Intestinal transport of azaserine and DON*

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THE antitumor agents azaserine (O-diazoacetyl-L-serine) and DON (6-diazo-5-oxo-L-norleucine)† are glutamine analogues, and hence might be expected to behave like amino acids in selected biologic systems. We wish to report that this is true for their transport by the hamster small intestine *in vitro*, and DON appears to be one of the more avidly transported amino acids. Despite their transport against a concentration gradient by the intestine, azaserine and DON were not effective inhibitors of the transport of an imino acid (L-proline) or an amino acid (L-glutamine); indeed, DON had a slight stimulatory effect on L-glutamine transport.

Everted sacs were prepared from the small intestine of adult golden hamsters (three per animal).[1] They were filled with 1 ml of the compound to be studied, dissolved in Krebs–Henseleit buffer, pH 7·4 (prepared without calcium or magnesium); the outer fluid was 5 ml of the same composition. Controls were sacs that had buffer alone both inside and out. Sacs were placed in stoppered flasks, gassed with 95% O_2 + 5% CO_2, and incubated at 37° for 1 hr in an oscillating water bath. Serosal fluid was drained from the sacs, and serosal and mucosal fluids were centrifuged to remove sloughed tissue. DON was assayed by absorbance at 275 mμ and azaserine by absorbance at 252 mμ (both corrected for controls). Both compounds were transported against concentration gradients. At an initial concentration of 3 μmoles of DON (65% L-isomer)/ml, the average 400-mg gut sac gained over 4 μmoles in the serosal fluid. For a 1-μmole initial concentration of azaserine (65% L-isomer)/ml, the sacs gained an average of 0·6 μmole in the serosal fluid.

The transport of 1×10^{-3} M L-proline (3,4-^{3}H, New England Nuclear Corp.), and 1×10^{-3} M L-glutamine (uniformly labeled with ^{14}C, Schwarz Bio-Research Corp.), was studied in separate experiments in both the absence and presence of 10×10^{-3} M azaserine or DON. The transport of L-proline (1·2 μmoles gain/sec) was not inhibited by either azaserine or DON. Serosal gain of L-glutamine (0·4 μmole gain/sac) also was not inhibited by either azaserine or DON. Indeed, in the transport experiments with L-glutamine, in the presence of DON, the mean serosal gain of L-glutamine (0·7 μmole) was greater than in untreated controls. The gut contains (at least in the rat) a glutaminase, which cleaves glutamine to glutamic acid.[2] It is known that glutamic acid is not transported by the everted gut sacs.[3] Whether DON is an inhibitor of intestinal glutaminase remains to be determined.

While azaserine and DON cannot be administered orally in unprotected form (because of degradation by gastric acidity), the possibility of introduction via intestinal tube remains. It is also known that azaserine and DON undergo concentrative uptake by tissues other than the intestine.[4] Azaserine has, interestingly, been reported to increase L-tryptophan transport in Ehrlich ascites cells.[5]

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REFERENCES

1. T. H. WILSON and G. WISEMAN, *J. Physiol., Lond.* **123**, 116 (1954).
2. R. P. SPENCER and N. ZAMCHECK, *Gastroenterology* 40, **423** (1961).
3. E. C. C. LIN, H. HAGIHIRA and T. H. WILSON, *Amer. J. Physiol.* **202**, 919 (1962).
4. J. A. JACQUEZ and D. J. HUTCHISON, *Cancer Res.* **19**, 397 (1959).
5. J. A. JACQUEZ, *Amer. J. Physiol.* **200**, 1063 (1961).