The Use of Autoradiographs in the Quantitative Determination of Radiation Dosages from Ca^{45} in Bone[1]

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BEFORE IT IS SAFE to introduce radioactive tracers into the human body, it is essential to know that the concentration will at no point become great enough to be a health hazard. The present safe dosage rate limit accepted by the Atomic Energy Commission is 0.1 rem (roentgen equivalent man) per day, or 0.1 rep (roentgen equivalent physical) per day for β-rays (quoted in *Nucleonics*, 1947, **1**, 4). The problem of determining local radiation dosages rates is therefore of vital importance in the use of many isotopes. Conventional counter measurements of activity are practically useless in this application, because they give information regarding average dosage rates over a large area, a value which can be several times smaller than the local dosage rates. Therefore, an autoradiographic technique capable of quantitatively measuring local dosage rates in tissue has been developed and is described below. It is currently being applied to the study of radioactive Ca^{45} dosages in bone.

The method consists of making an autoradiograph of the tissue being studied, producing on the same film calibration exposures from radioactive sources of known activity, developing the tissue and calibration exposures simultaneously, and comparing their blackening microphotometrically.

The technique is similar to that described by Axelrod and Hamilton (*1*) for the radioactive sulfur and arsenic studies of mustard gas and lewisite concentration in the skin and eye tissues. However, local radiation dosage is capable of being measured more simply than local isotope concentration.

Concentration deals with the distribution of the radioactive atoms; dosage deals with the distribution of the ionizing radiation from those atoms. Since in concentration studies the atoms are detected by means of their radiations, precautions must be taken to detect each β-ray or photon near its origin; i.e., the tissue section must be thin (microtome-sliced) and the distance between the film and tissue must be minimal. Because of self-absorption and backscattering effects (*5*) it is difficult to compare in a quantitative manner the intensity of radiation from a thick source with that from a thin source. Therefore in concentration studies the calibration source should be of the same thickness as the tissue section—a requirement not always easy to meet in the laboratory.

Dosage measurements, with which this paper is concerned, require tissue sections of dimensions greater than the maximum range of the radiation particles in order that all the radioactive atoms within the range contribute their share of dosage (and therefore film blackening) at the point in question. To be precisely correct, the tissue section should also be large as compared with the mean free path of any accompanying γ-rays, but for practical purposes this would generally be unnecessary. If the volumes of localization are small, and if local concentrations are high (compared with average concentration), the high specific ionization particles produce almost all of the localized dosage, the low specific ionization γ-rays almost none. Since the dosage rate within an infinite, homogeneous source can be readily calculated by the formula given below, such a calibration source, easily prepared, is desirable in dosage studies.[3] The penetration of the radiation is partly dependent on the atomic number of the surrounding elements; therefore it is important that the calibration source be composed of material whose atomic number distribution is the same as that of the tissue. It is obvious that the isotope in the tissue and in the calibration source should be the same.

The dosage *within* this infinitely thick source can be calculated by the formula (*3*):

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[3] Certain errors result from the fact that film blackening is not a function of reps of irradiation alone.

$$R = 60\ CE$$

where R = reps/day
C = μc/gm tissue
E = average energy of particles (approximately one-third their maximum energy) in Mev.

This dosage rate (R) within a thick source is our primary consideration. It is approximately twice the dosage rate at the surface, for when a block of material containing a radioactive substance is cut in half, the intensity of the radiation at a point on the cut surface is only one-half the intensity of radiation at this same point before the cut was made. This principle applies to the interpretation of autoradiographs made from tissue as well as calibration sources.

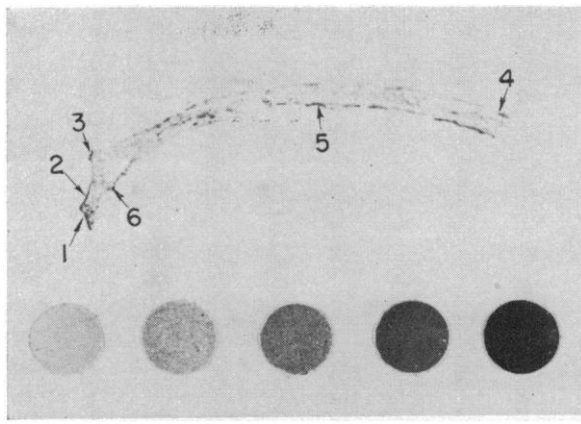

FIG. 1. Autoradiograph of dog rib resected 21 days after oral administration of radioactive Ca^{45}. The circles of graded blackening are calibration exposures increasing in magnitude by factors of 2, produced from plaster of Paris blocks containing known amounts of Ca^{45}.

The film characteristic most essential for accuracy is the contrast. Resolution is not of prime importance, since the scattering of the radiation in the thick source affects the film over a diameter comparable with the maximum particle range. Eastman No-Screen X-Ray Film was found to be a relatively good film to register the 0.26-Mev β-rays of Ca^{45} because of its high contrast and sensitivity. However, its resolution is rather low for the purpose, even though high resolution is not essential. It is also desirable to choose a film on which a given number of reps produce the same degree of blackening, regardless of the energy of the β-rays concerned. For energies less than 40 to 60 kev this requirement is not satisfactorily met by films now obtainable (*2*). This consideration is now being further investigated.

After the preparation of the tissue and calibration source, the procedure has been to make the autoradiograph and a set of five calibration exposures (each greater than the preceding by a factor of two) covering the range of exposures in which the film contrast is highest.[4] The blackening of the tissue autoradiograph is then microphotometrically compared with the blackening of the calibration spots, and the local radiation dosage rates in the tissue are thereby determined.

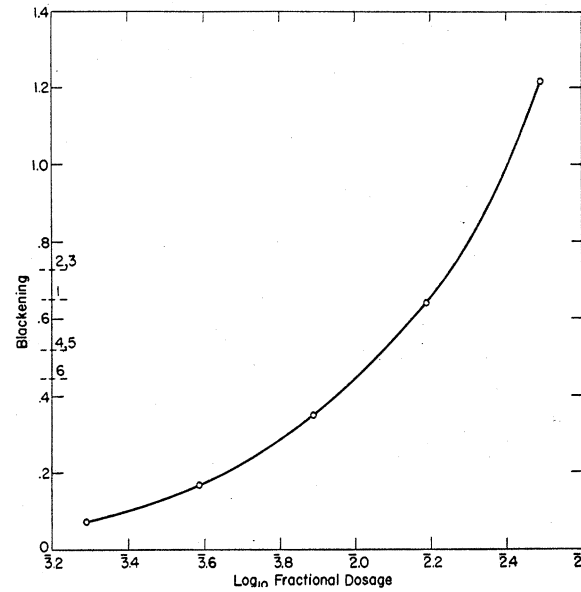

FIG. 2. Microphotometer record of calibration exposures and autoradiograph sites shown in Fig. 1. The points defining the curve are taken from the calibration exposures, and the dashed lines on the vertical scale indicate the blackening of the correspondingly numbered bone sites in Fig. 1. The activity of the calibration sources has been related to that of the dog's blood in such a way that the abscissa, essentially representing log (exposure), can be expressed in terms of log (fractional dosage).

An example of the work currently being done is shown in Figs. 1 and 2.[5] The study was made to determine the amount of dilution of radioactive Ca^{45} by normal calcium while passing from the blood to the bones of a growing dog. Fig. 1 shows the autoradiograph of a rib resected 21 days after the oral administration of the radioactive calcium, and five calibration exposures from artificial sources containing a known percentage of the peak blood activity. Fig. 2 shows the microphotometer record of the

(*Continued on page 342.*)

[4] Contrast is defined as the slope of blackening (also called density) vs. the log of the exposure. Blackening is defined as $\log \frac{I_0}{I}$, where I_0 and I are the intensity of the light transmitted through the clear film and the spot in question, respectively. Since to a good approximation, the microphotometer deflection $M \propto I$, the contrast is, closely, the slope of $\log \frac{M_0}{M}$ vs. log exposure.

[5] Complete biological results will be published later.

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(*Continued from page 328.*)

calibration exposures with the abscissa expressed in terms of the log of the fractional dosage. By fractional dosage is meant the ratio of the dosage rate in the calibration source to the bone dosage rate which would have existed had the bone been formed from the blood calcium at the instant of its highest specific activity. (It is assumed here that the *maximum* calcium concentration in bone is 36 percent by weight, the measured concentration in bone ash [*4*].) The blackening for each bone site labeled in Fig. 1 is indicated on the vertical scale of Fig. 2, from which it is seen that the maximum fractional dosage in the dog rib, corresponding to sites 2 and 3, is the antilog of $\bar{2}.26$, or .018.

The importance of this fact is its application to the possible use of radioactive Ca^{45} as a tracer in human beings. If such an experiment is to be carried on with reasonable convenience, this isotope will have to be present in the blood in a concentration of at least 1 μc/gm Ca. If bone were formed directly from such calcium, the dosage rate would be a forbidden 1.9 reps/day (again assuming 36 percent maximum calcium concentration). However, if the same dilution existed in humans as in the particular dog rib studied, the maximum local dosage rate would be a permissible value of $(.018)(1.9) = 0.034$ reps/day. It is important to notice, however, that this maximum local dosage rate is approximately six times higher than the average rate in the bone as a whole as determined by counter measurements on the digested rib.

Further work is planned in the quantitative study of film characteristics for different types and energies of radiation, and in the development of calibration sources which can be cut simultaneously with soft tissue, using a microtome technique.

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