it would be presumed that the antagonist was being metabolized in place of the missing amino acid. Growth was measured by titration of the acid formed in the culture.

An examination of the results in column 4 of Table 1 shows that under the test conditions used, NSC-1026 did not function as an antagonist of any of the 17 amino acids. In the case of *Lactobacillus arabinosus* 17-5 with L-glutamic acid, and *Leuconostoc mesenteroides* P-60 with glycine, there was evidence of some utilization of NSC-1026.

Ross[3] stated "the assumption could be made that NSC-1026 would function as an amino acid antagonist since it can be looked upon as an amino acid lacking the usual α-hydrogen atom". This hypothesis is not substantiated by the data presented in this report.

Acknowledgements—This work was aided in part by research grant CY-2479 from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service and, in part, by the Midwest Research Institute.

Division of Chemistry and Chemical Engineering,
Midwest Research Institute,
Kansas City, Missouri.

M. N. MICKELSON

REFERENCES

1. *Cancer Research*, **19**, p. 162, Part 2, Cancer Chemotherapy Screening Data (1959).
2. F. MARTEL and L. BERLINGUET, *Can. J. Biochem. Physiol.*, **37**, 433 (1959).
3. R. B. ROSS, *J. Chem. Ed.*, **36**, 368 (1959).
4. L. H. GOODSON, I. L. HONIGBERG, J. J. LEHMAN and W. H. BURTON, *J. Org. Chem.* In press.
5. M. N. MICKELSON and L. BARVICK, *J. Nat. Cancer Inst.*, **17**, 65 (1956).
6. J. L. STOKES and M. GUNNESS, *J. Biol. Chem.*, **157**, 650 (1945).
7. E. C. BARTON-WRIGHT, *The Microbiological Assay of the Vitamin B-complex and Amino acids* p. 138, Pitman Pub. Co., London (1952).
8. H. WILLIAMS, *Essential Amino Acid Composition of Animal Feedstuffs*, Cornell University Agricultural Experiment Station, Ithaca, N.Y., Memoir 337 (1955).
9. L. M. HENDERSON and E. E. SNELL, *J. Biol. Chem.*, **172**, 15 (1948).
10. L. R. HAC, E. E. SNELL, and R. J. WILLIAMS, *J. Biol. Chem.*, **159**, 273 (1945).

The inhibition of gamma aminobutyric-alpha-ketoglutaric acid transaminase *in vitro* and *in vivo* by amino-oxyacetic acid

(*Received* 15 *July* 1960)

THERE is abundant evidence (Roberts and Frankel, 1950; Awapara, *et al.*, 1950; and Roberts, 1956) that γ-aminobutyric acid (GABA) is found in high concentrations in the central nervous system. However, administration of GABA in large doses does not result in increased concentrations of this amino acid in brain, presumably because of its failure to cross the blood-brain barrier (Van Gelder and Elliot, 1958). Baxter and Roberts (1959) demonstrated that inhibition of γ-aminobutyric acid-α-ketoglutaric acid transaminase occurred in rats after administration of suitable doses of hydroxylamine, with concomitant increases of 100 per cent in brain GABA concentrations. This report is concerned with aminoöxyacetic acid (AOAA), a more effective inhibitor of this transaminase.

A transaminase preparation isolated from *E. coli* ATCC-26 was used for testing compounds as potential inhibitors using the following conditions: 40 μ moles of GABA, 40 μ moles of α-ketoglutaric acid (KGA), 50 μ moles of borate buffer, pH 8·2, enzyme sufficient to form from 3 to 4 μ moles of glutamate per hr, and water to 1·5 ml. Analysis for glutamic acid was carried out as described by Baxter and Roberts (1958). Under these conditions, AOAA inhibited the enzyme 100 per cent at a concentration of $3{\cdot}3 \times 10^{-4}$M and 40 per cent at $3{\cdot}3 \times 10^{-6}$M. Further studies with the bacterial preparation revealed that the inhibition was competitive for both substrates of the enzyme. When tested with a transaminase preparation from brain which, in contrast to the bacterial enzyme, requires supplementation with pyridoxal phosphate (Baxter and Roberts, 1958), a 92 per cent inhibition was observed at 1×10^{-5}M.

Jakoby and Scott (1959) described a preparation of adaptive enzymes from *Pseudomonas fluorescens* ATCC-13, 430 grown on a medium rich in pyrollidine which, in addition to GABA transminase, also has succinic semialdehyde dehydrogenase. This partially purified preparation could be used to assay either GABA or KGA, spectrophotometrically, by measurement of TPNH. An analogous system was found in *E. coli* ATCC-26 grown in nutrient broth (Difco) without special addition of substrates. An isolation procedure was developed for these enzymes which results in a preparation containing from 60 to 70 per cent of the initial activity of sonicates with a 1·5- to 2-fold purification. This preparation was used for all determinations of GABA in brain, as follows: to a Beckmàn DU cuvette were added 5·0 μ moles of 2-mercapto-ethanol, 6·0 μ moles of α-ketoglutaric acid, 600 μ moles of Tris buffer, pH 8·35, 1·0 mg of TPN, from 250 to 300 units of enzyme, and water to a final volume of 3·0 ml. An enzyme unit is defined as that amount of enzyme which will induce a change of 0·001 per min in the optical density, when assayed with 6·0 μ moles of GABA, in addition to the above components.

The rate of reaction was measured at 1-min intervals for 5 min at 340 m μ. The reading taken at zero-time was subtracted from the reading at 5 min in order to obtain the extent of TPNH formation. Standard curves in the range of from 0·1 to 0·6 μ moles of GABA were linear and the error on known standards was less than 3·0 per cent. Brain samples were prepared for analysis in a manner similar to that described by Roberts and Frankel (1950). The frozen brains were blended in 10 volumes of ethanol–water (75 : 25), and an aliquot of the supernatant fluid from this homogenate was dried *in vacuo* and made up in 0·1 volume of the aqueous ethanol for assay. Of the concentrated extract, 0·05 ml usually were taken for analysis.

By this means it was demonstrated that AOAA, administered in appropriate dosage to rats, mice, cats, dogs, and guinea pigs, elevated brain GABA levels 4- to 5-fold. The peak levels of GABA occurred 6 hr after AOAA administration. Thereafter, the GABA levels declined, but in most species above-normal levels were observed even 24 hr after administration of the inhibitor. As the GABA levels increased after AOAA administration, definite depression of the central nervous system ensued. This effect is under further study. The GABA levels were confirmed by paper chromatography.

DONALD P. WALLACH

Department of Pharmacology
The Upjohn Company
Kalamazoo, Mich.

REFERENCES

1. J. AWAPARA, A. J. LANDUA, R. FUERST and B. SEALE, *J. Biol. Chem.* **187**, 35 (1950).
2. C. F. BAXTER and E. ROBERTS, *J. Biol. Chem.* **233**, 1135 (1958).
3. C. F. BAXTER and E. ROBERTS, *Proc. Soc. Exp. Biol. Med.* **101**, 811 (1959).
4. W. B. JAKOBY and E. M. SCOTT, *J. Biol. Chem.* **235**, 937 (1959).
5. E. ROBERTS and S. FRANKEL, *J. Biol. Chem.* **187**, 55 (1950).
6. E. ROBERTS, in KOREY, S. R. and NURNBERGER, J. I. (editors), *Neurochemistry*, Vol. I. p. 11, Paul B. Hoeber, Inc., New York, (1956).
7. N. M. VAN GELDER and K. A. C. ELLIOT, *J. Neurochem.* **3**, 139 (1958).

Potentiation of carcinostasis by combinations of thioguanine and 6-mercaptopurine*

(*Received* 22 *August* 1960)

THE carcinostatic purine analogs 6-mercaptopurine and thioguanine are closely related in structure, and were at first thought to have similar mechanisms of action.[1] However, it has been shown by Greenlees and LePage[2] and Sartorelli *et al.*[3] that these drugs differ in their effects on purine metabolism in ascites tumors. In addition, their toxic effects are manifested in different tissues.[4] This report presents further evidence of the differences between 6-mercaptopurine and thioguanine by showing that combinations of these drugs produce a potentiation of the carcinostatic action. Furthermore, a tumor subline developed for resistance to thioguanine is only partially cross-resistant to 6-mercaptopurine.

* This work was supported by Contract No. SA-43-ph-3068 with the Cancer Chemotherapy National Service Center, National Institutes of Health, and by grants from the U.S. Public Health Service (CY-4551) and the Gustavus and Louise Pfeiffer Research Foundation.