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Inhibition of intestinal amino acid transport by dimethyl sulfoxide*

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TREATMENT of the malabsorptive state which is due to intestinal lesions is at present unsatisfactory. We have therefore begun a series of studies to evaluate the effect of agents that might increase the intestinal absorption of nutrients. One of the compounds tested, dimethyl sulfoxide (DMSO), had previously been utilized both as a vehicle for other compounds and as an anti-inflammatory agent because of its solvent and penetrant abilities.[1, 2] It has been noted that DMSO has a low order of systemic toxicity, but that it produces local toxic actions at high concentrations.[3] We wish to report that one of these undesirable effects, at high concentrations of DMSO, is inhibition of active amino acid transport. This raises doubts whether large quantities of DMSO could be used as a solvent to improve nutrient entry from the intestine in cases of malabsorption.

Transport of 1×10^{-4} M L-alanine was followed by means of the generally labeled ^{14}C compound (New England Nuclear Corp.). Everted intestinal sacs were prepared from adult hamsters of both sexes, which were allowed food and water *ad libitum*,[4] The initial condition was the same concentration of L-alanine in both mucosal (5 ml) and serosal (1 ml) solutions. After gassing with 95% O_2 + 5% CO_2, flasks were stoppered and incubated for 1 hr at 37°. Mucosal and serosal fluids were drained, centrifuged to remove sloughed tissue, and then assayed for radioactivity. Transport was expressed in terms of micromoles L-alanine gained in the serosal fluid per unit wet weight of tissue. All values were compared with that of a control (three sacs from each of three hamsters, with Krebs-bicarbonate buffer, pH 7·4, as the solvent). Various volumes of DMSO were used to replace the buffer, to produce concentrations up to approximately 5 M. The pH of such solutions was adjusted to 7·4 with a radiometer pH meter (although, because of nonaqueous DMSO effects, the actual pH may have been fractionally off this value; therefore, experiments were also run with the pH adjusted to 0·3 unit above and 0·3 unit below this value). Experiments were additionally performed with the buffer plus DMSO in which salts were added to readjust their concentration to that found in the original Krebs-bicarbonate buffer. Results of the transport experiments in these cases were identical with those in which the salt content was not readjusted (and slight variations in pH had no effect). Transport of L-alanine (nine sacs per experiment) was 52 ± 10 mμmoles/1·0 g sac (mean ± S.D.) in the controls. There was no significant interference with transport at DMSO concentrations below 0·3 M.

Inhibition of L-alanine transport against a concentration gradient was minimal at a DMSO concentration of 0·3 M (12 per cent inhibition) but was complete above 1 M. Preliminary studies have indicated that the kinetics were not classically competitive (raising the amino acid concentration did not increase transport). High local concentrations of DMSO, therefore, are inhibitory *in vitro* to amino acid movement by the small intestine, and this must be taken into consideration if the compound is to be used to facilitate nutrient uptake from the small intestine in cases of malabsorption.

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Enzymic hydroxylation of 5-fluoropyrimidines by aldehyde oxidase and xanthine oxidase

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SYNTHESES of the potential bacteriostatic and cancer chemotherapeutic agents, 4-hydroxy-5-fluoropyrimidine and 2-hydroxy-5-fluoropyrimidine, have been reported recently, the former by Buděšínský *et al.*,[1] and the latter by Helgeland and Laland[2] and also by the Czechoslovakian workers.[3] Preliminary studies[3] suggest that the 4-hydroxy compound is converted *in vivo* in both mammalian and bacterial systems to 2,4-dihydroxy-5-fluoropyrimidine (5-fluorouracil). On the basis of the observations of Lorz and Hitchings,[4, 5] which showed that xanthine oxidase (xanthine: O_2 oxidoreductase, EC 1.2.3.2.) possesses the ability to convert 4-hydroxypyrimidine to uracil, and the report by Debov[6] that the same enzyme can convert 4-hydroxy-5-bromopyrimidine to 5-bromouracil, it was logical to expect that xanthine oxidase might possess the ability to oxidize the 5-fluoro compounds. The ability of mammalian aldehyde oxidase (aldehyde: O_2 oxidoreductase, EC 1.2.3.1) to oxidize the 5-fluoropyrimidines was also investigated, since this enzyme has been shown recently to possess the ability to oxidize a number of non-aldehydic heterocyclic substrates, including purines[7] and pteridines.[8]

2-Hydroxy-5-fluoro- and 4-hydroxy-5-fluoropyrimidine were kindly supplied by Dr. Z. Buděšínský of the Research Institute for Pharmacy and Biochemistry, Prague; milk xanthine oxidase was purchased from Worthington Biochemical Corp.; aldehyde oxidase of rabbit liver was purified by the method of Rajagopalan *et al.*[9] The aldehyde oxidase preparation was free from xanthine oxidase activity, as indicated by its inability to convert hypoxanthine to uric acid (assessed by the method of Kalckar[10]).

With 2,6-dichlorophenolindophenol as an electron acceptor, 2-hydroxy-5-fluoropyrimidine did not serve as a substrate for xanthine oxidase; aldehyde oxidase, on the other hand, with either dichlorophenolindophenol or oxygen as an electron acceptor, catalyzed the oxidation of this compound. In Fig. 1, the aerobic conversion of 2-hydroxy-5-fluoropyrimidine to 5-fluorouracil by aldehyde oxidase is demonstrated spectrophotometrically. The reference cuvette contained all the constitutents of the reaction mixture except substrate; experimental details are given in the legend for the figure. This