5-Iodo-2′-deoxycytidine-^{3}H. A solution of tritiated 2′-deoxycytidine* in 50 per cent ethanol (2 ml, 2 mc, 1·82 μmole) was reduced to dryness at 40° and vacuum-dried at room temperature. To the dried residue were added non-radioactive 2′-deoxycytidine hydrochloride (2·23 mg, 8·46 μmoles) in sufficient amount to bring the specific activity of the final mixture to 2 mc/10.28 μmoles (194 μc/μmole), dilute hydrochloric acid (0·1 ml, 0·02 N), a solution of iodic acid in water (0·06 ml, 45 mg/ml), and a solution of iodine in carbon tetrachloride (0·1 ml, 20 mg/ml). The mixture was agitated at 40° for 3 hr and taken up in water (1 ml). The carbon tetrachloride layer was separated and washed with a little water (0·2 ml). The aqueous layers were combined and adjusted to about pH 11 with sodium hydroxide (1 N), divided into two equal portions, and put on two 30 × 1-cm Dowex-1 formate columns. These were eluted with water (1 l.) which was agitated magnetically and into which was introduced dropwise, in a continuous manner, 0·025 N formic acid. The eluate was collected in 1-ml fractions; yield, 1366 μg, specific activity, 76 μc/μmole.

* Purchased from Schwarz Bio-Research, Inc., Mt. Vernon, New York.

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Studies on the GABA pathway–II. The lack of effect of pyridoxal phosphate on GABA-KGA transaminase inhibition induced by amino-oxyacetic acid

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IT WAS reported previously[1] that amino-oxyacetic acid (AOAA) is an effective inhibitor of γ-aminobutyric acid (GABA, α-ketoglutaric acid (KGA) transaminase in the brain in five species of animals, inducing elevated levels of γ-aminobutyric acid. Further, it was reported that with a GABA–KGA transaminase preparation of *E. coli*, the kinetics of inhibition indicated that AOAA is a strictly competitive inhibitor for both substrates of the enzyme. Since the brain enzyme is also inhibited by AOAA, it was assumed that this agent was also competitively inhibiting this enzyme in a similar manner.

In animals it was found that toxic doses of AOAA cause convulsions which terminate in a respiratory death.[1] These convulsions can be prevented by the administration of a variety of different compounds which have in common an aldehydic or ketonic group.[2] One such compound of the series studied was pyridoxal phosphate. In common with other transaminases, GABA–KGA transaminase requires pyridoxal phosphate,[3] so that the question arose whether high concentrations of pyridoxal

phosphate might be capable of reversing the inhibition of GABA–KGA transaminase induced by AOAA.

As observed by Wallach, AOAA at a concentration of $3{\cdot}3 \times 10^{-2}$M has no effect on the succinic semialdehyde dehydrogenase from *E. coli* ATCC-26.[1] Thus, the coupled system of GABA–KGA transaminase and succinic semialdehyde dehydrogenase from this organism was a valid test system for these studies, and was composed in the following manner. To a Beckman DU Cuvette was added 600 μmoles of Tris buffer pH 8·35, 5 μmoles of 2-mercaptoethanol, 6 μmoles of KGA, 1 mg of TPN and 250–300 units of the enzyme preparation.* Additions of AOAA and pyridoxal phosphate were made in the concentrations to be indicated. In all cases, the final volume in each cell was 3 ml. After addition of the indicated components, the contents of the cell were well mixed by inversion, the reaction was started by addition of GABA (with an immediate second mixing of the cell contents), and an initial reading at 340 mμ was taken. Readings were made at 1-min intervals for 5 min. The initial reading was then subtracted from the reading at 5 min in order to determine the extent of TPNH formation.

As a control, the system was run at three concentrations of GABA, as shown in Fig. 1, Curve A. When AOAA was added at a final concentration of $6{\cdot}6 \times 10^{-6}$M, a 50 per cent inhibition of the reac-

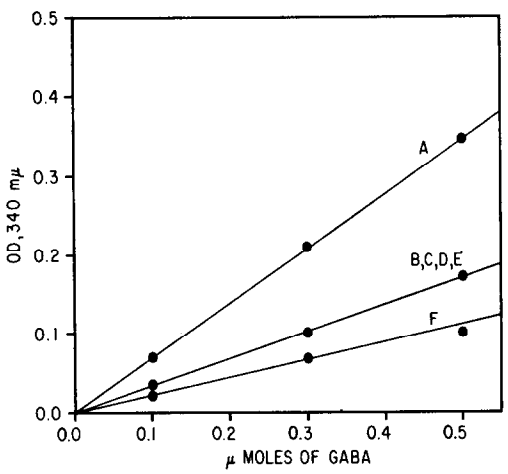

FIG. 1. The contents of each Beckman cuvette were as follows: 600 μmoles of Tris buffer, pH 8·35, 5 μmoles of 2-mercaptoethanol, 6 μmoles of KGA, 1 mg of TPN, 250–300 units of the coupled enzyme system from *E. coli*, and water to a final volume of 3 ml. Additions of AOAA and pyridoxal phosphate and GABA were as indicated in the text. The reaction was run at room temperature.

tion rate was observed, as shown in Fig. 1, Curve B; pyridoxal phosphate was then added to the cuvettes to produce final concentrations of $6{\cdot}6 \times 10^{-6}$M, $6{\cdot}6 \times 10^{-5}$M and $6{\cdot}6 \times 10^{-4}$M. Thus, there was present a 1-, 10- or 100-fold higher concentration of pyridoxal phosphate, as compared to the fixed concentration of inhibitor. The curves shown in Fig. 1, C, D, and E, were identical with Curve B, a circumstance which shows that at these concentrations there is no reversal by the cofactor of the AOAA-induced, inhibition of the enzyme. When the concentration of pyridoxal phosphate was increased to $6{\cdot}6 \times 10^{-3}$M, the enzyme was markedly inhibited, as shown in Curve F of Fig. 1. This concentration of the cofactor had no effect on succinic semialdehyde dehydrogenase. [This inhibitory effect of high concentrations of pyridoxal phosphate on GABA-KGA transaminase has also been

* A unit of the coupled enzyme preparation is defined as that amount of enzyme which will induce an OD change of 0·001 per min under the specified conditions of assay above and with the addition of 6 μmoles of GABA.

observed by C. F. Baxter (Private communication)]. Thus, no concentration of pyridoxal phosphate in the range employed reversed the inhibition induced by AOAA. These results are fully in agreement with the previously reported observation that the mechanism of the inhibition by AOAA of GABA–KGA transaminase from *E. coli* was of the strictly competitive type for both substrates of the enzyme. It was also definitive proof that under the conditions of the assay there was no significant degree of complexing between AOAA and pyridoxal phosphate, since a decreasing degree of inhibition would have been expected with increasing concentrations of the cofactor.

While it was most unlikely that AOAA, an inhibitor of both the bacterial and mammalian enzymes, could inhibit the two enzymes by different mechanisms, this point was checked, as is shown by the experiment of Table 1. It is evident not only that pyridoxal phosphate does not reverse the inhibition,

TABLE 1. THE NONREVERSAL OF GABA–KGA TRANSAMINASE DERIVED FROM BRAIN BY PYRIDOXAL PHOSPHATE (PY. PHOS.)

Tube contents	Complete system*	Complete system + 1×10^{-4}M (Py. Phos.)	Complete system + 1×10^{-3}M (Py. Phos.)	Complete system + 1×10^{-5}M U-7524	Complete system + 1×10^{-4}M (Py. Phos.) 1×10^{-5}M U-7524	Complete system + 1×10^{-3}M (Py. Phos.) 1×10^{-5}M U-7524
Concentration of Py. Phos. over U-7524	—	—	—	1 x	10 x	100 x
Percentage synthesis	100	110†	94‡	8	0	0
Reversal	—	—	—	—	0	0

* Complete system contains:
40 μmoles GABA
40 μmoles KGA
0·15 μmoles Py. Phos. (needed to supplement brain enzyme)
100 μmoles Borate pH 8·2
Enzyme from cat brain

† Note stimulation of system by pyridoxal phosphate at this concentration.

‡ Note depression of synthesis at this concentration of pyridoxal phosphate.

but also that excessive concentrations of the cofactor are of themselves somewhat inhibitory. This same effect has been reported previously for the mammalian transaminase by Baxter and Roberts.[3] The mechanism of the inhibition by pyridoxal phosphate has not been explained.

DISCUSSION

It seems reasonable to suggest that AOAA is a competitive inhibitor of GABA–KGA transaminase in brain. Although the mechanism of the convulsions induced by excessive doses of AOAA remains obscure, the fact that several compounds encompassing an aldehydic or ketonic function do reverse these convulsions points to either one of two possibilities. It is well known that AOAA complexes with carbonyl compounds (including pyridoxal phosphate at higher concentrations than those employed here) and at this time, a simple complex formation between this type of compound and AOAA cannot be ruled out. If the brain levels of one or more critical carbonyl compounds were drastically reduced by complex formation with AOAA, convulsions might well ensue. On the other hand, when excessive doses of AOAA are given to an animal, the GABA–KGA transaminase in the brain could be inhibited completely for a period of several hours. This should result not only in increased concentrations of GABA (and in fact this has been observed), but also a very marked depletion in the brain of the succinic semialdehyde which arises from the transamination of GABA. Since GABA and succinic semialdehyde are the two compounds which are unique to the GABA pathway, it is possible that there may be a reciprocal relationship between these two compounds in brain which has hitherto been overlooked. Accordingly, it is suggested that succinic semialdehyde may play a physiological role and that the convulsions seen with large doses of AOAA may result

from a depletion of succinic semialdehyde. The role of this aldehyde in the brain may be as important as GABA and investigation of its function may prove fruitful.

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5-Bromo-2′-deoxycytidine (BCDR)–II. Studies with murine neoplastic cells in culture and *in vitro*

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IN CONJUNCTION with studies of the stability of 5-bromo-2′-deoxycytidine (BCDR) to metabolic degradative attack *in vivo*,[1] the presently reported work was undertaken to investigate the toxicity, metabolism and mechanism of action of BCDR with mammalian neoplastic cells, both in culture and under conditions of incubation *in vitro*.

Effect of BCDR on murine neoplastic mast cells grown in culture. The culture medium,[2] cell line (murine neoplastic mast cells, P815Y),[3] and techniques[4] have been described previously, with the exception that the Coulter Cell Counter was used for determinations of the number of cells.

BCDR in graded concentrations, 1–400 μM ,in the medium progressively inhibited the growth of the cells, although complete inhibition did not occur even with 400 μM. With that level, cell reproduction was limited to only two divisions; while with the same level of 5-iodo-2′-deoxyuridine (IUDR) or 5-bromo-2′-deoxyuridine (BUDR), growth was limited to but a single division. Following exposure of the cells to BCDR for three cell-generations and subsequent transfer to drug-free medium, a progressive decrease in cell viability occurred with increasing concentration of the agent.

Inhibition of growth of BCDR could be prevented partially by addition to the medium of either thymidine (TDR) or 2′-deoxycytidine (CDR); however, much higher levels of CDR than of TDR were required for comparable degrees of prevention of growth inhibition, a finding which supports the concept that BCDR and TDR are handled analogously by the cells. However, complete reversal by TDR could not be obtained, since levels greater than about 60 μM are toxic to the cells, a phenomenon which has been described previously and explained.[5]

Both BUDR and BCDR partially prevented the inhibition of cell growth caused by otherwise totally inhibitory levels of 5-fluoro-2′-deoxyuridine (FUDR), but neither compound was as effective as TDR.

The uptake and metabolism of ^{3}H-BUDR and ^{3}H-BCDR *in vitro* were compared using suspensions of cells, 5×10^7 per ml, incubated with tracer quantities of nucleosides of high specific activity. After periods of incubation of 5, 15 and 30 min, respectively, the tubes were chilled, carrier BUDR and BCDR were added, and the cells were sedimented by centrifugation. The surface of the packed cells and the walls of the tube were washed once with fresh medium to remove most of the residual compound; the cell-pack was then extracted three times with cold 5% trichloroacetic acid (TCA), followed by extraction with 5% TCA at 90°C for 30 min. After the TCA was removed from the extracts with ether and water was removed *in vacuo*, the residues derived from the extractions with cold TCA were chromatographed on Whatman no. 1 filter paper in butanol:water (344:56, v/v). No significant