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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Department of Biochemistry,
Tennis Court Road,
Cambridge, England

R. A. PETERS
M. SHORTHOUSE

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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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of an acetone-dried powder of an antifolate-resistant subline (FR-8) of leukemia L1210 tumor was the enzyme source.[5] Dihydrofolate reductase assays were carried out as described elsewhere.[6, 7]

RESULTS AND DISCUSSION

The effect of 3′-iodoaminopterin and aminopterin on the activity of dihydrofolate reductase from leukemia L1210/FR-8 is shown in Fig. 1. The inhibition produced by equimolar concentrations of the two antagonists was found to be the same and was also consistent with the partly stoichiometric behavior expected in a "mutual depletion system".[8] Extrapolation of the straight-line portion of the titration curve to the abscissa yielded a value of $6{\cdot}5 \times 10^{-5}$ μmoles of inhibitor per unit of enzyme activity (μmoles of dihydrofolate reduced per hour), which is very close to the value reported earlier by Schrecker and Huennekens.[6]

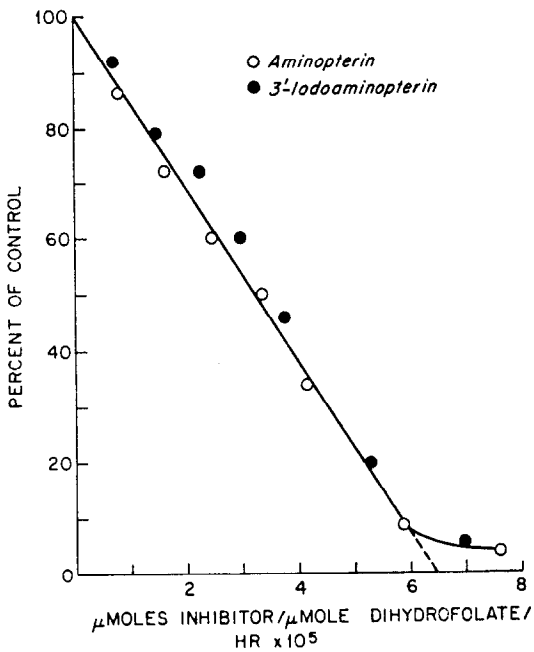

FIG. 1. Inhibition of L1210/FR-8 dihydrofolate reductase by variable amounts of aminopterin, ○—○, and 3′-iodoaminopterin, ●—●, at a constant enzyme level. An acetone-dried powder of the antifolate-resistant tumor was extracted with 0·05 M potassium phosphate buffer, pH 7·4, containing 0·01 M mercaptoethanol and 0·001 M EDTA. The extract (0·29 mg protein, sp. act. = 3·4 μmoles dihydrofolate reduced per hr/mg), inhibitor, and buffer mixture were preincubated 5 min at 37° before addition of reduced NADP and dihydrofolate. Enzyme activity (initial rate at 28°) is expressed as per cent of a control in which extract was preincubated in the absence of inhibitor.

On an equimolar basis 3′-iodoaminopterin, although considerably less toxic than the parent compound in mice, inhibited dihydrofolate reductase to the same extent as aminopterin. The introduction of the large halogen atom has apparently not altered the enzyme-inhibitory characteristics of aminopterin, reminiscent of the related observations[9] that aminopterin, methotrexate, and 3′,5′-dichloromethotrexate are equally effective as inhibitors of dihydrofolate reductase *in vitro*. The appreciable differences in the optimal therapeutic doses of the two compounds may indicate that there has been a reduction in the sensitivity of other enzyme systems to the halogenated derivative; however, permeability and drug metabolism may also be involved. In this instance it is apparent that halogenation has partially achieved a desirable aim. 3′-Iodoaminopterin is less toxic than aminopterin, and its

therapeutic effect is not reduced at optimal daily dose levels.[2] We have shown that the change in biological properties is not related to a difference in effect on the enzyme.

National Cancer Institute,
National Institutes of Health,
Bethesda, Md., and
Microbiological Associates, Inc.,
Bethesda, Md., U.S.A.

TI LI LOO*
J. A. R. MEAD
ANTHONY W. SCHRECKER
M. SHAFFER

* Present address: The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025.

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