COMMUNICATION

Direct Incorporation of Orally Administered Riboflavin Tetranicotinate into Portal Blood of Rabbit¹

HIROSHI OHKAWA, AKIRA KOTAKI AND KUNIO YAGI²

*Institute of Biochemistry, Faculty of Medicine,
University of Nagoya, Nagoya (Post No. 466)*

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From the pharmacological interest of fat-soluble riboflavin derivatives such as B₂-But (1) and B₂-Nic (2), it is important to show whether these compounds are, when administered orally, incorporated through gastro-intestinal tract into blood stream of mammals without losing their intactness of the ester bonds at 2',3',4' and 5' position. To test this, B₂-Nic may be suitable because it is fairly soluble in water despite its considerable lipophilic nature, and is less susceptible to pancreatic lipase action than B₂-But (3). In the present paper, evidence for the gastro-intestinal absorption of B₂-Nic is briefly described.

Male rabbit weighing about 3 kg was administered orally with 500 mg per kg body weight of B₂-Nic which was dissolved in a fixed volume of 0.5 N HCl. As a control run, another rabbit was administered with the same volume of the solvent. At 2 hours after the oral administration, rabbits were anesthetized with urethane, and heparin was introduced into blood stream from their ear vein.

Then, 10 ml of blood was collected from the portal vein of each rabbit and mixed with 40 ml of a mixture of 1 N HCl-CH₃COONa buffer (pH 2) and methanol (3:1, v/v) with

FIG. 1 TLC pattern of the phenol extract of portal blood from rabbit administered orally with 500 mg of B₂-Nic per kg of body weight

A: The extract, B: riboflavin, C: B₂-Nic. TLC plates were prepared by coating a slurry of a mixture of silica gel and alumina (1:2, w/w) and by activating at 120° for 2 hours. As a developer, CHCl₃-methanol-acetic acid (80:20:1, v/v/v) was used.

¹ Following abbreviations were used: B₂-But, riboflavin 2',3',4',5'-tetra-butyrate; B₂-Nic, riboflavin 2',3',4',5'-tetranicotinate; TLC, thin layer chromatography.

² 大川 博, 小瀧 祥, 八木国夫.

³ bp, 60°

stirring. The resulting mixture was heated at 80° for 15 min, and the precipitate formed was removed by centrifugation. The supernatant was saturated with ammonium sulfate and flavin compounds in the solution were extracted with phenol and transferred to water by shaking with a mixture of ethyl ether and petroleum ether³ (1:2, v/v). Extracted material was separated on a TLC plate (silica gel-alumina, 1:2, w/w) using CHCl₃-methanol-acetic acid (80:20:1, v/v/v) as a developer. A typical TLC pattern of the extract from rabbit portal blood is shown in Fig. 1. There are obviously two distinct yellow fluorescent spots on the plate when exposed to mineral light in the dark place. The *R_f* values of these spots are clearly different from the *R_f* value of free riboflavin, but the *R_f* value of the most rapidly moving band is identical with that of authentic B₂-Nic. In addition, such components could not be detected in the extract of portal blood from the control rabbit. Hence, it is reasonable to consider that the component having larger *R_f* value is B₂-Nic, and the component of smaller *R_f* value may be its partial hydrolysate.

Although at present the quantitative evaluation is lacking, this simple result would be direct evidence for the incorporation of orally administered B₂-Nic into the blood

stream of animal body without losing the intactness of the ester bonds. Certainly, free riboflavin and nicotinic acid liberated from B₂-Nic in the gastro-intestinal tract should also be incorporated, but it would be extremely difficult to consider that they can recombine with each other to produce B₂-Nic after being incorporated into the animal body.

Kotaki *et al.* (4) have recently found that the increase of lipoperoxides in the liver of rat suffering from fatty liver is prevented when rats are fed on B₂-Nic containing diet, whereas no prevention was observed when rats are fed on riboflavin containing diet. The result would now be well-explained by the direct lipoperoxide-reducing action of the iso-alloxazine part of B₂-Nic which would reach the liver without losing its lipophilic and deposit-type nature.

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