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Note upon the behaviour of rat brain tissue treated with fluoroacetate *in vitro*

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THERE is evidence to suggest that brain tissue does not readily convert fluoroacetate to fluorocitrate. Subarachnoid injections of fluorocitrate into rats and pigeons induce convulsions, whereas larger doses of fluoroacetate are without effect.[1] In the exposed cortex of the cat, Professor Szerb (personal communication) found that exposure to fluoroacetate (1·0 mM) was without effect on the electrical activity, whereas fluorocitrate soon abolished this. *In vitro*, the brain tissue of pigeons has been used to test for fluorocitrate in the presence of fluoroacetate.[2] Recently however[3] it has been stated that *in vitro* fluoroacetate (1·0 mM) interferes with ammonia metabolism in rat brain; and the authors have raised the possibility that this is due to a synthesis of fluorocitrate. It seemed advisable therefore to

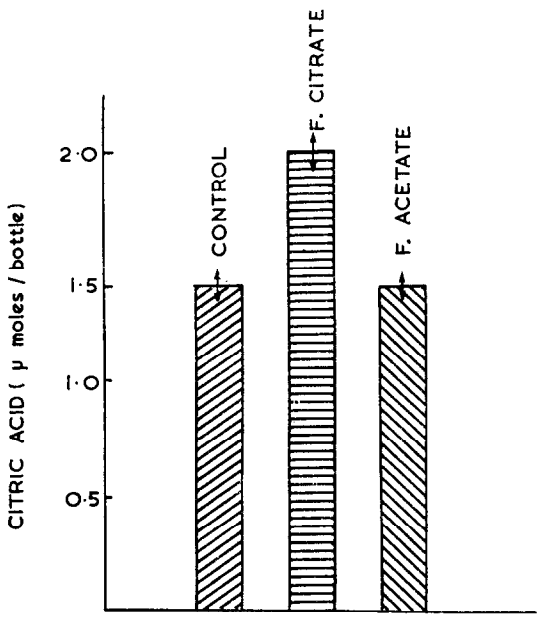

FIG. 1. Rat brain homogenates. Amount of citric acid present, μmoles/25 ml. Erlenmeyer flask, after shaking for 40 min in air at 37°. Average of duplicate estimations, indicated by the arrow.

The flasks contained 1·0 ml homogenate made with 0·25 M sucrose and containing 1/5 vol. M/2 phosphate (pH 7·4); Na pyruvate 0·1 ml (30 μmoles) Na fumarate 0·1 ml (9·4 μmoles), $MgCl_2$, $6H_2O$ 0·1 ml, (0·49 μmoles), Na-ATP 0·1 ml (4·8 μmoles), with additions of Na fluorocitrate 0·1 ml (50 μg) or Na fluoroacetate 0·1 ml (100 μg) together with 1·0% KCl to a total vol. of 3·0 ml. At the end of the experiment 25% trichloracetic acid (1·0 ml) was added to each flask, and after centrifuging the citric acid content was determined in the supernatant by Taylor's method.[4]

Note. A large excess of the synthetic (crude) Na fluorocitrate, not activated, was used in this experiment.[5] From other experiments we know that less than 10 μg fluorocitrate produces large increases in citric acid.

test whether mitochondrial homogenates of rat brain showed any block in citrate metabolism in presence of fluoroacetate. This would be expected if the rat homogenates synthesized fluorocitrate, as guinea pig tissue homogenates from kidney readily synthesize fluorocitrate *in vitro*.[2] The experiment reported here shows that no increase was observed in rat brain homogenate by addition of fluoroacetate, whereas 50 μg of a synthetic specimen of fluorocitrate gave the expected rise of citrate. We

could not therefore demonstrate an effect of fluoroacetate *in vitro*, and conclude that any synthesis of fluorocitrate must be very slight, if it occurs at all.

EXPERIMENTAL

Chemicals have been AnalaR where possible. Na pyruvate was a specimen recrystallized from a commercial sample (Light). The rat brain homogenates were made as soon after death as possible, the heads being placed in ice. The brains were homogenized in a hand homogenizer in 0·25 M sucrose pH 7·4 solution, a vol. of 3 ml being used for each brain. The homogenate was centrifuged at 600 *g* for 15 min, and the supernatant used direct.

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The effect of 3′-iodoaminopterin on dihydrofolate reductase*

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HALOGENATION of folic acid antagonists elicits significant changes in their biological effectiveness; thus, in mice, 3′, 5′-dichloromethotrexate has a greater antileukemic effect and is less toxic than the parent methotrexate.[1] The syntheses of 4-amino-4-deoxy-3′-iodopteroylglutamic acid (3′-iodoaminopterin) and 3′-iodopteroylglutamic acid (3′-iodofolic acid) have been described recently.[2, 3] The activity of 3′-iodoaminopterin against murine leukemia L1210 has been determined.[2] The compound was able to increase the survival time of the tumor-bearing mice to the same extent as aminopterin, although the optimal dose was about ten to twenty times greater than that of aminopterin. It thus became of interest to study the effect of 3′-iodoaminopterin on dihydrofolate reductase.

MATERIALS AND METHODS

3′-Iodoaminopterin was synthesized as previously described.[2, 3] Aminopterin, supplied by the Lederle Division of American Cyanamid Co., was purified as described previously.[4] The molarity of solutions of these compounds was determined spectrophotometrically according to published values of molecular extinction coefficients of chromatographically pure materials. A phosphate buffer extract

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