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TRITIUM RADIOTHERAPY

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SUMMARY

Three properties of tritium will perhaps make it a useful agent for radiotherapy:

1. Tritium β radiation is the weakest known; its action can be localized to a single cell with little harm to the neighbouring ones;
2. being an isotope of hydrogen, nearly all organic compounds can be labelled with it;
3. its specific activity of 30 Curies per milliatom-gram enables tritiated compounds to be made with enough activity to kill cells which have taken up very little of them. Tritium is also cheap, which makes the preparation of these highly radioactive compounds economically feasible.

Tritium is the radioactive isotope of hydrogen. It is a very soft β emitter: the maximum energy of its β rays is only 18,000 eV and the mean value 5,690 eV. The half life of ^3H is about 12.5 years and the corresponding activity of pure tritium 30 Curies per milliatom-gram.

Specific tritium labelled precursors are powerful tools with which to study the metabolism of cells by the autoradiographic technique. By reason of the low energy of tritium's β rays, the radioactive constituents of the cell can be localized within less than 1 μ; this definition is unequalled by other β emitters. The autoradiographic work with tritium done before 1956 used unspecific precursors like tritiated water or acetate (Fitzgerald *et al.*, 1951; Eidinoff *et al.*, 1951; Chapman-Andresen, 1953); the pictures obtained illustrated the accurate localization given by tritium but were of little value for the study of the cellular biochemistry. In 1956, Firket and Verly in Belgium and Hughes in the U.S.A. thought that thymidine, a very specific precursor of DNA, labelled with tritium would be of much greater interest. Verly and Hunebelle (1957) prepared a tritium-labelled thymidine chemically and radioactively pure and found that the radioactivity of the compound prepared by catalytic exchange was entirely bound to the pyrimidine moiety.

The first results published, using tritiated thymidine in cytological work, were the beautiful autoradiographs obtained by Taylor *et al.*, (1957) on chromosome duplication in *Vicia faba*, those of Firket (1957)

on DNA synthesis in chicken fibroblasts cultivated *in vitro* and those of Ficq and Pavan (1957) on the DNA synthesis in puffs of polytene chromosomes of Diptera. Since then, people using tritiated thymidine for cytological studies have increased exponentially and tritium has become the preferred radioisotope of the cytologists because of its unmatched accuracy.

The trajectory of a tritium electron has a mean length of 1 μ (with a maximum of 8 μ) in a medium of density 1; the tritium autoradiographs are only a striking illustration of this fact. The dimensions of a cell are of an order of magnitude greater than the length of a tritium electron path. If it were possible to localize tritium in one cell, this cell should be irradiated with little harm to the neighbouring ones. This makes a *qualitative* difference between tritium and the other β emitting radioisotopes: the radiological action of ^{32}P or ^{131}I is at the scale of the organ, while that of tritium is at the scale of the cell.

Considering the radiological toxicity of tritium for the cell, it is necessary to take the discussion one step further. Tritium can be homogeneously distributed in the cell for instance as tritiated water, but more often it will show some subcellular localization and its toxicity will depend on this localization. If we agree that DNA is the most sensitive molecule of the living cell to the action of radiations, tritium incorporated into the nucleus or the chromosomes must be much more toxic than the same amount of tritium fixed in the mitochondria. To evaluate its toxicity, it will thus be necessary to multiply the concentration of tritium in the whole cell by a coefficient of biological efficiency depending on the heterogeneous distribution of the isotope. This consideration has clearly much importance for the problem of tritium radiotherapy.

Theoretically and schematically, tritium radiotherapy must enable one to kill cells of kind A *intimately* mixed with cells of kind B and with *little harm* to the latter ones. To attain such a goal, it is necessary to find a radioactive compound taken up by A cells and very little or not at all by B cells. When the problem is considered from this angle, tritium appears to have the great advantage of being an isotope of hydrogen. Firmly bound hydrogen is present in nearly all organic compounds, so that nearly all organic compounds can be labelled with tritium and be candidates for tritium radiotherapy; the quasi infinite number of these compounds gives tritium a high probability of achieving, in one chemical form or another, a selective radiological action at the cellular level. These organic compounds can be anything: normal metabolites or foreign molecules, toxic substances (such as chemotherapeutic agents) or non-toxic substances.

The high specific activity of pure tritium makes it possible to prepare

compounds with sufficient radioactivity to kill quickly a cell which has taken very little of it (an example will be given later on). The low cost of tritium makes the synthesis of such compounds economically feasible.

Two cases of tritium irradiation must be considered:

1. The tritium remains indefinitively because the cellular constituent in which it is integrated has no turnover. Here is an all-or-none action: the cell which has picked up the radioactive compound must die. A differential action between two cell populations appears if many cells of one kind and very few of the other have assimilated the tritiated compound.

2. More often the tritium progressively disappears from the cell. Tritiated compounds should be chosen which are taken in greater quantity or retained longer by A cells than by B cells; the quantity to give should be such that the dose of radiation received by A is lethal while the dose received by B is not too harmful. The rate of removal of the tritiated compounds must be taken into account in order to calculate tritium-hours integrals per unit volume; the result so obtained must be multiplied by a factor of effectiveness depending on the subcellular localization (which was discussed earlier).

Because the turnover of DNA is very slow or nil, tritium irradiation from tritiated thymidine belongs to the first category. Lubin (1959) cultivated a mixture of wild-type and mutant *Escherichia coli* in a minimal medium containing tritiated thymidine: the wild-type cells multiplied and incorporated tritium into their DNA while the mutants did not; next, they were cooled in a refrigerator to stop any further division. During this period, the wild-type *E. coli* were irradiated and killed; the mutants, however, survived to the same extent as when cooled down to the same temperature during the same time but without pre-treatment with tritiated thymidine.

Lubin's experiment is only a model and human cancer radiotherapy is, unhappily, not such a simple case; there are, in the human body, many different kinds of cells. In spite of the results of Painter *et al.*, (1958) who showed that tritiated thymidine can decrease the viability of HeLa cells *in vitro*, the labelled deoxynucleoside is surely not a panacea against human cancer because there are so many cells which multiply much more frequently than the cancerous ones. It is, however, possible that tritiated thymidine might be useful in some cases of fast-growing tumours which could be treated by local perfusions.

To deliver 500 rep to a cell nucleus of $100\mu^3$, 400 disintegrations of tritium are necessary. Before dividing, a mammalian cell utilizes 5×10^{-12} millimole of thymidine. It is easy to calculate that to irradiate the nucleus with 500 rep in a single day, the specific activity

of thymidine in the cellular pool must be 0.03 Curie per millimole; in other words, only 1 molecule of thymidine per 1000 need contain tritium. Synthesis of compounds containing several tritium atoms per molecule could be achieved; because of self-irradiation, these compounds would be very labile, but the problem of keeping them for a short time is not insoluble.

An example of the second category, where tritium progressively disappears from the cell, will now be discussed. As mentioned earlier, the rate of tritium disappearance is as important as the initial total intake; tritium-hours must be computed and corrected for radiological effectiveness depending on the subcellular localization.

In our laboratory, Koch (1959) has prepared tritiated myleran (dimethylsulphonate of butanediol-1,4) with a high specific activity and we have started experiments to see whether the tritium from this compound is concentrated by some tissues in normal and leukemic mice. Kenis and Verly (1960) making a gross examination on organs and assaying total tritium on the water of combustion found no great concentration in any organ. Of course, a mean value so obtained can mask important localizations. The usefulness of autoradiography to detect these localizations will soon be discussed.

A more general attempt to understand the biochemical action of myleran was made. The experiments were performed on germinating seeds of *Vicia faba* in which the cytological action of the drug has been fully described by the Moutschen-Dahmens (1958). Seeds were incubated 3 hr. in water saturated with tritiated myleran, washed and left to germinate for 57 hr.; the growing root tips were cut, fixed with alcohol:acetic acid, squashed and autoradiographed. The cytoplasm was free of radioactivity, while some nuclei were labelled (Moutschen-Dahmen *et al.*, 1960). These autoradiographic observations together with the mutagenic action and the effect of myleran on the morphology of chromosomes, prompted us to see to what extent the deoxyribonucleoprotein was alkylated *in vivo* by the myleran. With Dewandre and Dulcino, we found that very little of the radioactivity found in the whole cell was bound to the DNA (1960).

For tritium radiotherapy, it is necessary to know the amount of tritium in each kind of cell (the Gaussian distribution curve specific for each kind of cell) and its rate of disappearance. Gross tritium determination on the organ does not give the answer. But obviously, the usual autoradiographs do not either: they only show the tritium incorporated into constituents which are not washed away in the course of the preparation (in the experiment with myleran, for instance, free tritiated myleran and small molecules alkylated by it are removed

during fixation). To be a useful guide for tritium radiotherapy, special methods retaining all the tritium *in situ* are necessary. But this is not yet the whole story since the number of silver grains developed in the nuclear emulsion laid on top of a tissue slice is not a measure of the amount of tritium in the cell below. The β radiation of tritium is so soft that the absorption by a few microns of tissue is not at all negligible. This is strikingly illustrated by a result of Firket (1957) on fibroblast nuclei: more silver grains are found above flat nuclei than above round ones; put into coordinates, the data yield a true self-absorption curve. To use tritium for radiotherapy, it is necessary to find methods of determining the absolute amount of tritium present in a single cell.

If the problem of the absolute counting of tritium can be solved, the accuracy of the autoradiography to localize tritium will make the determination of the efficiency factor depending on tritium subcellular localization within the reach of experimental work. It is a very important problem of cellular radiobiology.

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DISCUSSION

KINOSITA: The range of the β-particle emitted by tritium is very short. Is not this a limitation for radiotherapy? You would have to get it exactly to the right spot.

VERLY: This is a matter of choosing the right quantity for the most radio-sensitive intracellular spot.

HEIDELBERGER: There is evidence from the Brookhaven laboratories that tritiated thymidine in tissue-culture caused a prolongation of the mitotic time of the cells and that chromosome breaks were produced.

LURIA: One can envisage two types of damage caused by the tritium. The first is by radiation and the second by the disruption of a macromolecule by disintegration of the ^{3}H atoms in it. This has been observed, for example, in DNA labelled with ^{32}P.

HARRIS: We must try and persuade our physicist colleagues to devise methods of detecting tritium disintegrations with the resolution of the electron-microscope.

HEIDELBERGER: Have you thought of labelling certain synthetic dyes etc., which are already known to show selective localization in the tumour cell.

VERLY: We haven't done this yet.

ELSON: We have prepared tritium-labelled dimethylmyleran, and Dr. Lajtha, of Oxford, has been studying its uptake by bone-marrow. He felt that myleran should show affinity for myeloid rather than lymphoid cell elements, but found no specific localization of dimethylmyleran in the different types of bone-marrow cells. It would be very useful to have a ^{3}H-nitrogen mustard.