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BROOKHAVEN NATIONAL LABORATORY

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TO: Committee for the Use of Isotopes in Human Beings.

FROM: V. P. Bond, R. A. Conard, E. P. Cronkite, W. L. Hughes, J. R. Rubini, H. Quastler, J. S. Robertson

SUBJECT: A Study of the Metabolism of Tritium Labeled Thymidine and Tissue Uptake in Human Beings.

1. Isotope. Tritium has a physical half life of 12.4 years. It emits soft beta particles with a maximum energy of approximately 18 Kev. and an average energy of 5.7 Kev. No gamma radiation is emitted. Consequently it presents no external radiological hazard to personnel.

2. Compound. Tritium ~~labeled thymidine, as first prepared by Hughes~~, is now available commercially from Schwarz Laboratories, Inc. in sterile solution with a specific activity of several hundred millicuries per millimole.

3. Radiation Exposure. The very low kinetic energies of the beta particles restrict their effects to a short range around the tritium atom. Robertson and Hughes have calculated that 53% of the energy of tritium is delivered within a radius of 1 micron from the source and 82% within 2 microns. Moreover, the average dose to a sphere of 1 micron radius will be 11 rads/disintegration whereas the second micron shell will receive only 1 rad. Thymidine is only incorporated into DNA and as such will label chromosomes and nuclei. There are no data on tritiated thymidine in human beings. Hughes et al., have shown in mice that following an intravenous injection of tritiated thymidine the plasma activity of thymidine tritium approaches zero within a few minutes, and there is a concurrent appearance of tritium within cells. This cellular activity appears to be in thymidylic acid. After a few minutes there is a progressive fall in the tissue concentration

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of alcohol - soluble (thymidylic) activity which is accompanied by the formation of tritiated water. Tritium oxide formation reaches a maximum within an hour and accounts for slightly over half of the administered radioactivity. The remaining activity must then be incorporated into a non-metabolizing form - presumably DNA of the cell nuclei of proliferating tissues.

Using autoradiographic techniques in mice nuclear uptake of the labeled thymidine is evident in normoblasts and lymphocytes, 15 minutes after injection. At one hour we have shown that the generative compartment of the gastrointestinal tract is labeled. From the above it is seen that this compound offers a way to tag the nuclei of cells and in particular this compound will be taken up by dividing cells where 11 rads will be delivered per disintegration with a radius of 1 micron from the source of the disintegration. However the meaning of this "average" dose, and the biological significance of this localized dose to such a small sphere is vague. The above suggests that tritium labeled thymidine might well be explored as a potential therapeutic agent in proliferative disorders such as cancer and leukemia and in fact was developed by Hughes with this in mind. Quoting from Siri's book, page 541,

> " If artificial radioactivity is ever to prove a vital tool in the cure of malignancy, the only way of accomplishing this end would seem to be the discovery of organic substances that undergo highly selective localization in various types of cancer cells, in contrast to those of normal tissue and that can be labeled with a suitable radioisotope. Tritium with low penetrating power of its beta particles and (its rapid turnover in the body) is particularly promising because if it can be put in relatively stable positions in localizing compounds, it will expend most of its ionizing power within the concentrating cells. Potentially it is an ideal radiotherapeutic agent."

There is no data on the toxicity of tritium labeled thymidine. There is every reason to be cautious in its use because the labeled DNA will remain in cells for their own specific life. In some instances this will

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be very short - hours to days depending upon the turnover of the particular cellular system. There is no positive evidence that DNA or its breakdown products are reutilized in new cell production. Even if this were true the dilution in the total pool would be great. The greatest potential danger would seem to lie in its uptake in the gonads. Subsequent fertilization by labeled sperm might constitute a definite genetic and or embryologic hazard. Accordingly, its use in young human beings, who may later procreate, is not justified. In respect to a late somatic danger there is no data and a meaningful analysis would depend upon knowledge of the nature of cell proliferation that is not now available. If cells of the generative compartment of each tissue have a synchronous regular division and maturation the labeled DNA should be promptly diluted out of the generative compartment where carcinogenesis is in theory possible. If instead of synchronous division the process is random and measured interphase is a mean value with a large variance, primitive cells may remain behind with significant levels of labeled DNA that might be a more potent direct cause of cancerous change. On the positive side, no histologic evidence of radiation injury has yet been observed by us in mice injected with 0.7 μc of labeled thymidine per gram of mouse and observed for ten days after injection. Further studies on the toxicity of H^3 thymidine in animals are underway.

If one considers the thymidine activity as tritium oxide, assuming uniform distribution and a biologic half life of 19 days, the infinity whole body dose to a 50 kilo individual is 0.18 rep following the injection of 1 mc.

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Maximal dose rate in reps/day = $2.3 \times \frac{\mu c}{Grm} \times E_{av}$

$2.3 \times \mu c \times E_{av}$ / Grm

$6.6 \times 10^{-3} = 2.3 \times \frac{1000}{70,000} \times .006 \times .24$

$6.6 \times 10^{-3} = \frac{2.3 \times 1000 \times .006}{70,000}$

Integrated Total dose = dose rate $\times \frac{T_{eff}}{.693}$

10^{-3}

$= 6.6 \times 10^{-3} \times \frac{19}{.693} = .18$ rep

During the first week the dose would be approximately 45×10^{-3} rep.

This calculated dose is the maximum hazard to any personnel around patients per millicurie of material used since they could obtain only a small fraction of the expired tritium oxide.

4. Specific Proposal It is proposed to administer H^3 thymidine to patients under the AEC recommendation and requirements, section 5E,

> "It is recognized however that special circumstances may arise which indicate the desirability or necessity for the use of long lived radio isotopes in human subjects where prior animal data are not available. Consideration of such proposals shall be limited to patients suffering from diseased conditions of such a nature (life expectancy of one year or less) that there is no reasonable probability of radioactivity employed producing manifest injury."

Initially patients in this category (terminal brain tumors) will be given 5 mc of H^3 thymidine intravenously (i.e. at 1/8 of the body concentration used in studies in mice) ~~and the plasma clearance~~, urinary and fecal excretion will be measured. Catabolism to body water will also be followed as perhaps the best method of estimating retention. Chemical determination of ~~fixed tritium in appropriate tissue such as leukocyte~~ concentrates will be attempted. Serial blood smears, bone marrow smears, skin biopsies exfoliative cytology and testicular biopsies will be performed for histoautoradiographic studies.

The dose of H^3 thymidine will be progressively increased (up to 50 mc if necessary) by regular increments until positive histoautoradiographs

are obtained.

When sufficient data has been accumulated on the metabolic behaviour of this material, therapeutic studies involving doses in excess of the maximum doses considered here for metabolic purposes will be considered in selected patients.

5. General Comments. There are many uncertainties in respect to the use of tritiated thymidine. It is reasonable to assume on the basis of the preceding calculations that there is no hazard to personnel from the catabolism of the tritiated thymidine to tritium oxide. There is obviously no external hazard to radiation. The probability of any hazard to personnel are minimal providing ordinary precautions are taken to prevent ingestion of the material. The probable major hazard is a genetic injury. The gonadal dose is 0.18 rep plus the dose from the discrete sources incorporated into DNA. Since the concentration and the microscopic distribution of the H^3 thymidine is not known neither the localized nor its average dose can be calculated. Similar problems exist in respect to all of the proliferative tissues and the possible hazards are discussed earlier.

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