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Memorandum

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To: Committee on Use of Radioactive Isotopes in Patients

From: L. V. Hankes

Title: Metabolism of Tryptophan Project No. H-69

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Tryptophan, a widely distributed naturally occurring essential amino acid, has been the subject of a great deal of biochemical investigation. It is converted to many other compounds of biological significance, some of which no longer retain the indole nucleus. This amino acid is now recognized as the precursor of such diverse compounds as quinolinic acid, nicotinic acid, xanthommatin, xanthurenic acid, serotonin, CoI, and many others. Many points concerning the biosynthesis of these biologically important compounds are still obscure. (1,2) Tryptophan balance data on growing animals and the distinct requirement for tryptophan for maintenance in the adult, indicates that a fair amount of carbon from this amino acid is disposed of through the lung and kidney each day. Only a small part of the injected indole nucleus is accounted for in the various excretion products of normal mammals. Tryptophan, like other essential amino acids, must be disposed of by a major route leading to aliphatic compounds and finally to CO_2. There is evidence that tryptophan is converted to niacin in man. Niacin deficiency in man has been specifically cured by niacin or tryptophan (3-5) and blood pyridinenucleotides were restored to normal levels by supplements of tryptophan after depletion on niacin-deficient diets (6). A number of intermediary metabolites of tryptophan have been isolated or identified in human urines. Makino and coworkers reported that 3-hydroxykynurenine was responsible for the positive diazo reaction frequently observed in severe tuberculosis (7). Musajo, Benassi and Panpajola (8) isolated kynurenine and 3-hydroxykynurenine from urine of patients with various pathological conditions but could not isolate these compounds from normal human urine. Tuberculosis patients studied by Musajo, Spada and Coppini (9) excreted 3-hydroxyanthranilic acid in significant amounts whereas normal urine contained only traces. Xanthurenic acid has been identified in urine from vitamin B_6-deficient subjects (10,11). It has been called an abnormal metabolite in human urine, however, Price and Dodge (12) were able to identify xanthurenic acid along with kynurenic acid and the 8-methyl ether of xanthurenic acid in normal human urine.

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Of current interest in the metabolism of tryptophan is the possibility that certain of its metabolites may cause bladder cancer in man. The chemical similarities between the known bladder carcinogens and tryptophan metabolites are apparent, in that several of the metabolites of tryptophan normally found in urine are aromatic amines and aminophenols. Reports from England indicate that 3-hydroxyanthranilic acid, 3-hydroxykynurenine, 2-amino-3-hydroxyacetophenone and xanthurenic acid 8-methyl ether were weakly carcinogenic in the bladders of mice. Analyses of the urine of bladder tumor patients has shown that only half of 41 patients studied had abnormal quantities of certain tryptophan metabolites, chiefly kynurenine, acetylkynurenine, hydroxykynurenine and kynurenic acid. No definite correlation could be made between the degree of abnormal tryptophan metabolism and type or number of tumors, recurrence, rate, age at onset or other clinical findings. Studies on patients with other forms of cancer or with a variety of other non-neoplastic diseases, 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 (14). At the present time the significance of tryptophan metabolism in neoplastic diseases is not clear. The quantity of ingested tryptophan which is metabolized through the kynurenine pathway in man has not been adequately studied. In animals various isotope experiments have indicated that very significant amounts of tryptophan, kynurenine and hydroxyanthranilic acid are metabolized to carbon dioxide (14,15,16) thru the breakdown of 3-hydroxyanthranilic acid (17). That a large amount of ingested tryptophan may also enter this pathway in man is suggested by quantitative studies of the urinary excretion of tryptophan metabolites by subjects who excrete elevated amounts of metabolites.

In rats 48 per cent of tryptophan-2-C^{14} is excreted in $C^{14}O_2$ and 12 per cent in the urine in a 24-hour period. The remainder of the activity is bound in proteins in the body organs, in enzymes, and in the various retained metabolites.

The proposed study will determine the percentage conversion of labeled tryptophan into $C^{14}O_2$, and tryptophan metabolites, as measured by urinary excretion, also the percentage incorporation of tryptophan and its metabolites into blood fractions and other body components, such as coenzyme I. These mechanisms will be studied in all types of neoplastic disease patients. It is planned that a dose of 50 μc. of carbon-14-labeled tryptophan-2-C^{14} or tryptophan-7a-C^{14} will be given orally in a gelatin capsule. However, in terminal patients it is possible that a 100 μc. dose may be advisable to obtain sufficient activity in the various fractions for measurement.

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A simple formula for beta dosimetry, assuming uniform distribution in a volume large in relation to the range of beta particles (18) is

d_β (day) = 51.2 C $\bar{E}_\beta$ rads where C = concentration of isotope in $\mu c/gm$

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

substituting,

$$d_\beta = 51.2 \times \frac{50}{70,000} \times 0.05 = 0.0018 \text{ rads/day.}$$

This dose of 1.8 m rads/day or 0.66 rads/year would be sustained if there were complete retention indefinitely and is therefore a maximum estimate for whole-body dosage. The known metabolism of labeled tryptophan does not suggest any critical organs except the liver, and even in this case the finding of less than 6% of the ingested dose in the whole liver after 24 hours does not indicate any organ to be more critical than the whole body.

The patients will be given tryptophan-2-C^{14} with a specific activity of 0.62 mc/mMole. This compound was obtained from Tracerlab and it has no radioactive components other than the C^{14}.

From the use of 50 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.

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