The structural relation of several potent carcinogenic hydrocarbons to cyclopentenophenanthrene, which forms the cyclic portion of cholesterol, bile acids, and steroid hormones, has given rise in recent years to various speculations concerning the rôle which cholesterol and other steroid derivatives may play in tumor metabolism. However, there are also numerous carcinogenic aromatic hydrocarbons, of which the skeleton bears no obvious resemblance to cyclopentenophenanthrene. The obvious contrast between carcinogenic hydrocarbons and aromatic nitrogen compounds of carcinogenic action (\textit{p}-aminoazotoluene, \textit{\beta}-naphthylamine, etc.) in the route of invasion and in their mechanism of action speaks against a fundamental concatenation between steroid metabolism and tumor growth.

Yet, cholesterol is linked in some peculiar manner with tumor metabolism. According to numerous authors (1) tumor tissue is richer in cholesterol than any parenchymatous organ, exclusive of the brain perhaps.\footnote{1}

The cholesterol level in blood and serum of cancerous individuals has been the subject of innumerable studies (3), but owing to the effects of emaciation and cachexia and to the specific influence of the state of various organs on cholesterol metabolism, also owing to the prevailing ignorance concerning the physiological function of cholesterol, the results of such investigations have not yet found a conclusive and uniform interpretation.

The cholesterol excretion through the kidney is minute under normal conditions, but considerable amounts are found in the

\footnote{1}{Roffo (2) asserts that increase of cholesterol in the integument, brought about by irradiation, increases the susceptibility for tumor growth.}
urine of nephritic patients (4-6) and in chylous urine (7). Evidence for this occurrence of cholesterol is based on colorimetric determinations (5, 6) and on gravimetric (digitonide) findings (4). There exists the possibility of enhanced cholesterol excretion in cases in which bile acids are forced through the kidneys. In other conditions, the amount of cholesterol in the urine was so negligible that no significance was attributed to it. However, the recovery of female and male sex hormones from large volumes of urine has not only stimulated the development of efficient methods of extraction, but has also emphasized the possibility that small, but significant concentrations of substances, which may be considered water-insoluble for most practical purposes, may occur regularly in urine. Butenandt and Dannenbaum (8) in the isolation of androsterone from normal male urine obtained cholesterol from mother liquors in a pure state and crystalline form; they failed, however, to isolate more than 1.3 mg. of pure cholesterol acetate per 100 liters of urine and in another batch 4.4 mg. of cholesterol benzoate per 100 liters. However, since their method of purification involved big losses, they estimated the original cholesterol content at 50 to 70 mg. per 100 liters. 10 times this concentration had previously been found by Butenandt (9) in pregnancy urine.

In a study of unsaponifiable fractions of urine extracts, we observed a cholesterol content in urine from normal individuals of the same order of magnitude; namely, 30 mg. in 100 liters. In the lipid extract of 2000 liters of urine from cancer patients an average of nearly 400 mg. per hectoliter was obtained, most of which crystallized spontaneously from a concentrated methanol solution after saponification and removal of the fatty acids. Minor amounts were obtained from the mother liquors by precipitation with digitonin.

As it seems plausible that cholesterol as well as other “water-insoluble” ingredients of the urine may be bound to albumin, or would in any event be coprecipitated with it during the extraction process, the precipitate in the urines was not removed, but extracted together with the bulk of the urine. This procedure introduces a factor of uncertainty, as the urinary sediment contained cellular material, such as white and red blood cells and epithelial cells. However, the amounts of cellular material were not sig-
significant; moreover, the lipid content of the precipitate as a whole was remarkably low. A batch of pooled cancer urine without turbidity or sediment gave the same picture of lipid extractives, qualitatively and quantitatively, as the turbid batches.

The rise of urinary cholesterol to more than 10 times the normal value may not be characteristic for cancer urine but a symptom

**Table I**

*Cholesterol Content of Urine*

<table>
<thead>
<tr>
<th>100 liter lots; source of urine</th>
<th>Initial residue</th>
<th>Unsoapifiable residue</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer wards, Welfare Island</td>
<td>4.8</td>
<td>4.9</td>
<td>490</td>
</tr>
<tr>
<td>Cardiac wards, Montefiore Hospital</td>
<td>5.4</td>
<td>5.5</td>
<td>430</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>5.5</td>
<td>5.6</td>
<td>360</td>
</tr>
<tr>
<td>Tuberculosis wards, Montefiore Hospital</td>
<td>2.9</td>
<td>3.0</td>
<td>20‡</td>
</tr>
<tr>
<td>Cardiac wards, Montefiore Hospital</td>
<td>4.7</td>
<td>4.8</td>
<td>50‡</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>4.5</td>
<td>4.6</td>
<td>30‡</td>
</tr>
</tbody>
</table>

* Digitonide not included.
† Clear specimen of urine.
‡ Precipitated as digitonide and determined colorimetrically.

common to any group of cachectic patients. Therefore, controls were run on urine from cardiac and consumptive patients; these gave figures of 50 and 20 mg. of cholesterol per 100 liters respectively, figures which correspond with the values for normal subjects, as shown in Table I.

The study of such quantities of urine as were used in the present investigation necessitates the collection of pooled batches. Any unusual findings in pooled specimens naturally raise the question
whether these deviations are general and typical for the entire group or whether they should be ascribed to deviations in the same direction, but of much greater degree, occurring in individual patients, due, for example, to hypercholesterolemia in disease processes involving the urinary tract. However, the constant occurrence of cholesterol values of between 300 and 500 mg. per 100 liters in urine from a large number of tumor patients on the wards, where the urine was collected, renders this possibility remote.2

As a more general explanation of our results, we are inclined to attribute the high cholesterol level in cancer urines to the continuous destruction of tumor tissue. The study of urines collected from individual patients will throw light on the question whether choledochuria is an expression of abnormal cholesterol metabolism or merely of increased catabolism of cholesterol-rich tissue.

**EXPERIMENTAL**

2000 liters of urine, collected from the wards of the New York Cancer Institute, Welfare Island, were extracted in 25 liter lots. Preliminary extraction experiments had been carried out by Dr. Walter Marx with urine concentrated in vacuo to one-fifth or even one-tenth its original volume, with butyl alcohol or benzene as solvent. Urine, completely desiccated in vacuo and mixed with an equal amount of anhydrous sodium sulfate, was extracted in a Soxhlet apparatus with benzene. A comparison of various methods tried resulted in the selection of the procedure described below, with butyl ether as solvent and non-concentrated urine stirred vigorously at room temperature. 25 liters of acidified urine were agitated with a total of 4 liters of n-butyl ether in three portions over 8 hours. The filtered extracts were concentrated in vacuo to about one-half the original volume and extracted exhaustively in a separatory funnel successively with 15 per cent sodium carbonate, 20 per cent sodium hydroxide, and 5 per cent hydrochloric acid. The washed and dried extracts were freed

2 We did not find androsterone or dehydroandrosterone accompanying cholesterol, as described by Butenandt and Dannenbaum (8), because the urines had not been hydrolyzed before extraction. Thus androgenic substances were present mostly in conjugated form and escaped extraction.
from solvent in vacuo, in the last stage by heating on a boiling water bath, and the combined residues from 100 liters of urine were refluxed with 50 cc. of 20 per cent methyl alcoholic potassium hydroxide for 2 hours. The unsaponifiable fraction was separated from the soaps by several extractions with benzene. After removal of the benzene the residue was taken up in 30 cc. of warm methanol, from which cholesterol invariably crystallized in typical platelets. The yield was slightly increased by recovery of minor amounts of cholesterol from the mother liquor as digitonide. The mother liquors of the digitonin precipitation were worked up for other substances, discussed elsewhere. The cholesterol was obtained from the digitonide by dissociation in pyridine-ether (10). The total amounts obtained in the last thirteen lots which had undergone a uniform procedure are given in Table I.

These cholesterol fractions were purified by repeated crystallization from methanol. The melting point of the purified product was 147° (corrected); its optical rotation $[\alpha]_2^22 = -28.5° (c = 0.500$ in methanol), $[\alpha]_5^22 = -37.5° (c = 1.112$ in chloroform). The acetate, which was prepared by heating with acetic anhydride, melted at 114° (corrected) and had a rotation of $[\alpha]_2^22 = -47° (c = 0.500$ in methanol). No other crystalline products could be obtained from the mother liquors. Cholestanol may be present, but its quantity must be minute, as we were not able to isolate it by the method of Schoenheimer (11).

Our thanks are due to Dr. S. S. Goldwater, Commissioner of Hospitals, New York, and to Dr. T. I. Price, Superintendent of the New York Cancer Institute, Welfare Island, and his staff, whose kind cooperation enabled us to undertake this work, and also to Dr. H. R. Miller of Montefiore Hospital for his aid in obtaining control specimens. Mr. Lewis R. Fibel rendered valuable and conscientious assistance in the extraction of the urines.

SUMMARY

Urines from cancer patients contain about 10 times as much cholesterol as from normal controls. Cachectic patients with other diseases, such as tuberculosis and heart disease, show normal cholesterol values, whereas hypercholesteroluria is known to occur in kidney disease and perhaps during pregnancy.
Two possible causes for the appearance of cholesterol in urine in cancer are discussed. The explanation by destruction of tumor tissue, rich in cholesterol, is given preference.

BIBLIOGRAPHY
