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Letter to the Editor

Determination of coproporphyrin isomer contents in urine of tumour patients

Sir,

We would like to present some data on coproporphyrin isomer contents in the excretions of tumour patients, since no data of this type have ever been reported in the literature. We believe that our data may provide valuable information for the understanding of porphyrin metabolism disturbances that occur in the organisms as the tumours grow.

Up to now, an abundance of material on quantitative and qualitative porphyrin contents in biological materials has been accumulated and analysed [1—4]. These problems are most extensively studied by example with porphyria patients. Much consideration is also given to metabolism disturbances caused by chemical substances. As a rule, the metabolism disturbance is characterized by an increase in the total porphyrin contents in erythrocytes, bile and urine. In many instances, there is a change in the ratios of the individual components and the qualitative content. An important role is played by isomers in the metabolic processes. Their ratios can also vary in pathology studies. In particular, coproporphyrin I and III isomer contents in urine can vary with heavy metals and drugs, poisoning in liver diseases, as well as with porphyrias.

Many good high-performance liquid chromatography (HPLC) systems have been reported for the analysis of coproporphyrin isomers in urine [5—9]. We have separated coproporphyrin isomers I and III of methyl ethers and acids. A Du Pont 8800 chromatograph has been used. The chromatographic system consisted of a reversed-phase Zorbax column (250 × 4.6 mm I.D., 5 μm) and a protective column filled with Permaphase ODS. The flow-rate of the mixture was 1 cm^3/min at a pressure of 82 bar and a temperature of 35°C. The UV detector was set at a wavelength of 300 or 345 nm. The eluent used for the analysis of coproporphyrin tetramethyl ethers was prepared by mixing acetonitrile and 0.1 *M* sodium acetate buffer (pH 5.16). Effective separation of isomers was achieved in 11 min with components in the ratio 85:15. Methanol and a mixture of sodium hydrophosphate buffer (0.02 *M*) with potassium dihydrophosphate buffer (0.04 *M*) served as the eluent for chromatography of the coproporphyrin acids. The pH of the mixture was 6.52. Separa-

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tion was also achieved in 11 min with a mobile phase component ratio of 40:60.

The method was applied to the analysis of urine isomers in 30 subjects. The known method of porphyrin level estimation [2] provides us with the total coproporphyrin isomer contents in urine. It is possible to determine the percentage of each isomer by measuring the peak areas belonging to the individual coproporphyrin isomers I and III. Fig. 1 gives examples of the pattern observed for normal subjects and cancer patients.

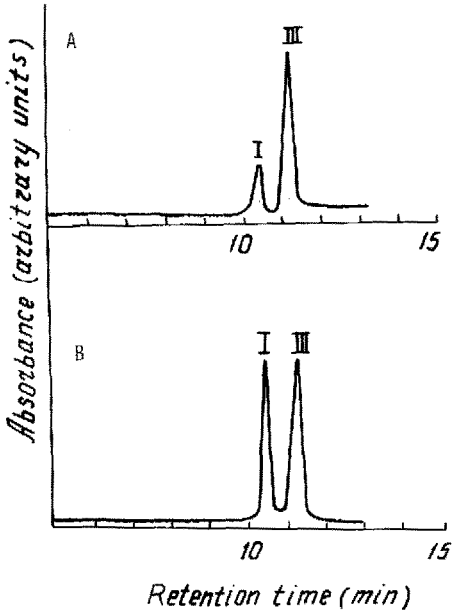

Fig. 1. Separation of isomers I and III of tetramethyl coproporphyrin ethers in urine of (A) normal subjects and (B) malignant tumour patients.

We have shown previously that the coproporphyrin contents in urine for tumour patients is lower than for normal subjects [10]. As seen from Fig. 1, the proportion of two isomers also differs for these groups of people. Coproporphyrin III per total coproporphyrin found in urine for normal individuals varies from 60 to 80%, which is in agreement with the value reported by Meyer et al. [5] and Udagawa et al. [8].

This value remains practically unchanged for nine patients with innocent tumours. Eighteen patients had malignant tumours and for the majority of them the above ratio varied from 50 to 60%. A comparison of cases with a similar total coproporphyrin contents in urine (22—27 μg per day) enables one to expect a tendency of the content of isomer III to decrease.

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