

# COMPARATIVE EVALUATION OF NONESTERIFIED AND TOTAL URINARY CHOLESTEROL IN PAPILLOMA AND CARCINOMA OF THE BLADDER

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Urinary excretion of nonesterified (NEC) and total cholesterol (TC) has been determined in 137 healthy individuals with a range (2 SD) of 0.26–2.2 mg/24 hours NEC and 0.3–3.0 mg/24 hours TC. 264 patients with various internal diseases revealed normal values of NEC and TC; neoplasias, diseases of the kidney and prostatic adenoma with residual urine had been excluded with reasonable certainty. There was no correlation between urinary cholesterol excretion and cholesterol plasma levels. In papilloma of the bladder ( $n = 16$ ) NEC hyperexcretion was present in 56%; elevated levels of TC were determined in 68%. In carcinoma of the bladder,  $T_1N_0M_0$ – $T_2N_0M_0$  ( $n = 28$ ), hyperexcretion of NEC occurred in 50% and of TC in 64%. In advanced clinical stages of the disease,  $T_3N_0M_0$ – $T_4N_4M_{1d}$  ( $n = 21$ ), elevated values of NEC were detected in 76% and of TC in 90%. Since all macroscopically blood contaminated urinary samples had been excluded, a determination of total urinary cholesterol excretion may be valuable in the diagnosis of papilloma or carcinoma of the bladder in the absence of macrohematuria. Occult blood in urine was present in 33 of the 65 patients with papilloma or carcinoma of the bladder, which was associated in 26 cases with elevated urinary total cholesterol. 32 patients revealed a negative test for occult blood in urine. In 22 of these, hyperexcretion of urinary total cholesterol was observed, indicating diagnostic sensitivity of this parameter for papilloma and carcinoma of the bladder even in the absence of microhematuria. However, one has to regard that elevated urinary cholesterol levels could also occur in other carcinomas of the urogenital system, prostatic adenoma with residual urine and kidney diseases.

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**H**YPEREXCRETION OF URINARY CHOLESTEROL in cancer has been first reported by Bloch and Sobotka in 1939 and confirmed in later studies.<sup>2,3,4,5,6,8,9,11,12,14,19,20</sup> Determination of this parameter seems to be of diagnostic value in the detection of carcinomas of the urogenital system, although elevated levels could occur in kidney diseases and prostatic adenoma with residual urine too.<sup>3,13,15,17,21</sup> However, there are only a few reports about urinary cholesterol excretion in papilloma and carcinoma of the bladder. Only the study Frick and Spittler has demon-

strated marked hyperexcretion of urinary cholesterol in four patients with carcinoma of the bladder in advanced clinical stages.<sup>14,20</sup> Therefore it was necessary to study urinary cholesterol excretion, especially in regard to early clinical stages of carcinoma of the bladder and precancerous lesions as papilloma. A comparative evaluation of nonesterified and total urinary cholesterol was undertaken to investigate which parameter would be of higher diagnostic sensitivity for these diseases.

## MATERIALS AND METHODS

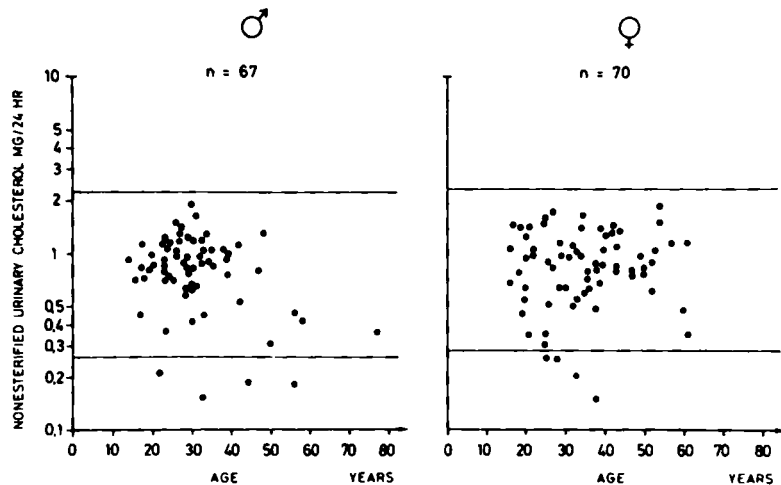
Nonesterified and total urinary cholesterol were analyzed in 2 ml aliquots of 24 hours urine with a gas-liquid chromatographic assay using 4-androstendione as internal standard and peakheight ratio technique for quantitation. This method has been described in detail previously.<sup>15</sup>

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FIG. 1. Urinary excretion of nonesterified cholesterol in normal individuals.



### Clinical Material

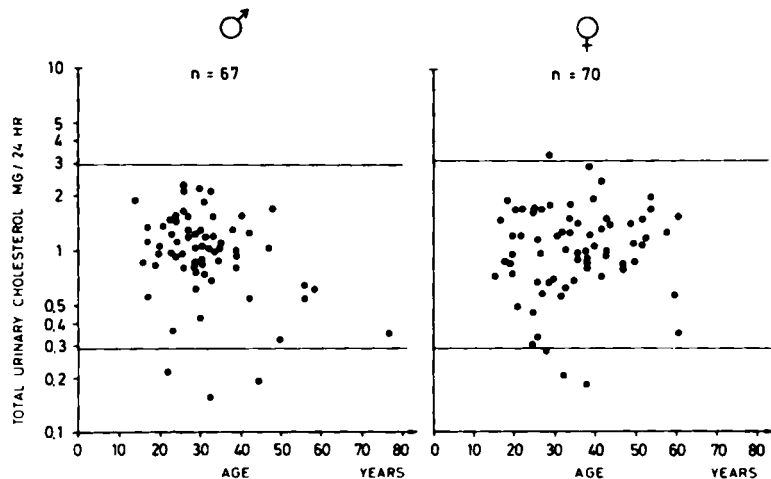
Normal range for NEC and TC excretion was calculated using standard procedures and obtained from a group of clinically healthy and biochemically normal individuals. The 264 patients with various internal diseases consisted of 75 with endocrine and metabolic, 68 with cardiovascular, 78 with gastrointestinal, 27 with respiratory and 16 with hemopoetic disorders. The presence of neoplasias, kidney diseases and prostatic adenoma with residual urine had been excluded in this second control group with reasonable certainty.

In all patients with papilloma or carcinoma of the bladder diagnosis was proven histopathologically after resection. Only urinary samples without macroscopically blood contamination were included in this study. When urinary cholesterol determinations were performed, patients did not receive any treat-

ment. A diagnosis had been established in most cases at the time of the investigation.

Patients with carcinoma of the bladder were classified according to the TNM system. For assessment of T categories, clinical examination, urography, cystoscopy, bimanual examination under full anesthesia and biopsy or transurethral resection of the tumor prior to definitive treatment were performed. Classification of N categories based on clinical examination, lymphography and urography and of M categories on clinical examination, chest x-ray and biochemical tests, in more advanced primary tumors radiographic or isotope studies had been done. Accordingly, we divided patients with carcinoma of the bladder in two groups;  $T_1N_0M_0$ - $T_2N_0M_0$  and  $T_3N_0M_0$ - $T_4N_4M_{1d}$ . Patients with  $T_1$ - $T_2$  carcinoma of the bladder and node involvement or metastasis were not present in this study.

FIG. 2. Urinary excretion of total cholesterol in normal individuals.



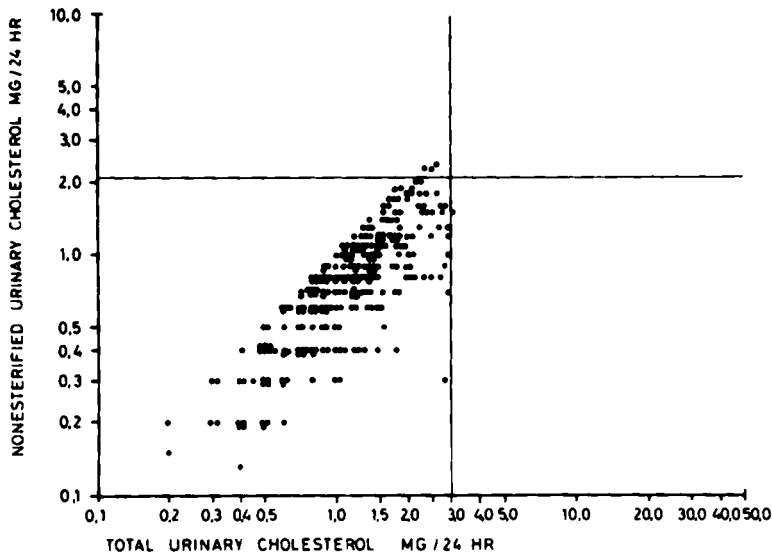


FIG. 3. Correlation of urinary nonesterified and total cholesterol in patients with various internal diseases without evidence of neoplasia or kidney disorders.

## RESULTS

### Normals

This group comprised 137 clinically healthy, asymptomatic and biochemically normal individuals, 67 males and 70 females, covering an age range of 14 to 76 years.

The excretion values for NEC are shown in Fig. 1 and those for TC in Fig. 2. Based on the statistical analysis of the results, we obtained a mean excretion of 0.76 mg/24 hours NEC and 0.92 mg/24 hours TC in males and 0.84 mg/24 hours NEC and 0.98 mg/24 hours TC in females.

The range for normal values was calculated as the mean  $\pm$  2 SD including 95% of the population. On the basis of these results, an

upper normal limit of 2.2 mg/24 hours of NEC and 2.9 mg/24 hours of TC was determined in males and 2.3 mg/24 hours of NEC and 3.1 mg/24 hours of TC in females. It is important to note that values of urinary cholesterol excretion were independent of age and presented a log normal distribution. For practical purposes, we defined hyperexcretion of NEC as any result exceeding 2.2 mg/24 hours and hyperexcretion of TC any result exceeding 3.0 mg/24 hours, since there were only minimal differences of urinary cholesterol excretion between the male and female group.

In 264 patients with various internal diseases, urinary excretion of NEC and TC were within the normal range, except 3 cases with

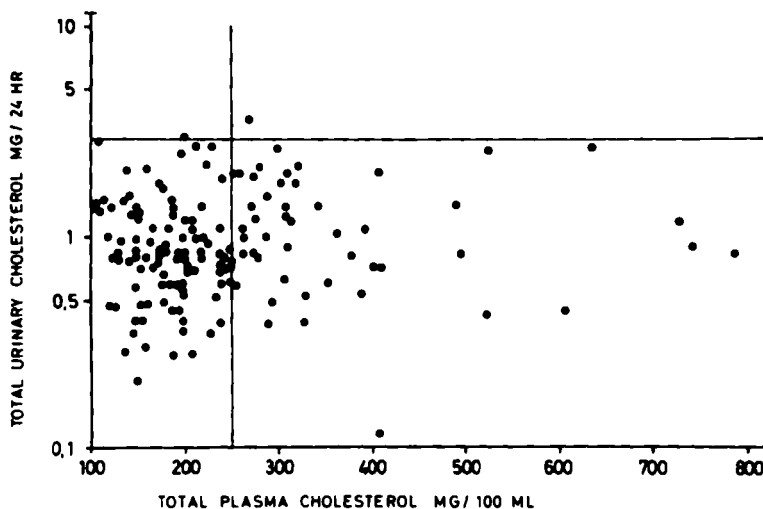
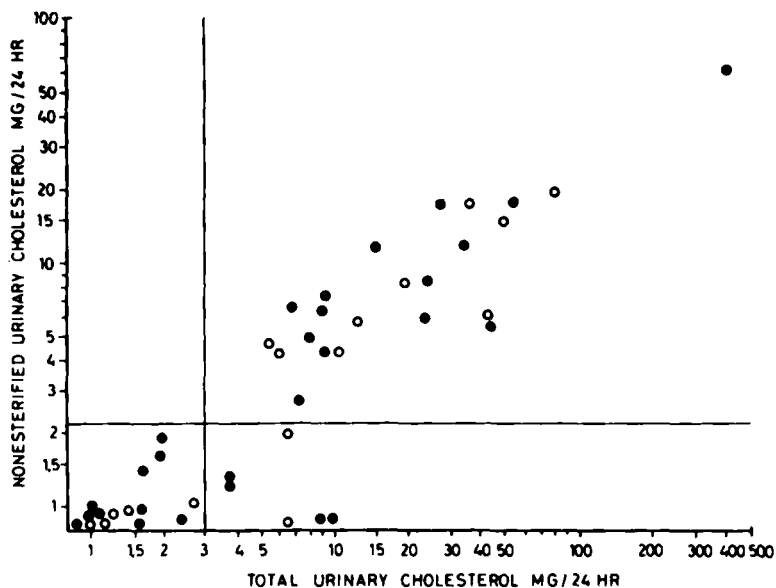


FIG. 4. Correlation of urinary total cholesterol and plasma total cholesterol in patients with various internal diseases without evidence of neoplasia or kidney disorders.

FIG. 5. Comparative evaluation of nonesterified and total urinary cholesterol in papilloma (○) and carcinoma (●) of the bladder, T<sub>1</sub>N<sub>0</sub>M<sub>0</sub>-T<sub>2</sub>N<sub>0</sub>M<sub>0</sub>.



slightly elevated levels of NEC and one patient with elevated TC (Fig. 3). There was not a constant ratio between NEC and TC; in most cases nonesterified cholesterol was the major component of total urinary cholesterol. No correlation between urinary cholesterol excretion and cholesterol plasma levels was obtained; even in patients with marked hypercholesterolemia urinary cholesterol values were normal (Fig. 4). In papilloma of the bladder (n = 16) NEC hyperexcretion was

present in 56%, elevated levels of TC were determined in 68% (Fig. 5). In carcinoma of the bladder, T<sub>1</sub>N<sub>0</sub>M<sub>0</sub>-T<sub>2</sub>N<sub>0</sub>M<sub>0</sub> (n = 28), hyperexcretion of NEC occurred in 50% and of TC in 64% (Fig. 5).

In advanced clinical stages of the disease, T<sub>3</sub>N<sub>0</sub>M<sub>0</sub>-T<sub>4</sub>N<sub>4</sub>M<sub>1d</sub> (n = 21), elevated levels of NEC were detected in 76% and of TC in 90% (Fig. 6). Urinary cholesterol excretion values did not correlate with histological findings. NEC-TC ratios varied considerably; in most patients with hyperexcretion, cholesterol esters were the major component of total urinary cholesterol. In 33 of the 65 patients with papilloma or carcinoma of the bladder, occult blood in urine was present, which was associated in 26 cases with elevated urinary total cholesterol levels. 32 patients had negative test results for occult blood in urine; in 22 of these hyperexcretion of urinary cholesterol was observed.

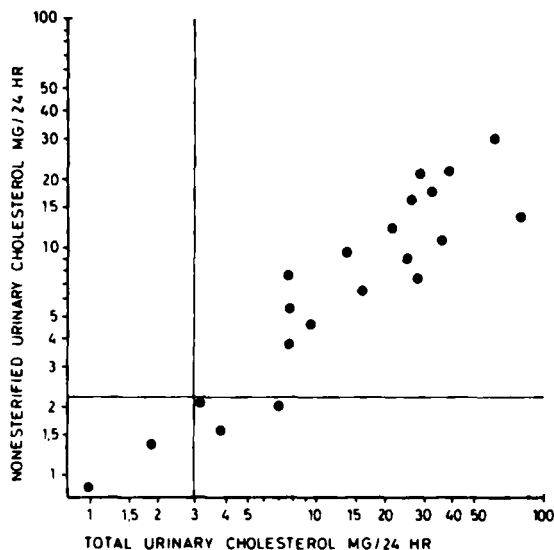


FIG. 6. Comparative evaluation of nonesterified and total urinary cholesterol in carcinoma of the bladder, T<sub>3</sub>N<sub>0</sub>M<sub>0</sub>-T<sub>4</sub>N<sub>4</sub>M<sub>1d</sub>.

DISCUSSION

Elevated levels of urinary cholesterol in cancer had been determined by Bloch and Sobotka and confirmed in later studies.<sup>8,9,11,12,18,19</sup> Although the scientific value of these findings was limited due to the nonspecific analytical techniques using colorimetric methods, elevated nonesterified urinary cholesterol (NEC) had been demonstrated recently by Acevedo in patients with carcinomas of steroid hormone-producing glands and their main

target tissues with specific gas-liquid chromatography.<sup>2,3,4,5,6</sup> In a previous publication we stated that determination of nonesterified or total urinary cholesterol may be of value in the diagnosis of prostatic cancer, since elevated levels of both could be demonstrated even in early clinical stages of this disease.<sup>15</sup> To date only few studies exist about urinary cholesterol excretion in malignant diseases of the bladder. Frick and Spitteller demonstrated slightly elevated urinary cholesterol values in three cases of papilloma and in four patients with carcinoma of the bladder using specific mass spectrographic analysis. A marked hyperexcretion of urinary cholesterol, exceeding 10 mg/l, was observed in four cases with advanced clinical stages.<sup>14,20</sup>

Our results demonstrated pathologic values in papilloma of the bladder of NEC in 56% and of TC in 68%. In early clinical stages of carcinoma of the bladder, hyperexcretion of NEC was present in 50% and in 64% of TC. The percentage of elevated values increased to 76 of NEC and 90 of TC in advanced clinical stages, corresponding to the observations of Frick and Spitteller. Only in 2 patients of this group (n = 21) was urinary excretion of total cholesterol normal. Diagnostic sensitivity of TC determination for the detection of papillomas or carcinomas of the bladder was considerably higher in comparison to determination of NEC. Combination of both assays did not increase the possible detection rate for these diseases, since in most cases with hyperexcretion, cholesterol esters were the major component of urinary total cholesterol.

Discussing the clinical value of these findings, one has to consider that early diagnosis of carcinoma of the bladder is difficult because macrohematuria is an inconsistent symptom. Since macroscopically blood contaminated urinary specimens had not been included in this study, a special determination of urinary total cholesterol could be valuable in the diagnosis of asymptomatic carcinoma of the blad-

der. Additionally, papillomas of the bladder could be detected in a considerable percentage of patients, which is of interest due to their potential malignancy. Occult blood in urine was present in 33 of the 65 patients with papilloma or carcinoma of the bladder, which was associated in 26 cases with elevated urinary total cholesterol values. 32 patients revealed a negative test for occult blood in urine. In 22 of these patients, hyperexcretion of TC was observed, indicating diagnostic sensitivity of this parameter in papilloma and carcinoma of the bladder even in the absence of microhematuria.

Other common conditions of urinary cholesterol hyperexcretion are neoplasias of the steroid hormone-producing glands and their main target tissues, prostatic adenoma with residual urine and kidney diseases, especially the nephrotic syndrome.<sup>7,10,17,21</sup> These had been excluded with reasonable certainty in the 264 patients with various internal disorders; except for 1 patient, all revealed normal values of urinary total cholesterol. These results demonstrate the real practical value of our cutoff limit—3.0 mg/24 hours for TC—which is based on the statistical analysis of TC levels in the normal group.

The physiologic mechanisms of urinary cholesterol excretion are not known. Acevedo *et al.* suggested an excretion as a protein-bound complex on the basis of ultrafiltration studies.<sup>1</sup> Enhanced production of glomerular filtrable lipoproteins by the malignant cells are being discussed regarding the possibility of their causing hyperexcretion of urinary cholesterol in cancer of steroid hormone-producing glands or their main target tissues. In the case of papilloma or carcinoma of the bladder, we suggest that urothelial lesions may be responsible for the observed elevated urinary cholesterol values, similar in nature to the finding of increased urinary cholesterol in patients with prostatic adenoma in advanced clinical stages with residual urine.

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