Glucuronide formation in the active transport of amino acid and thyroxine analogues by rat intestine

It has recently been demonstrated that L-[^{131}I]MIT is actively transported *in vitro* from the mucosal to the serosal side of everted sacs of rat small intestine[1]. Subsequent experiments with ^{14}C-labeled amino acids have suggested that MIT is transported by a mechanism which is also responsible for the transport of the naturally occurring amino acids.

In the course of this investigation it has been found that the propionic and acetic acid analogues of thyroxine and triiodothyronine are likewise transferred from the mucosal to the serosal side of the small intestine against a concentration gradient. The active transport of one of these compounds, [^{131}I]TRIAC, has been studied extensively. All the TRIAC which accumulates on the serosal side of the intestinal wall is in the form of a conjugate. This conjugate is acid-labile, alkali-stable, and does not form a hydroxamate derivative when incubated with NH_2OH[2]. The conjugate is cleaved by incubation for 2 h at 37° with β-glucuronidase (250 units/ml); this breakdown can be prevented by the addition to the incubation medium of 1,4-saccharolactone, a specific inhibitor of β-glucuronidase[3]. The conjugate is not altered by incubation for 18 h with Taka-diastase, an enzyme which cleaves a sulphate conjugate[4]. A positive reaction is given by the naphthoresorcinol method for determining glucuronic acid[5]. This evidence suggests that the conjugate is an ethereal glucuronide linked through the phenolic hydroxyl of the TRIAC. This glucuronide of TRIAC is not itself actively transported by the rat intestine. Indeed, the mucosal membrane is apparently as impermeable to TRIAC glucuronide as it is to salicyl glucuronide[6]. This relative impermeability to the conjugate offers a reasonable basis for its accumulation on the serosal surface of the everted gut sac.

These findings with thyroxine analogues prompted a re-examination of the inner fluid from gut sacs incubated with MIT. It had been reported[1] that a portion of the radioactivity in the inner fluid from sacs incubated with MIT migrates in *n*-butanol–acetic acid with an R_F of less than 0.1 (*i.e.*, with an R_F less than that of either iodide or MIT). Subsequent experiments have demonstrated that this radioactive material is in a conjugate of MIT and that from 5–35 % of the MIT inside the sac may be recovered in this form. As with TRIAC, the conjugate of MIT is acid-labile and alkali-stable; it does not form a hydroxamate derivative with NH_2OH; it is broken down by β-glucuronidase and gives a positive reaction with naphthoresorcinol. It is concluded that the conjugate is a glucuronide, probably linked through the phenolic hydroxyl of MIT. As with the TRIAC glucuronide, the MIT glucuronide is not actively transported; indeed, the gut wall is also relatively impermeable to this conjugate.

The mechanisms responsible for the active transport of MIT and of TRIAC by everted gut sacs are apparently similar, if not identical. Both systems have the same pH optima, are stimulated by the same substrates, and inhibited by the same inhibitors. It is of particular interest that the transport of TRIAC is inhibited by amino acids; as can be seen in Fig. 1 for L-phenylalanine, this inhibition is competitive in nature. Studies of MIT transport have shown that the α-amino group is the one part

Abbreviations: MIT, monoiodotyrosine; TRIAC, triiodothyroacetic acid.

of the molecule which is absolutely essential for its transport[1]. These findings suggest that the active transport of MIT may be accomplished through the production of two glucuronides. Evidence for the formation of an ethereal glucuronide has been presented. It has not been possible to obtain direct evidence for the production of

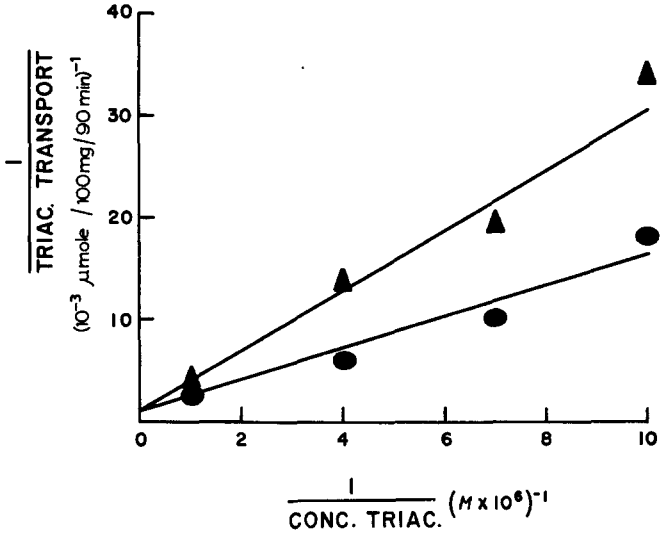

Fig. 1. Lineweaver–Burk plots of TRIAC transport in the presence and absence of inhibitor. ●, no inhibitor; ■, 0.04 *M* L-phenylalanine.

an N-glucuronide of MIT; however, all of the evidence suggests that such a conjugate is formed. Those N-glucuronides which have been previously described have been so labile that they could not be recovered by chromatographic techniques used in this study[7]. Such glucuronide formation would offer an attractive explanation for the active transport of MIT and other amino acids by rat intestine.

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Department of Medicine, Columbia University College of Physicians and Surgeons, New York, N.Y. (U.S.A.)

D. F. TAPLEY
R. HERZ, JR.*
J. E. ROSS
T. F. DEUEL
L. LEVENTER

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* Fellow of the National Foundation.

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