Measurement of Ionizing Radiations *in Vivo*[1]

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It is well known that the intensity of roentgen rays can be quantitatively measured by using a photomultiplier tube to pick up the light from a fluorescent screen exposed to these rays. It has occurred to various investigators that the light from the screen can be transmitted by various types of optical systems to the photocell. The optical system does not have to produce an image; in its simplest form it is an internally reflecting tube or light pipe. For roentgen-ray intensities used in therapy the amplification of the photomultiplier tube is far greater than is necessary for convenient measurement. This leeway suggested the possibility that the system would still be sensitive enough if the light pipe had a very small diameter and the fluorescent screen occupied a very small area at the end of the light pipe. It seemed possible that the light from the screen could be piped through a hypodermic needle or through small diameter Lucite or quartz rods for insertion in body cavities. Both these possibilities have been realized, permitting direct measurements of roentgen-ray depth doses *in vivo*. The first system tried used Lucite rods of ¼-in. and ⅛-in. diameter and about 10 in. long. With the view in mind that ultimately these rods could be used to transmit separate scintillation pulses instead of a steady light level, the ends of the rods were turned in a parabolic section as shown in Fig. 1; a small hole was drilled to the focus of the parabola and a very small amount of luminescent zinc sulfide phosphor was pushed into the bottom of the hole. The rods were then aluminized by evaporation and coated with a black varnish. The total internal reflection from an air interface could not be depended upon, since in use the rods are frequently immersed in water. This arrangement would give a much smaller number of scintillations than could be had by cutting the end of the rod square and coating it with a layer of fluorescent crystals, but much more light from a single scintillation could be collected and reflected along the inside of the rod. This arrangement still had plenty of reserve sensitivity for roentgen-ray dose measurements, thus permitting a very fine-grained resolution of isodose curves to be determined.

To illustrate the potentialities of this arrangement the isodose curves were plotted in a pail of water subjected to a beam of 65-kv roentgen rays. Fig. 2 shows the arrangement used. As the ⅛-in. rod still had plenty of reserve sensitivity, work was started to pipe light from a speck of fluorescent material through a hypodermic needle to the photocell. This worked very well with a No. 15 needle. An internally polished needle gave more than twice the response of an unpolished one. The needles were polished internally by running them back and forth on a stretched copper wire charged with polishing compound. In these tests the needles were not Lucite-filled. A few grains of zinc cadmium sulfide phosphor (Patterson type B) were stuck on a tiny tapered Lucite plug, which was forced in a short distance up from the point of the needle. The needle could be inserted into an animal in the usual manner. It was inserted into the abdomen of an anesthesized rat to measure the abdominal depth dose for 60-kv roentgen rays. The dose measured in the abdomen in this case was found to be about 60% of the surface dose. The wall of the needle absorbed an undetermined amount of radiation, but this had no effect on the relative readings or on the ability of the device to be calibrated for any particular voltage quality of radiation. The luminosity of the screen per r-unit per minute depends on voltage quality and falls off considerably for radium γ -rays. A low atomic number phosphor showing only Compton scattering absorption should have a calibration independent of voltage quality but will produce a much smaller total luminosity than the highly efficient known phosphors containing higher atomic number elements. However, zinc sulfide should be considerably better than zinc cadmium sulfide in this respect, since for voltages higher than the 100-kv range the K energy level of Zn is only about 9.5

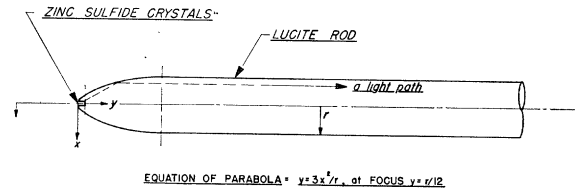

FIG. 1.

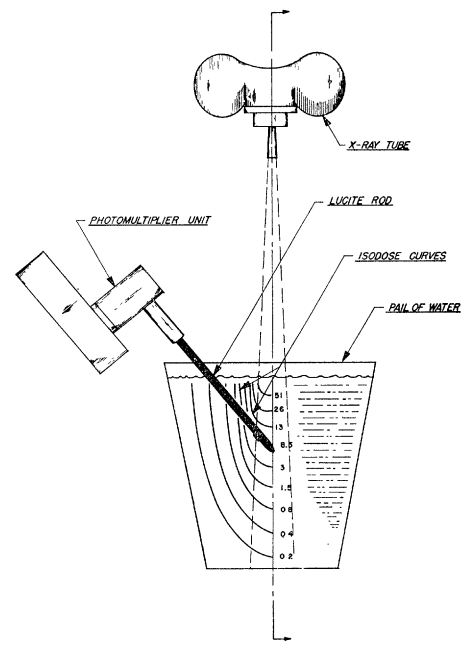

FIG. 2.

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kv. Although the photomultiplier tube (1P21) is more sensitive to the blue light from zinc sulfide than from the yellow-green light from zinc cadmium sulfide, the latter gave more response at 60 to 80 kv, presumably because of its greater absorption.

The experiments with the long Lucite rods were performed without any amplifier added to the photomultiplier tube. An amplifier of current gain of about 10^5 was constructed and used to observe the alpha particle pulses. This amplifier was not a d-c amplifier, so that it could not be used for measuring a steady luminosity level unless a mechanical chopper were used to interrupt the light entering the photomultiplier. However, it was easy to observe the half-wave pulses of X-rays from self-rectified X-ray tubes, even at extremely low levels. The intrinsic noise level, mostly due to shot effect from the thermionic emission of the photocathode of the photomultiplier tube, was easily observable with the full gain of the added amplifier. The extra gain is necessary to pick up alpha particle scintillations through the hypodermic needles and scintillations caused by beta rays.

Alpha particle scintillations on the zinc cadmium sulfide phosphor were so high as compared with the noise level that it was fairly easy to observe scintillations through the polished No. 15 needle. Further work is in progress to improve this technique and investigate the possibilities of measuring the local concentration of beta activity of radioisotopes *in vivo*. It has been found possible to turn Lucite rods of sufficiently small diameter to slide inside a No. 15 needle. These are coated with evaporated aluminum and a few crystals of fluorescent material are stuck on one end. The method of insertion is to slide them into the needle so that the screen end is slightly retracted from the beveled end of the needle. The needle is inserted to proper depth and then the Lucite rod is pushed on so that the screen end slightly protrudes into the tissue. One-mm-diam optically clear quartz rods gave even better results than the Lucite, but they have the disadvantage of being fragile.

Concerning the Specificity of Chicken Pancreas Conjugase[1]

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In a previous paper of this series (*1*), the conclusion was reached that chicken pancreas conjugase should be classified as γ-glutamic acid carboxypeptidase. The minimum requirements for the length of the peptide chain of the substrate could not have been established at that time. While it was suggested that pteroyl-γ-diglutamate was the end product of the reaction, the possibility of premature termination of the reaction, due to inhibition by the products formed, was not excluded.

We were able to study some recently synthesized derivatives of pteroylglutamic acid.[3] The methods used were the same as those previously described (*1*). The results are summarized below:

Substrate	*Equivalents of glutamic acid recovered*
Pteroyl-α-diglutamate	0 (or traces)
Pteroyl-γ-diglutamate	0 (or traces)
Pteroyl-α-α-triglutamate	0.0–0.2
Pteroyl-γ,γ-triglutamate	0.4–1.0
p-Aminobenzoyl-γ-γ-triglutamate	0.5–1.0

Since pteroyl-γ-diglutamate was not attacked by the enzyme, it represents the end product of digestion of the substrate by chicken pancreas conjugase. These data confirm our previous hypothesis and are in agreement with the findings of J. J. Pfiffner[4] who isolated the diglutamate from a digest of pteroylheptaglutamate with this enzyme. The minimum requirement for the number of glutamic acid residues for the substrate was, therefore, established at *three*.

In three out of five experiments with α,α-triglutamate, free glutamic acid was recovered. But the speed of hydrolysis of the alpha linkage was less than one-half that for the gamma linkage. Contrary to our previous conclusion, it appears that the chicken pancreas conjugase hydrolyzes the gamma linkage *preferentially*, but not *specifically*. The final decision must be withheld, however, until a purer enzyme is available.

The rate of liberation of the microbiologically active substance at various stages of purification of the enzyme was also studied. The ratio of the units of activity found with heptaglutamate[5] as substrate divided by the units found with triglutamate varied from 1: 2 to 1: 8, with an average value of 1: 4.2. The calculated value for the diglutamate as the end product of the reaction is 1: 5; for the monoglutamate, 1: 3. In spite of considerable variation in experimental results, there was no tendency for a change in this ratio with increasing purity of the enzyme. This indicates that the liberation of microbiologically active substance from heptaglutamate is achieved by a single chicken pancreas conjugase, in contrast to the hog kidney conjugase, which has two components (*2*).

References

1. Kazenko, A., and Laskowski, M. *J. biol. Chem.*, 1948, **173**, 217.
2. Mims, V., and Bird, O. D. Vitamin Symposium, AAAS Conference. Colby College, August 1948.

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[2] On leave of absence from the Agricultural Institute, Pulawy, Poland.

[3] Obtained through the courtesy of E. L. R. Stokstad, Lederle Laboratories Division, American Cyanamid Company.

[4] Discussion at the Federation Meeting, Atlantic City, 1948.

[5] Heptaglutamate was obtained through the courtesy of Dr. J. J. Pfiffner, Parke, Davis and Company.