

## Nicotinamide in Adenocarcinoma 755 and in the Milk of Mice Carrying the 'Agent' of Spontaneous Mammary Tumor

The presence of pyridine nucleotides and of nicotinamide in a variety of normal tissues and in certain neoplastic growths, either of spontaneous origin or resulting from implants in laboratory animals, has been described in the literature<sup>1</sup>. This is a brief report of the identification of nicotinamide in implanted mammary adenocarcinoma 755 in C57Bl mice and in the milk of Paris RIII mice with mammary carcinoma acquired in infancy through the nursing influence referred to as the 'milk agent'. Identification was made by means of (1)  $R_f$  values, (2) light-absorption curves and (3) conversion to nicotinic acid. In the course of this work a previously unreported color reaction of nicotinamide with ninhydrin was observed and will be described.

The tumors were dropped into liquid nitrogen immediately on removal from the ether-killed mice, disintegrated and dried from the frozen state. The dried tissue was treated exhaustively with ether and the extract stirred vigorously with acidified water. The last step was repeated. The combined water solutions were concentrated under reduced pressure and used for paper chromatographic analysis. Despite precautions it is possible that some of the nicotinamide was derived from splitting of pyridine nucleotides.

**$R_f$  Values.** Four solvent systems were used. (1) 60 ml *n*-butanol and 10 ml 0.7 *M*  $\text{NH}_4\text{OH}$ ; (2) 77 ml *n*-butanol, 10 ml formic acid and 13 ml water; (3) 72% phenol and (4) 40 ml *n*-butanol, 10 ml acetic acid and 10 ml water. The  $R_f$  values were 0.64, 0.41, 0.92 and 0.64, respectively. They were the same, within 5%, for sample of known nicotinamide.

**Light-Absorption.** To free it of possible contamination before making measurements the compound was eluted from the chromatogram with *N*/100 HCl, the solution concentrated under reduced pressure and respotted using a different solvent system. The eluted compound from the second chromatogram had the same ultraviolet light-absorption curves in *N*/100 HCl and at pH 9 as those of corresponding solutions of known nicotinamide similarly processed<sup>2</sup>.

**Conversion to Nicotinic Acid.** When the eluates from chromatograms of tumor extracts were evaporated to dryness and the residues taken up in a small volume of *N*/1 HCl and heated for 2 h at 100° the nicotinamide was converted to nicotinic acid. Using solvent systems 1, 2 and 4 the  $R_f$  values of the product were 0.18, 0.17 and 0.22; they were the same for acid-treated, known nicotinamide. Also the ultraviolet light-absorption curves in *N*/100 HCl and at pH 9 of the eluted product were the same as those of the corresponding solutions of eluted, known nicotinic acid<sup>3</sup>.

Nicotinamide was similarly extracted from the kidneys, livers and spleens of the tumor-bearing mice.

**Reaction with Ninhydrin.** The tissue-extracted compound gave a pale-rose color similar to that obtained with

very small amounts of glycine when paper chromatograms on which it was present were sprayed with a solution of ninhydrin (0.1% in absolute ethanol). An authentic sample of nicotinamide gave the same color; the intensity resulting from 40  $\mu$  was about equal to that given by 4  $\mu$  of glycine.

**Mouse Milk.** The fat-free milk was dried from the frozen state and then treated as was the tumor tissue. A chromatogram showed the presence of a compound having the same ultraviolet light-absorption curve as that of nicotinamide and responding to the above-described color test with ninhydrin; its  $R_f$  value, however, was 0.14 (solvent 2). Evaporation of a solution in *N*/100 HCl with mild heating gave a compound with  $R_f$  values of 0.64 and 0.41 (solvents 1 and 2) which are the same as those of the tumor-extracted compound and of known nicotinamide. This compound also had the typical nicotinamide absorption curve in the ultraviolet and formed nicotinic acid when heated with *N*/1 HCl at 100°.

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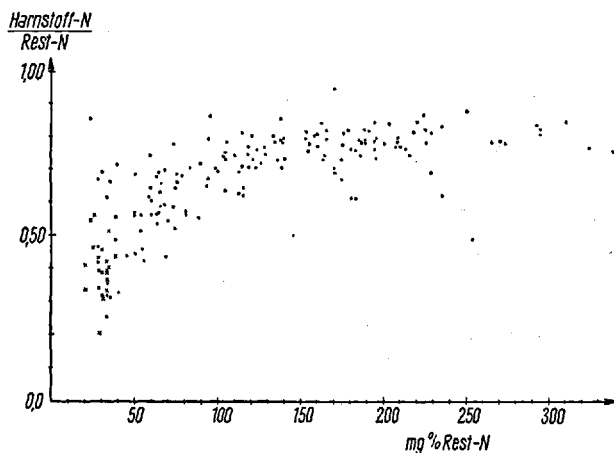
Department of Biochemistry, College of Physicians and Surgeons, Columbia University and Francis Delafield Hospital, New York City, April 23, 1957.

### Résumé

L'auteur montre que la nicotinamide se trouve dans l'adénocarcinome 755 et aussi, sous forme d'un dérivé non-identifié, dans le lait de souris porteuses du «milk agent». Il décrit en outre une réaction colorée de la nicotinamide avec la ninhydrine.

## Das Verhalten des Quotienten Harnstoff-N:Rest-N bei normalen und nephrektomierten, urämischen Ratten

Der Quotient Harnstoff-N:Rest-N beträgt bei 22 leber-, herz- und nierengesunden Menschen 0,39. 156 Bestimmungen bei 36 nierenkranken Patienten mit N-Re-



Harnstoff-N/Rest-N bei 36 Nierenpatienten (•) und 22 herz-, leber- und nierengesunden Kontrollpersonen (×)

tention ergaben einen Quotienten von 0,69 im Durchschnitt (Abb. 1). Theoretisch kann die Erhöhung des Harnstoff-N-Anteils am Rest-N hepatogen oder nephrogen sein.

<sup>1</sup> F. BERNHEIM and A. V. FESOVANYI, *Science* 81, 76 (1940). - H. V. EULER, F. SCHLENK, H. HEIWINKEL, and B. HÖGGER, *Z. physiol. Chem.* 266, 208 (1938). - C. CARRUTHERS and V. SUNTZEFF, *Arch. Biochem. Biophys.* 45, 140 (1953); *J. Cancer Res.* 12, 879 (1952). - B. CHANCE, *Trans. N. Y. Acad. Sci.* 16, 74 (1953).

<sup>2</sup> H. H. G. JELLINEK and M. G. WAYNE, *J. phys. Chem.* 55, 173 (1951).

<sup>3</sup> R. F. EVANS, E. F. G. HERINGTON, and W. KYNASTON, *Trans. Faraday Soc.* 49, 1284 (1953).