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BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

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DATE: 22 June 1961

TO: Committee on the Use of Radioactive Isotopes in Patients

FROM: L. V. Hankes

SUBJECT:

TITLE: Metabolism of Tryptophan Metabolites: 3-Hydroxyanthranilic Acid and Anthranilic Acid.

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Tryptophan, an essential amino acid, has been the subject of a great deal of biochemical investigation. It is now recognized as the presursor of such diverse compounds as quinolinic acid, 3-hydroxyanthranilic acid, anthranilic acid and kynurenine. A number of intermediary metabolites of tryptophan have been isolated or identified in human urines. Tuberculosis patients studied by Musajo, Spada and Coppini (1) excreted 3-hydroxyanthranilic acid in significant amounts, whereas normal urine contained only traces. Musajo, Benassi and Panajola (2) isolated kynurenine and 3-hydroxykynurenine from the urines of pathological patients, but could not isolate the compounds from normal human urine.

Of current interest in the metabolism of tryptophan is the possibility that certain of its metabolites may cause bladder cancer in man. Reports from England indicate that 3-hydroxyanthranilic acid, 3-hydroxykynureine, 2-amino-3-hydroxyacetophenone and xanthurenic acid-5-methyl ether were weakly carcinogenic in the bladders of mice. Using different methods of analysis, Boyland and Williams reported, in ten bladder tumor patients, abnormal tryptophan metabolism consisting of elevated urinary levels of anthranilic acid, 3-hydroxyanthranilic acid, kynurenine and hydroxykynurenine (3). Analyses of the urine of bladder tumor patients in this country has shown that only half of 41 patients studied had abnormal quantities of certain tryptophan metabolites, chiefly kynurenine, acetylkynurenine, hydroxykynurenine and kynurenic acid. Studies on patients with other forms of cancer or with a variety of other non-neoplastic diseases, have indicated that many of these subjects had abnormal tryptophan metabolism and that there were a number of clinical conditions, including bladder cancer, in which disorders of tryptophan metabolism occurred (4). At the present time, the significance of tryptophan metabolism in neoplastic diseases is not clear. In animals various isotope experiments have indicated that very significant amounts of tryptophan, kynurenine, hydroxykynurenine and hydroxyanthranilic acid are metabolized to carbon dioxide (5,6,7,8) thru the breakdown of 3-hydroxyanthranilic acid (9). In animals labeled anthranilic acid is metabolized

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to CO_2 to a limited extent with the majority of the labeled material excreted in the urine. In rats 60-65% of 3-hydroxyanthranilic acid-carboxyl-C^{14} is excreted in $C^{14}O_2$ and 20-30 per cent in the urine in a 24-hr. period. The remainder of the activity is bound in enzymes and in the various retained metabolites. In rats one per cent of anthranilic acid-carboxyl-C^{14} is excreted in $C^{14}O_2$ and 95% in the urine in a 24-hr. period. The remainder of the activity is bound in enzymes and in the various retained metabolites.

The proposed study will determine the percentage conversion of labeled 3-hydroxyanthranilic acid or labeled anthranilic acid into $C^{14}O_2$ and 3-hydroxyanthranilic acid metabolites or anthranilic acid metabolites, as measured by urinary excretion. We also hope to determine the percentage incorporation of these labeled compounds into Coenzyme I. These mechanisms will be studied in all types of neoplastic disease patients. It is planned that a dose of 100 μc. of carboxyl-C^{14}-labeled 3-hydroxyanthranilic acid or carboxyl-labeled anthranilic acid will be given orally in a gelatin capsule. A simple formula for beta dosimetry, assuming uniform distribution in a volume large in relation to the range of beta particles (10) is

$d_\beta(\text{day}) = 51.2\ C\ \bar{E}_\beta$ rads where C = concentration of isotope in μc/gm.

$\bar{E}_\beta$ = average energy per distintegration in MEV

substituting

$$d_\beta = 51.2 \times \frac{100}{70{,}000} \times 0.05 = 0.0036 \text{ rads/day.}$$

This dose of 3.6 m rads/day or 1.32 rads/yr would be sustained if there were complete retention indefinitely, and is therefore a maximum estimate for whole-body dosage. The known metabolism of labeled 3-hydroxyanthranilic acid or anthranilic acid does not suggest any critical organ.

The patients will be given carboxyl-labeled 3-hydroxyanthranilic acid with a specific activity of 0.56 mc/mMole or carboxyl-labeled anthranilic acid with a specific activity of 0.2 mc/mMole. These compounds have been prepared in this laboratory and they have no radioactive components other than the C^{14}. No more than one gram of these compounds will be administered.

From the use of 100 μc. of C^{14} there will be no radiological hazard to attendant personnel. As part of the study the patients' breath will be collected for analysis during the first few hours and continuously evacuated. Blood and urine samples will be collected for analysis in the research laboratories. Any samples sent to the clinical labs on the first day will be marked "radioactive." No monitoring procedure, except in case of accident, and though no isotope accountability is necessary, experimental design will provide such accountability. Patients will be above 40 years of age or they will have a life span estimated to be less than two years. It will not be given to patients under 18 years of age.

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