L'électrophorèse permet donc d'obtenir des fractions solubles douées de pouvoir antigénique. Sauf dans un cas, la solubilisation du matériel antigénique de départ n'a toutefois été que partielle. L'analyse chimique des fractions solubles actives est superposable à celle des fractions obtenues par chromatographie sur phosphate calcique par OTH ET CASTERMANS[3]. Ces faits suggèrent que le pouvoir antigénique pourrait être supporté par une glycoprotéine, dont la forte charge positive expliquerait les caractères particuliers de solubilité et peut être aussi l'affinité pour l'ARN (ou l'ADN[7] dans certains cas).

Ce travail a été réalisé avec l'assistance technique de Melles C. HENIN et M. PROTIN.

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Laboratoire de la Clinique Chirurgicale, Université de Liège et Fonds National de la Recherche Scientifique (Belgique) ANDRÉ CASTERMANS

Received July 16th, 1960

Biochim. Biophys. Acta, 43 (1960) 136–137

Elimination by the urine of orotic acid and orotidine in man after application of 6-azauracil

The principal pathway of biosynthesis of the pyrimidine components of nucleic acids can be characterized by the sequence of the intermediates:

aspartic acid → ureidosuccinic acid → dihydroorotic acid → → orotic acid → orotidylic acid → uridylic acid,

the key positions being occupied by derivatives of orotic acid[1,2]. Their isolation from biological material, however, is quite complicated. Orotic acid[3] and later even dephosphorylated orotidylic acid, orotidine[4], have been isolated only from some mutants of *Neurospora crassa*, deficient with respect to decarboxylase of orotidylic acid. The older detection of orotic acid in milk can be considered nowadays rather as a curiosity[5].

New possibilities were provided by the application of 6-azauracil which—as was found subsequently—is transformed to 6-azauracilriboside 5'-phosphate[6,7] and as a such inhibits the decarboxylase of orotidylic acid[8]. Inhibition of *Escherichia coli* cultures by 6-azauracil resulted in an accumulation of orotic acid[6] or—with some strains—of orotidylic acid[9] in the medium. At the same time, it was established that orotic acid and orotidine are eliminated en masse by the urine of normal and tumour-bearing mice treated with 6-azauracil or with its riboside[7,10], which served actually as the first direct proof of existence of a similar type of compounds in higher animals. Later, the compounds were identified even in acid extracts of tumours of animals which were treated with 6-azauracilriboside[11]. The present communication contains

new information on the elimination by the urine of considerable amounts of orotic acid and orotidine by healthy subjects as well as by cancer patients, following application of 6-azauracil, which indicates that the derivatives of orotic acid occupy an important position in the biosynthesis of the pyrimidine components of nucleic acids even in man.

The urine of 4 patients suffering from stomach cancer and treated subcutaneously with 100 mg 6-azauracil/day and of a healthy man after a single dose orally of 100 mg of the analog was analysed. Pyrimidines, purines and their derivatives were adsorbed from acidified urine on active carbon, desorbed by aqueous alcohol–ammonia and analysed by descending paper chromatography in *n*-butanol–water. In order to achieve separation of substances with low R_F values the chromatograms were run for 48–72 h. The nucleic acid components were detected by photographing in u.v. light (Fig. 1). After elution of the individual compounds with 0.1 *N* HCl orotic acid was identified by its characteristic u.v.-absorption spectrum with a minimum at 243 mμ, a maximum at 280 mμ and the ratio of extinctions at 280 and 260 mμ

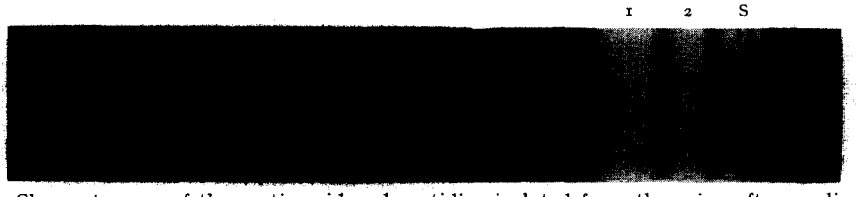

Fig. 1. Chromatogram of the orotic acid and orotidine isolated from the urine after application of 6-azauracil. Whatman No. 1 paper, developed by *n*-butanol saturated with water for 72 h, photographed by u.v. light. S, start; 1, orotic acid; 2, orotidine.

equal to 1.78. Orotidine displayed a minimum at 234 mμ, a maximum at 267 mμ and the ratio of extinctions at 280 and 260 mμ equal to 0.76. After hydrolysis in 4 *N* H_2SO_4 the hydrolysate was shown to contain orotic acid with a typical absorption spectrum in the u.v. region. The ratio of nitrogenous base: pentose: phosphorus was found to be equal to 1.00:0.93:0.02.

During the first 12 h after a single application of 100 mg 6-azauracil the healthy man eliminated approximately 75 mg orotic acid and 100 mg orotidine in the urine; later on, the two compounds could not be detected in the urine.

Charles University Medical Faculty,
Institute of Medical Chemistry,
Plzeň, Karlovarská 48 (Czechoslovakia)

VLASTIMIL HABERMANN

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Received July 20th, 1960