor, in conjunction with the massive tumor stores of cholesteryl ester. If the primary metabolic lesion in renal cell carcinoma is the elevated ACAT activity, then a reduction in release of cholesterol to HDL could be expected to result from preferential shunting of free cholesterol into storage esters. However, since free cholesterol content of this tumor is also modestly increased, it is possible that excessive free cholesterol serves as the stimulus to induce ACAT enzyme activity. In that case, a primary flaw in the release of free cholesterol would be accompanied by a secondary increase in ACAT activity to enable handling of free sterol. More detailed studies of cell metabolism will be required to distinguish between primary defects in ACAT enzyme activity versus impaired efflux of cholesterol from these tumor cells.

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