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

marked difference between the ultraviolet absorption spectrum of 2-hydroxy-5-fluoropyrimidine and that of 5-fluorouracil permitted the use of a direct spectrophotometric assay for the measurement of reaction rate. Details of the direct assay are given in the legend for Fig. 2A; the molecular extinction coefficient at 310 mμ was accepted as 4,400, as reported by Helgeland and Laland.[2] From the rate of decrease in absorbance at 310 mμ, the K_m for the oxidation of 2-hydroxy-5-fluoropyrimidine by aldehyde oxidase was found by the Lineweaver-Burk method to be $3{\cdot}4 \times 10^{-4}$ M. The reaction was completely inhibited by menadione (5×10^{-6} M), a specific inhibitor of aldehyde oxidase.[9]

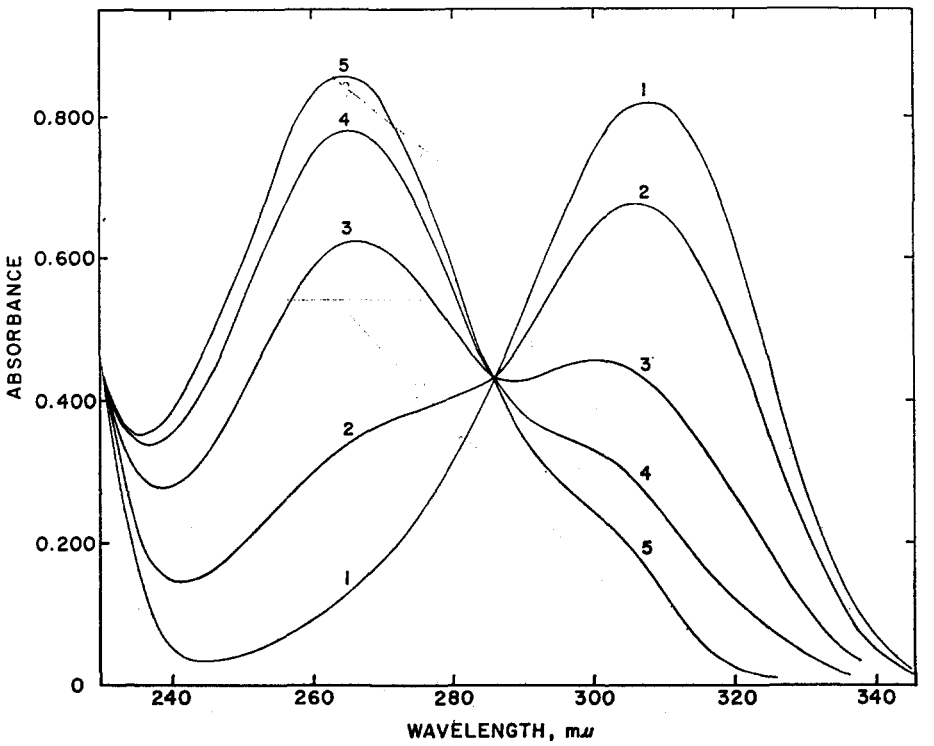

FIG. 1. Spectrophotometric demonstration of the enzymic conversion of 2-hydroxy-5-fluoropyrimidine to 2,4-dihydroxy-5-fluoropyrimidine (5-fluorouracil). The reference cuvette contained partially purified aldehyde oxidase, 200 μg; sodium phosphate buffer, pH 7·8, 500 μmoles; Versene Fe-3, 20 μg; and ammonium chloride, 600 μmoles, in a total volume of 3 ml. The sample cuvette contained the same constituents, and, in addition, 0·54 μmole of 2-hydroxy-5-fluoropyrimidine, in a total volume of 3 ml. Ultraviolet absorption spectra were recorded at 23° at 0 min (1), 20 min (2), 40 min (3), 60 min (4), and 80 min (5).

In contrast to 2-hydroxy-5-fluoropyrimidine, the corresponding 4-hydroxy compound is a very poor substrate for aldehyde oxidase. When assayed with dichlorophenolindophenol as electron acceptor, only a trace of oxidation was detected; on the other hand, it was an extremely good substrate for xanthine oxidase. Because of the similarity between the ultraviolet absorption spectra of 4-hydroxy-5-fluoropyrimidine and 5-fluorouracil, a direct spectrophotometric assay was not practical, and the rate of reaction was followed by measuring the rate of bleaching of dichlorophenolindophenol at 600 mμ. Details of the reaction mixture are given in the legend for Fig. 2B; the K_m under these conditions is $3{\cdot}8 \times 10^{-4}$ M.

The rates of oxidation of the two fluoropyrimidines were compared with the rates of oxidation of substrates for which more information is available, hypoxanthine in the case of xanthine oxidase,

and N-methylnicotinamide in the case of aldehyde oxidase. With dichlorophenolindophenol as an electron acceptor, 2-hydroxy-5-fluoropyrimidine was oxidized by aldehyde oxidase at 56% of the rate of oxidation of N-methylnicotinamide, while 4-hydroxy-5-fluoropyrimidine was oxidized by xanthine oxidase at 18 per cent of the rate of oxidation of hypoxanthine. Neither enzyme utilized 5-fluorouracil as a substrate.

The results permit the suggestion that xanthine oxidase may be responsible for the conversion of 4-hydroxy-5-fluoropyrimidine to 5-fluorouracil that has been observed in human subjects by the Czechoslovakian workers.[3] The situation is less clear-cut in the case of 2-hydroxy-5-fluoropyrimidine; preliminary studies indicated that, in man, metabolic alteration of this compound does not take

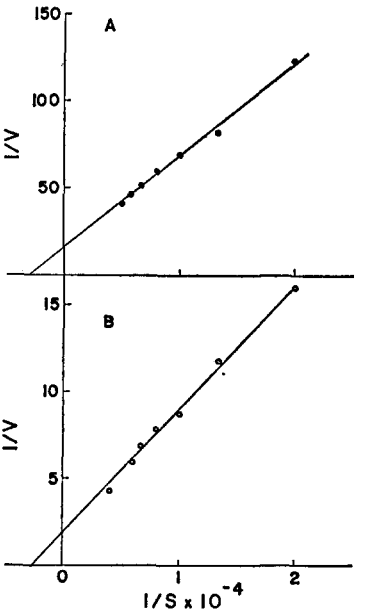

FIG. 2. A. Reciprocal plot of the enzymic oxidation of 2-hydroxy-5-fluoropyrimidine by the aldehyde oxidase of rabbit liver. Reaction rates were determined from the decrease in absorption at 310 mμ, as described in the text. Sample cuvettes contained partially purified aldehyde oxidase, 500 μg; sodium phosphate buffer, pH 7·8, 80 μmoles; Versene Fe-3, 3 μg; ammonium chloride, 400 μmoles; and substrate as indicated; total volume, 1 ml (v = μmoles 2-hydroxy-5-fluoropyrimidine oxidized/min/ml).

B. Reciprocal plot of the enzymic conversion of 4-hydroxy-5-fluoropyrimidine to 2,4-dihydroxy-5-fluoropyrimidine (5-fluorouracil), with dichlorophenolindophenol as an electron acceptor. Reaction rates were determined by measuring the decrease in absorption of dichlorophenolindophenol at 600 mμ. Each cuvette contained xanthine oxidase, 210 μg; sodium phosphate buffer, pH 7·8, 80 μmoles; Versene Fe-3, 3 μg; dichlorophenolindophenol, 30 μg; and substrate as indicated; final volume, 1 ml (v = $\triangle$ O.D$_{600m\mu}$/min).

place. As Knox[11] pointed out some years ago, aldehyde oxidase from rabbit liver, while almost identical with the aldehyde oxidase of other species in its ability to utilize aliphatic and aromatic aldehydes as substrates, differs significantly from this enzyme in other species in its much greater ability to utilize non-aldehydic heterocyclic compounds as substrates. The reason for this species difference and, indeed, the physiological function of aldehyde oxidases in general, remain unknown.

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Variation in gastric histamine levels and effects of histidine decarboxylase inhibition in rats from different sources

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IN AN earlier communication[1] it was reported that both 4-bromo-3-hydroxybenzyloxyamine (NSD-1055) and a hydrazino analog of histidine (MK-785) were potent inhibitors of specific histidine decarboxylase *in vitro*. Parenteral administration to rats of either of these substances was followed by rapid depletion of a rapidly turning-over pool of histamine in gastric mucosa.

Subsequently, in a different laboratory, the author has continued to investigate the effects of these drugs on histamine metabolism. Preliminary experiments failed to confirm the results of the previous report; neither NSD-1055 nor MK-785 produced any change in histamine levels in stomach or any other tissue studied. Experiments designed to assess possible influences of conditions of animal housing, diet, environmental temperature, seasonal variation, methods of preparing and administering the drugs, and methods of processing tissue for histamine assay failed to yield an explanation for the unexpected results. The last factor to be considered was the source of the experimental animals. In rats obtained from each of two different breeders it was possible to demonstrate that, as reported previously, administration of NSD-1055 was followed by rapid depletion of histamine levels in gastric tissue.

It is generally known that animals of different species differ importantly in several aspects of metabolism and in their responses to various drugs. Furthermore, different strains within the same species may have metabolically or physiologically unique properties. The purpose of this communication is to emphasize that closely related animals within a species may differ greatly and that this may introduce an important, and sometimes overlooked, source of experimental variation.

The animals used in the studies reported in this paper were female rats weighing 150 to 200 g; all were derived originally from Sprague-Dawley stock. They were obtained from the following sources: "Sprague-Dawley" rats from Blue Spruce Farms, Inc., Altamont, N.Y. (BSF); "CFE" rats from