Possible Usefulness of Substituted Amino Acids for Tumor Growth Inhibition.*† (18040)

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The avidity of transplanted sarcoma in rats for labelled tyrosine(1) and rat hepatoma for labelled alanine and glycine(2) suggested the possibility that amino acids containing a toxic substituent might prove useful for the inhibition of tumor growth. Although N-acetylation of tryptophane does not prevent its utilization as a growth promoter(3). N-iodoacetyl derivatives of L-tryptophane, L-leucine and D,L-phenylalanine were considered suitable for an exploratory study because the iodoacetyl group, an inhibitor of certain respiratory enzymes, is a toxic residue. Accordingly, these derivatives were prepared from corresponding chloroacetyl derivatives by treatment with sodium iodide in acetone(4). For preparation of the radioactive analogues NaI^{131} was substituted for sodium iodide(4). For assessment of their distribution in tissues, their general toxicity and their specific toxicity for tumor tissue, control observations with iodoacetamide were necessary. Radioactive iodoacetamide was similarly prepared from chloroacetamide(2).

The distribution of radioactivity in tumor, liver and blood of Swiss mice bearing transplanted sarcoma 37 was determined at intervals after injection of the radio-active analogues of the three amino acid derivatives and of iodoacetamide. With the possible exception of radioactive iodoacetamide in tumor (Fig. 2, 3, 4) there were no consistently significant differences among the 4 compounds in the concentrations of radioactivity in liver, blood and tumor. Although the concentration of radioactivity in blood and tissues in the initial stages is not significantly different after administration of these 4 compounds than it is after the administration of NaI^{131}, the radioactivity from the latter disappears within 4 hours(5) while from the former small but significant amounts of radioactivity persist for considerably longer periods. This persistence may reasonably be explained by the assumption that at least a small fraction of radioactive iodoacetamide and iodoacetylamino acid derivatives had reached tissue cells.

Significant differences in systemic toxicity of the 4 compounds were observed (Fig. 1). At the LD_{50} on a molar basis, iodoacetamide was more toxic than iodoacetyltryptophane and about twice as toxic as iodoacetylleucine and iodoacetylphenylalanine.

The effect on the growth of the sarcoma 37 in Swiss mice was studied by tumor assay (6). The compounds were administered intraperitoneally in aqueous solution in ten daily doses in groups of from 15-25 mice bearing 3-day-old sarcoma 37. Control animals received saline in equivalent volume. On the 14th day, the tumors were excised and weighed. Two sets of experiments were performed. In the first experiment, in which the

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1. Winnick, T., Friedberg, F., and Greenberg, D. M., *J. Biol. Chem.*, 1948, v173, 189.

2. Zamecnik, P. C., Frantz, I. D., Loftfield, R. B., and Stephenson, M. D., *J. Biol. Chem.*, 1948, v175, 299.

3. Berg, C. P., Rose, W. C., and Marvel, C. S., *J. Biol. Chem.*, 1929-30, v85, 207.

4. Friedman, O. M., and Rutenburg, A. M., *J. Am. Chem. Soc.*, 1950, v72, 3285.

5. Seligman, A. M., Rutenburg, A. M., and Friedman, O. M., *J. Nat. Cancer Inst.*, 1949, v9, 261.

6. Seligman, A. M., Milden, M., and Friedman, O. M., *Cancer*, 1949, v2, 701.

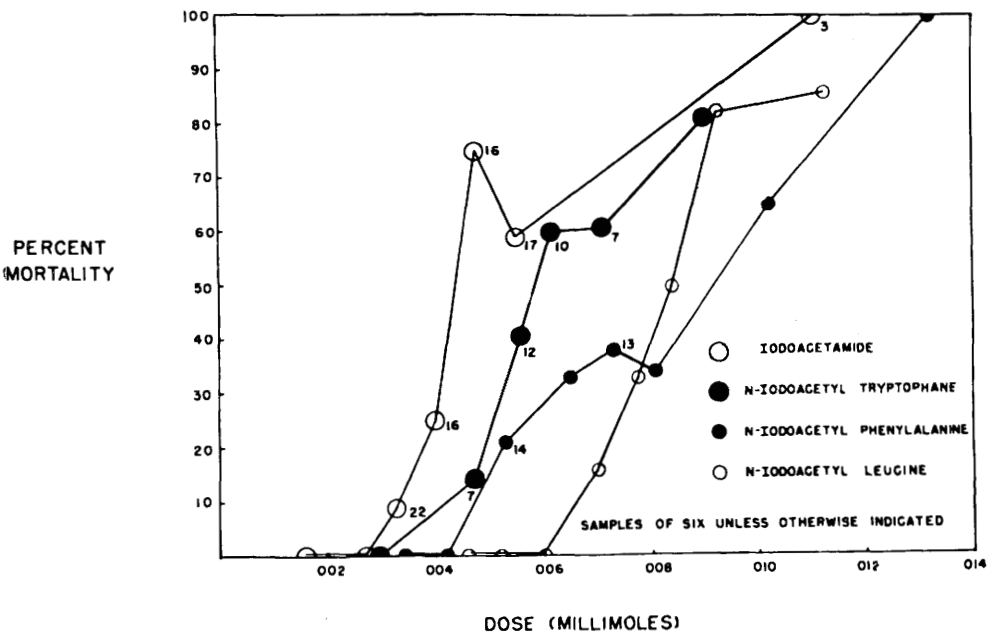

FIG. 1.
Toxicity in Swiss mice after intraperitoneal injection of compounds in saline solution. Bicarbonate used with amino acid derivatives. Per cent mortality based on 10-day survival.

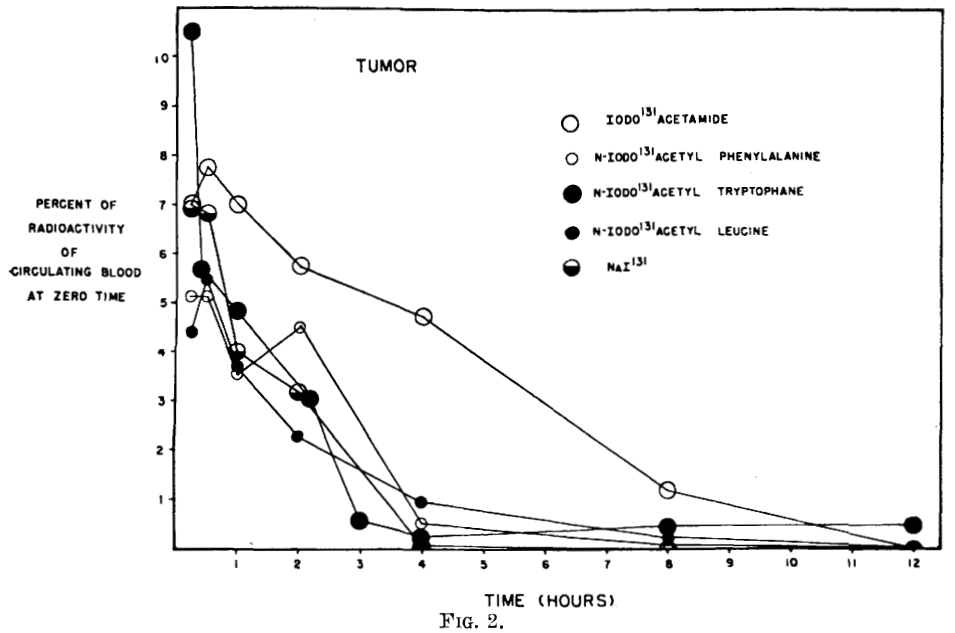

FIG. 2.
Sarcoma 37 in Swiss mice. Compounds administered intravenously in saline solution, bicarbonate being used with amino acid derivatives. Curves represent the average of 2 experiments.

average tumor weight of the untreated mice was 0.88 g the inhibition ratios (*i.e.* the ratio of the mean tumor weight of the untreated to that of the treated mice) was 1.6 for iodoacetylphenylalanine, 1.4 for iodoacetamide, 1.3 for iodoacetylleucine and 1.1 for iodoacetyltryptophane. Even the highest of these ratios is not more than of borderline sig-

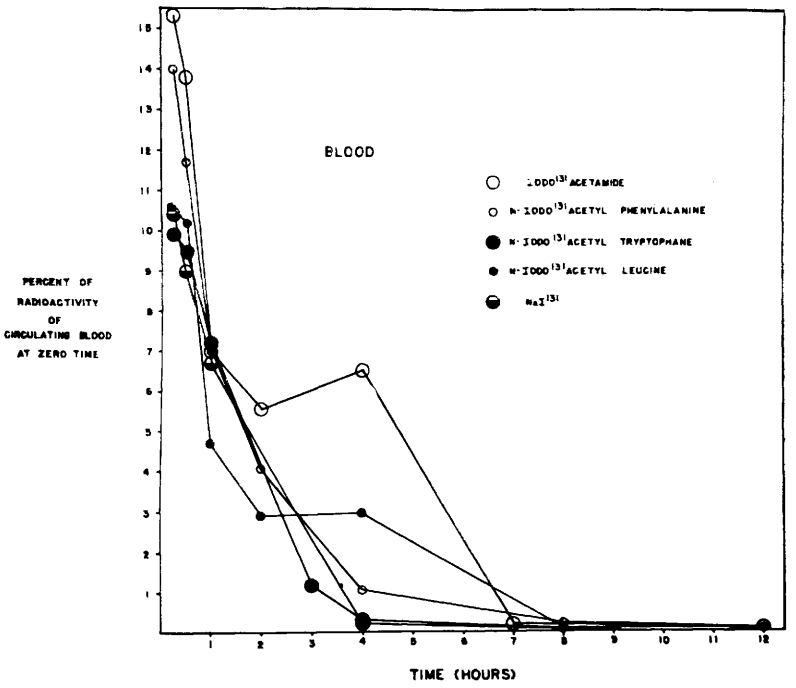

FIG. 3.
Swiss mice bearing sarcoma 37. Compounds administered intravenously in saline solution, bicarbonate being used with amino acid derivatives.

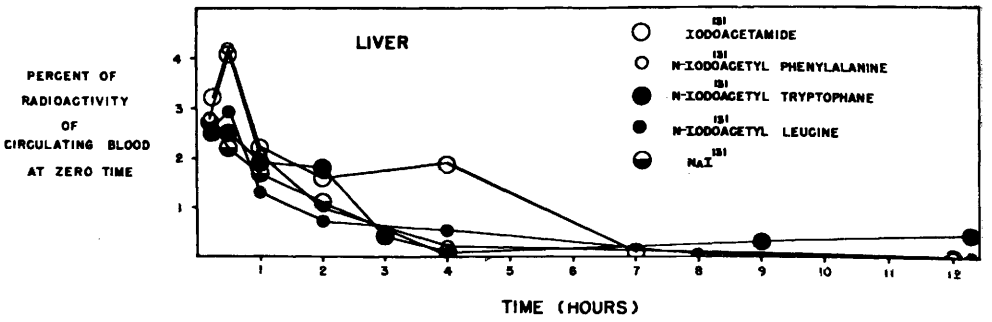

FIG. 4.
Swiss mice bearing sarcoma 37. Compounds administered intravenously in saline solution, bicarbonate being used with amino acid derivatives.

nificance. In the second experiment, however, the average tumor weight of the untreated mice was 1.75 g, the inhibition ratios were 5.2 for iodoacetylphenylalanine, 3.7 for iodoacetamide, 1.9 for iodoacetylleucine and 1.6 for iodoacetyltryptophane. The first two of these ratios are statistically significant. Although these results are not conclusive with regard to the use of these compounds therapeutically, because of limitations inherent in this type of bioassay(6), they do indicate that the tumor growth inhibitory effect of these compounds bears no quantitative relationship to their systemic toxicity. Possibly the nature of the particular derivative rather than the common iodoacetyl radical alone is responsible for whatever inhibitory action is produced. Further investigation of the phenylalanine derivative, therefore, might be justified.

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