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 $\mu$ moles of 2-mercapto-ethanol, 6·0 $\mu$ moles of $\alpha$-ketoglutaric acid, 600 $\mu$ 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 $\mu$ 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 $\mu$. 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 $\mu$ 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.

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

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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.

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Female Swiss mice, 25–30 g, were inoculated with $2 \times 10^6$ cells of the Ehrlich ascites carcinoma or a thioguanine-resistant subline of this tumor which has been described previously.[3] The drugs were administered intraperitoneally. Therapy was initiated 24 hr after tumor implantation and daily injections were continued for six days. All data are averages of results from two experiments.

The results of treatment of Ehrlich ascites carcinoma with eight combinations of thioguanine and 6-mercaptopurine are presented in Table 1A. Several combinations prolonged the average survival time markedly beyond that produced by any dose of either drug alone. These combinations also resulted in mice surviving 50 days; almost all of the surviving animals were tumor-free at that time. The thioguanine-resistant subline of the Ehrlich ascites carcinoma showed some cross-resistance to 6-mercaptopurine, but this resistance was not as complete as that to thioguanine (Table 1B). Treatment with combinations of thioguanine and 6-mercaptopurine, which showed potentiation in the parent line, here showed some increased toxicity.

TABLE 1. TREATMENT OF EHRLICH ASCITES TUMOR LINES WITH COMBINATIONS OF THIOGUANINE AND 6-MERCAPTOPURINE

| Dose (mg/kg) Thioguanine | 6-mercaptopurine | Average survival time (days) | 50-day survivors/ total no. of animals | Average weight change (g) |
|---|---|---|---|---|
| (A) Ehrlich ascites | | | | |
| 0 | 0 | 10·6 ± 2·0* | 0/15 | +1·5 |
| 0 | 30 | 18·8 ± 1·8 | 0/15 | +0·8 |
| 0 | 15 | 16·0 ± 2·5 | 0/15 | +3·0 |
| 0 | 7·5 | 12·6 ± 0·9 | 0/15 | +2·6 |
| 0 | 3·75 | 11·6 ± 3·0 | 0/15 | +1·4 |
| 1 | 0 | 20·4 ± 1·9 | 0/15 | +4·0 |
| 0·5 | 0 | 18·6 ± 2·6 | 0/15 | +2·2 |
| 1 | 30 | 30·8 ± 5·4 | 6/15 | −0·9 |
| 1 | 15 | 23·4 ± 3·0 | 3/15 | +2·4 |
| 1 | 7·5 | 13·4 ± 1·8 | 0/15 | +0·4 |
| 1 | 3·75 | 36·2 ± 6·0 | 9/15 | +10·0 |
| 0·5 | 30 | 24·0 ± 3·9 | 3/15 | +0·8 |
| 0·5 | 15 | 21·0 ± 4·5 | 3/15 | +1·4 |
| 0·5 | 7·5 | 24·0 ± 1·6 | 0/15 | +2·4 |
| 0·5 | 3·75 | 26·0 ± 5·1 | 3/15 | +2·8 |
| (B) Ehrlich-thioguanine resistant subline | | | | |
| 0 | 0 | 10·7 ± 0·9 | 0/10 | +2·97 |
| 0 | 30 | 14·3 ± 2·3 | 0/10 | +4·76 |
| 0 | 15 | 14·2 ± 1·9 | 0/10 | +5·7 |
| 1 | 0 | 8·1 ± 0·8 | 0/10 | +5·3 |
| 0·5 | 0 | 10·2 ± 1·0 | 0/10 | +4·2 |
| 1 | 30 | 8·1 ± 1·9 | 0/10 | −3·1 |
| 0·5 | 15 | 9·4 ± 2·0 | 0/10 | +4·0 |

* Average deviation from mean.

The mechanism of action of neither of these drugs is known. These biological data, however, support implications of other workers that their carcinostatic activity must involve different biochemical reactions.

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