ACRH-5

ARGONNE CANCER RESEARCH HOSPITAL
950 EAST FIFTY-NINTH STREET • CHICAGO 37 • ILLINOIS

Semiannual Report to
THE ATOMIC ENERGY COMMISSION

MARCH 1956

LEON O. JACOBSON, M.D.
Editor

ANTREEN PFAU
Associate Editor

OPERATED BY THE UNIVERSITY OF CHICAGO
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THE REACTION OF BRAIN TISSUE TO BETA RADIATION*†

By

P. V. Harper, T. B. Rasmussen,‡ T. T. Kennedy,‡ and A. Y. Gerol‡

INTRODUCTION

The reaction in tissue surrounding an implanted β -ray source consists of a roughly spherical volume of completely necrotic tissue surrounded by a very narrow transition zone beyond which is histologically normal tissue.⁽¹⁾ The dose of β radiation required to produce necrosis may be estimated by measuring the distance from the source to the border of the necrotic zone and utilizing the dose distance relationships determined by means of a lucite phantom.⁽²⁾ In this study we have investigated the effect of β -ray sources of different half-lives to determine the dose required to produce necrosis in brain tissue.

METHODS

Beta-ray sources of Pd^{109} , Y^{90} , P^{32} , and Sr^{90} - Y^{90} that have been described elsewhere^(2,3) were implanted into the brains of cats. Sr^{90} - Y^{90} sources were implanted in the undersurface of a bone button using bone wax because these sources had to be removed. The bone button was then replaced in the trephine hole with the source in contact with the underlying cortex. The other sources were all implanted with hollow needles into the brain substance. The animals were sacrificed at various intervals, and the brains were fixed by perfusion with formalin. Microscopic sections were made of the lesions, and the average diameter was determined by direct measurement. Shrinkage artifacts and swelling or shrinkage during the process of irradiation could not be controlled and were ignored. While all the sources of a given isotope were not of the same strength, the dose required to produce necrosis did not appear to be affected by this, and for purposes of graphical presentation it was assumed that, for instance, the effects of a 1-mc Y^{90} source at 4 mm and of a 1.5 mc source at 5.4 mm could not be distinguished in tissue.

RESULTS

Representative lesions are shown in Figures 1 to 5, and the progress of these lesions with time is shown graphically in Figures 6 to 9. The lesions increase in size during the period of irradiation and then shrink as the radiation ceases and scarring takes place. Taking the maximum size of the lesion as the end point, the dose required to produce necrosis

* Based on a paper presented at the Conference on Rare Earths in Biochemical and Medical Research, Oak Ridge National Laboratory, October 1955.

† This study was aided by grants from The Damon Runyon Memorial Fund and The Douglas Smith Foundation for Medical Research of The University of Chicago.

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Figure 1. Tissue reaction in cat brain surrounding a 5-mc Pd^{109} source. Animal sacrificed 14 days after implantation. Mag. 10 X (Hematoxylin and phloxine).

is about 35,000 rep for Pd^{109} ($T_{1/2} = 13$ hrs), 75,000 rep for Y^{90} ($T_{1/2} = 62$ hrs), and 150,000 rep for P^{32} ($T_{1/2} = 14.3$ days). As the time of irradiation increases, the dose required to produce necrosis continues to increase, as shown in the Sr^{90} - Y^{90} data, leveling off at 2,000,000 rep after 2 to 3 months. These figures do not take into account swell-

Figure 2. Tissue reaction surrounding a 1.6-mc Y^{90} source. Animal sacrificed at 8 days. Mag. 10 X (Hematoxylin and phloxine).

ing or shrinkage that occurs during irradiation, shrinkage artifact caused by fixation, and in the case of the Sr^{90} - Y^{90} , possible movement of the brain inside the skull. The net result of these factors is probably to make the absolute values of the figures somewhat high. Their relative values are certainly more nearly correct.

Figure 3. Lesion surrounding a 0.13-mc P³² source. Animal sacrificed at 16 days. Mag. 10 X (Hematoxylin and phloxine).

Figure 4. Lesion produced by a 0.19-mc Sr⁹⁰-Y⁹⁰ point source in contact with surface of cortex. Animal sacrificed at 8 days. Mag. 10 X (Hematoxylin and phloxine).

Figure 5. Lesion produced by a 0.19-mc $\text{Sr}^{90}\text{-Y}^{90}$ point source in contact with surface of cortex. Animal sacrificed at 112 days. Mag. 10 X (Hematoxylin and phloxine).

Figure 6. Size of lesion produced by Pd^{109} (5 mc) as a function of time. The indicated dosage is for complete decay of the isotope as calculated from film dosimetry data.⁽²⁾ The decay of the isotope is indicated by the dotted area on the chart.

Figure 7. Data for Y^{90} (1 mc) presented as in Figure 6.

Figure 8. P^{32} (100 μ c) data presented as in Figure 6.

Figure 9. Sr^{90} - Y^{90} data presented as in Figure 6, except that the cumulative dosage at the border of the lesion is indicated because there is no appreciable decay of the isotope.

DISCUSSION

The radiation dosage that was found to cause necrosis in brain tissue is of the same order of magnitude as that observed by Quimby⁽⁴⁾ for radon γ radiation in rabbit muscle, which was about 100,000 r. This also fits the time scale: 75,000 rep for Y^{90} , half-life 62 hours; 100,000 r for radon, half-life 4.85 days; and 150,000 rep for P^{32} , half-life 14.3 days.

CONCLUSION

The immediate necrosis produced in brain tissue by β radiation is strongly dependent on the dose rate.

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A PLANE INTERSTITIAL ISOTOPE APPLICATOR FOR IRRADIATION OF THE MEDIASTINUM^{*†}

By

P. V. Harper, W. E. Adams,[†] E. E. Schwartz,^{**} K. A. Lathrop,
and R. W. Harrison[†]

INTRODUCTION

Although it is usually impossible for a patient to tolerate cancericidal radiation dosage to the mediastinum from external sources, it is possible to reach very high superficial dose levels with a plane γ -ray source applied to the mediastinum. The rapid fall-off of the radiation field surrounding such an applicator reduces the radiation to the vulnerable trachea, esophagus, and lung to tolerable levels. The technical details of such an approach have been worked out in experimental animals and form the basis for this report.

METHODS

Applicators were constructed using blotting paper of appropriate dimensions sealed in envelopes of polyethylene sheeting. These were implanted in experimental animals against the mediastinum (Figure 1). Several days later, the blotting paper was saturated with a solution of a suitable isotope introduced through fine polyethylene tubing sealed into the envelope.

Envelopes constructed of 0.002-inch polyethylene sheeting were satisfactory but had to be handled very delicately to avoid making perforations and leaks. These were readily distorted by contraction of scar tissue. Envelopes made with 0.01-inch sheeting were substantial but somewhat less pliable. Very fine polyethylene tubing (Clay Adams PE-10) was sealed into the envelope for subsequent introduction of the isotope, and the applicator was tested for leaks by evacuating it and submerging it overnight in Zephiran solution. No further sterilization was used. Various methods of fixing the envelopes in place were found to be satisfactory. For example, wide borders were left on the envelope through which sutures were passed. Perforations in the borders made this process easier, and the sutures were sealed directly into the borders of the envelope.

The end of the polyethylene tubing was brought out through a stab wound and left in a

* Based on a paper presented at the meeting of the Surgical Forum, American College of Surgeons, Chicago, Illinois, November 1955. The paper was also given at the Conference on Rare Earths in Biochemistry and Medical Research, Oak Ridge National Laboratory, October 1955.

† This study was aided by grants from The Damon Runyon Memorial Fund and The Douglas Smith Foundation for Medical Research of The University of Chicago.

‡ The Department of Surgery, The University of Chicago.

** The Department of Radiology, The University of Chicago.

1A

1B

Figure 1. Antero-posterior and lateral roentgenograms of dog with 3- x 15-cm applicator against mediastinum. Note Sievert chambers in esophagus. The applicator is saturated with strong potassium iodide solution containing 45 mc of I^{131} .

small coil under the skin. When it was time to introduce the isotope, the end of the tubing was exposed under local anesthesia, and the isotope solution was injected by means of a shielded syringe in a sufficient volume to saturate the blotting paper evenly with a sufficient isotope density to give the desired dosage. The tubing was cut off, sealed, and left beneath the skin. It was found that an isotope solution containing a substantial amount of carrier could be removed to any extent at any time by repeatedly washing the envelope via the tubing. With reasonable care, radiation and isotopic contamination were negligible.

The biologic effects of two isotopes were investigated. The first was I^{131} . This was chosen because of its availability, suitable half-life, and γ radiation. The second isotope chosen was Cs^{131} ,⁽¹⁾ which has a 10-day half-life. It decays by K-capture consequently emitting the soft characteristic X rays of xenon (29 kv). The range of this radiation in tissue is much less than that of hard γ radiation, the true absorption being about 17 per cent per cm as compared with 3 per cent per cm for I^{131} and radium γ rays. Dose measurements were made surrounding point sources of these isotopes in a water phantom using a small ion chamber. From these data, using methods of graphical integration, isodose surfaces were calculated surrounding applicators of various sizes and shapes.

RESULTS

Three- x 15-cm applicators were placed against the mediastinum in 8 animals (Figure 1). They were well tolerated and none became infected. There was no hematologic depression in any of the animals subjected to a radiation dosage of 5000 r at 1 cm from the center of the applicator. In one animal with an I^{131} implant, Sievert chamber measurements in the esophagus confirmed the calculated radiation dosage within the limits of error.

It was found that within 3 to 4 cm of both I^{131} and Cs^{131} sources in the water phantom, the region with which we are most concerned in implant work, scattered radiation compensated for absorption almost completely, as is true of the radiation from radium and cobalt-60.⁽²⁾ The I^{131} radiation field resembles closely that produced by radium. In the Cs^{131} field close to the source, scattered radiation increased the dose rate about 20 per cent above the air dose. The field falls off with a half-value distance of about 4 cm due to absorption in the water (Figure 2). Figure 3 shows the isodose curves in a vertical plane through the center of a 10- x 10-cm applicator. Immediately adjacent to the applicator, the radiation field of both isotopes is extremely high, producing essentially a local cautery of the tissue. The effects of this on adjacent lung tissue are shown in Figure 4. The fibrous sheath formed about the applicator is relatively acellular in the case of the I^{131} implant because of the very high β -radiation dosage. This was not the case with Cs^{131} . Both applicators produced about the same degree of damage in the subadjacent lung tissue. At a distance of 10 cm from the applicator on its central axis, the Cs^{131} field falls to one-fifth of the I^{131} field.

DISCUSSION

It appears that the present method could be used clinically to deliver intense radiation

Figure 2. Attenuation due to absorption and scatter surrounding a point source of I^{131} and Cs^{131} in a water phantom. Curve for radium⁽²⁾ is given for comparison.

Figure 3. Isodose curves through center of 10- x 10-cm plane applicator showing contrast between Cs^{131} and I^{131} fields at a distance from the applicator and the similarity close to the applicator. Isotope density 0.7 mc/cm² for I^{131} ; 2.6 mc/cm² for Cs^{131} . Dosage is calculated to complete decay for each isotope.

Figure 4. Effect of plane applicator on lung tissue at 6 weeks after insertion. In A, I^{131} density equals 1 mc/cm²; in B, Cs^{131} density equals 1.7 mc/cm². Note complete destruction of cellular elements adjacent to I^{131} applicator due to the intense β -ray field. Damage to subadjacent lung tissue appears about the same in both cases.

B A

to large areas in the mediastinum to a depth of a centimeter or so without producing serious reaction in the patient and without radiation hazard to the surgeon. Applicators of suitable sizes could be kept sterile and available for use at any time. Isotope administration may be carried out at leisure after the patient has recovered, after a bronchial stump has had time to heal, etc. The patient may even be sent to another institution for isotope administration if necessary. The use of Cs^{131} , although it is not available at present, shows great promise of allowing more extensive implants than can be tolerated using ordinary radiation because of the much more marked localization of the radiation field.

It is believed that a sufficient number of cases of carcinoma of the lung might have small superficial mediastinal metastases accessible to this type of radiation field to make its prophylactic use following pneumonectomy worthy of clinical trial.

SUMMARY

A general method is presented for using isotopes in solution in plane interstitial applicators. Dosimetric considerations for I^{131} and Cs^{131} are discussed, and experimental observations of such applicators in the mediastinum have been made in dogs. The method appears practical from a clinical standpoint.

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IRRADIATION OF THE POSTERIOR OCULAR SEGMENT WITH YTTRIUM-90*

By

F. W. Newell,[†] P. V. Harper, and A. Koistenen[‡]

The delicate functional structure of the eye is an excellent medium for investigating the effects of superficial radiation with β rays that do not penetrate deeply. The results of studies of focal area β irradiation of the anterior segment of the globe have been reviewed in recent years by Hughes,⁽¹⁾ Wilson,⁽²⁾ and Iliff.⁽³⁾ Stallard⁽⁴⁾ has described the effects of radon seeds, radium discs, and cobalt-60, upon the posterior ocular segment in eyes containing retinoblastomas. There have been no reports concerning the effects of a pure β -ray emitter upon the posterior ocular segment. Such a study is of clinical application because of the possibility of using point sources of radiation for the treatment of small retinoblastomas and retinal tumors of angiomatosis.

Prior to the invention of the cyclotron in 1931, and more recently nuclear chain reactors and piles, β -ray emitters were present only as naturally occurring sources. Now, a wide variety of elements with different physical and radioactive properties is available.

Yttrium-90, which was used in this study, is most familiar to ophthalmologists as the therapeutically effective portion of radioactive strontium. Strontium-90 decays to yttrium-90 by the emission of a β particle having a penetration of but 0.35 mm in tissue. Yttrium-90 decays to zirconium-90, a stable isotope, by emitting a β particle with a maximum energy of 2.3 Mev and a mean energy of 0.90 Mev. Penetration in tissue is about 10 mm. Yttrium-90 has a half-life of 62 hours so that it reaches 90 per cent saturation activity after irradiation in the pile for 1 week. With a neutron flux of 3×10^{12} neutrons per cm^2 per second, this activity is about 0.5 mc per mg of Y_2O_3 .

TECHNIQUE

Yttrium pellets, prepared and calibrated as described by Rasmussen *et al.*,⁽⁵⁾ were superficially attached to the sclera in a series of male, mongrel, pigmented rabbits. The animals were anesthetized with intravenous pentobarbital and topical tetracaine hydrochloride (Pontocaine). A medial and lateral canthotomy was performed, and the lids were retracted with sutures. The superior rectus muscle was then grasped, the eye rotated

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downward, and an incision was made through the conjunctiva, exposing the sclera. In a few animals, a hinged scleral flap was made to contain the pellets, but in the majority, the pellets were placed underneath Tenon's capsule, and the conjunctiva was closed with no particular provision for holding the yttrium pellet in contact with the sclera. The radioactive pellet was placed in position by means of a spinal puncture needle that was shielded with lead and equipped with a stylet through which the pellet was expelled. Ophthalmoscopic examination was carried out at weekly intervals post-operatively. The animals were sacrificed with intravenous pentobarbital at various time intervals following the implantation, and the eyes were removed.

The eyes were fixed in formalin solution. Sections, 17 microns thick, were made, and every tenth section was stained with hematoxylin and eosin.

RESULTS

Forty-one eyes were studied histologically. In 11 of these there were no abnormal findings. It is probable that in these eyes the yttrium pellet was dislodged and the posterior ocular segment received either too little radiation to cause histologic changes or that the changes were too minute to be recognized.

Acute radiation injury was observed in the eyes enucleated within 14 days after the insertion of the yttrium pellet. Graduated doses of radiation were not administered so that the minimal effective amount was not observed except by measurement of the area of involvement in the affected eyes. However, fixation of the eye causes unequal shrinkage of the choroid and retina so that such measurements may be extremely inaccurate.

The eyes of animals to which an 0.8-mc pellet had been applied for 24 hours showed acute radiation damage. The muscle and the sclera immediately adjacent to the pellet were necrotized markedly. The entire area was infiltrated with large numbers of polymorphonuclear cells that were also necrotic. In point of fact, the reaction was so severe that the sclera perforated in some animals. The choriocapillaris layer, which in most normal animals is relatively inconspicuous, became markedly dilated, and there was rupture of the blood vessels. No recognizable structure was evident in the retina in the area of the most intense reaction. The layer of rods and cones was entirely destroyed and replaced by a homogeneous pink staining fluid. Adjacent to the area of acute necrosis, the external nuclear layer showed pyknosis and karyorrhexis which, however, were not as marked as in the internal nuclear layer. The ganglion cell layer, histologically at least, appeared the most radioresistant of the retinal layers. Nonetheless, similar changes were observed in the nuclear layers but to a lesser degree. The retina was entirely destroyed; individual layers could not be recognized in an area 2 mm in diameter. Injured cells were observed in an area approximately 6 mm in diameter. The area of acute injury to the sclera was 4 mm in diameter, and necrotic cells were evident in an area 8 mm in diameter.

The application of an 0.8-mc pellet for a 7-day period led to similar changes. At this interval, however, vascular engorgement of the choroid was not marked and the blood vessels were obscured by the cellular debris. Many necrotic polymorphonuclear leucocytes

were present in the sclera, choroid, and retina. Many cystic areas were present in the retina in cases where the choroid and sclera were relatively uninvolved. In areas adjacent to the site of most intense damage, choroidal pigment proliferation was evident and the choroidal vessels were dilated.

Fourteen days after the application of an 0.8-mc pellet, the acute process appeared to be subsiding and the polymorphonuclear leucocytes in the area were no longer necrotic. Many macrophages were scattered throughout the necrotic sclera and retina. The sclera was considerably thinned and only occasional collagenous bundles were present. These were not interwoven in the normal fashion. In many places, the retina was detached with a protein-rich fluid separating it from the choroid. In the area of most acute inflammation, the retina was destroyed entirely. Injury was not observed beyond the internal nuclear layer and ganglion cell layer. In many eyes, there was spontaneous perforation of the sclera, or the trauma incident to enucleation caused the ectatic area to rupture.

The ophthalmoscopic observations of the eyes paralleled the histologic findings. Hemorrhages, which frequently surrounded and obscured the optic disc, usually appeared first. Pigment proliferation was evident by the second week and was very marked by the third week. In the final stages, an area of retinal and choroidal atrophy was evident; presumably in the area of the most intense irradiation. This was surrounded by a ring of pigment. Frequently, pigmentary proliferation was very marked.

In eyes removed 25 to 75 days after the application of comparable amounts of yttrium-90, the histologic changes were similar. In some, the sclera was ruptured and the intraocular contents had escaped. The acute inflammatory reaction had cleared entirely and the adjacent areas of sclera appeared relatively normal. At the site of the most intense reaction, the choroid and retina were entirely destroyed. Adjacent to this was an area of pigmentary proliferation, and there was a gradual appearance of normal choroid. Generally speaking, the histologic changes in the retina were more conspicuous and more widespread than those in the choroid.

The area of complete destruction of the retina measured about 5 mm in diameter. Partial destruction included an area of almost 9 mm in diameter. It is of interest to note that retinal destruction appeared more extensive than choroidal, although the retina is composed entirely of neural tissue. This resulted very likely from the damage occurring in the retina because radiation, per se, combined with the interference with the choroidal blood supply. Moreover the choroid, composed essentially of vascular channels, regenerates in time in the peripheral area of radiation injury, while the retina remains unchanged.

Cataract formation was not seen in these animals either clinically when examined with the ophthalmoscope nor histologically. This may be accounted for by the relatively short time the animals were observed or by the fact that the animals were adults, making them less sensitive to radiation-induced cataract formation.

SUMMARY

Ophthalmoscopic and histologic studies were made of the eyes of mature rabbits fol-

lowing irradiation by means of a radioactive yttrium pellet attached to the sclera. The initial reaction was one of acute necrosis in which the polymorphonuclear cells were destroyed also. Proliferative activity was evident in the choroid after 2 weeks. In the area of acute injury, the choroid and retina were destroyed entirely.

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EXPERIMENTAL MYOCARDIAL INFARCTION PRODUCED BY LOCAL BETA RADIATION^{*†}

By

P. V. Moulder,[‡] R. W. Harrison,[‡] W. E. Adams,[‡] and P. V. Harper

Surgery of the coronary artery has a short history⁽¹⁾ and has had only a few faithful workers: There have been two formidable problems: 1) the experimental and clinical evaluation of the procedure employed; and 2) the selection of patients for this surgery. The second will remain unanswered until the first is well established. All of the studied and discussed operations for coronary artery diseases have as their purpose the induction or production of extracoronary cardiac vascular communications. It has been anticipated that the additional blood supply would protect against infarction when a major channel was occluded; that it would afford enough blood to the infarcted area to prevent it from becoming a "trigger zone" for the development of ventricular fibrillation; and that it would afford sustenance for the myocardium while intercoronary anastomoses were enlarging.^(1,2)

Experimental evaluation of the protective effects has shown that:

1. Mortality from acute ligation of a major coronary artery (anterior descending branch of the left coronary artery)⁽³⁾ is decreased.
2. The size of the infarct formed after acute ligation⁽³⁾ is decreased.
3. There is retrograde flow in an artery after its division (circumflex branch of left coronary artery).⁽⁴⁾
4. There is an extracoronary myocardial blood supply.⁽²⁾

The study described in this paper was undertaken to find means for producing standard experimental subjects for evaluation of coronary operations. It was believed that a method for inducing coronary occlusion in the unanesthetized, intact animal was needed and that a control should be an infarct without major coronary vascular occlusion. Initial studies have shown that thrombosis of the anterior descending branch of the left coronary artery can be produced by the implantation of radioactive yttrium (Y^{90}) pellets along the side of the vessel. Furthermore, pellets implanted in the myocardium will produce small, but relatively standard areas of necrosis and subsequent fibrosis.

METHODS

The production of the Y^{90} pellets has been described, and the irradiation data have

* Based on paper presented at the meeting of the Surgical Forum, American College of Surgeons, Chicago, Illinois, November 1955.

† This study was aided by grants from The Damon Runyon Memorial Fund and The Douglas Smith Foundation for Medical Research of The University of Chicago.

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been delineated.⁽⁵⁾

A lead-protected needle is loaded with the radioactive pellet under water. After the needle is introduced into the myocardium or pericoronary fat, the obturator is introduced rapidly, forcing the pellet into the tissue. A pack of wet gauze placed over this affords protection while preparations are being made for the next insertion.

Three groups of dogs were studied:

- Group 1. Implantation of single pellets into a localized site to demonstrate the amount of the destroyed myocardium. (Note that multiple separate pellets were introduced into the same heart at one and/or subsequent operations.)
- Group 2. Implantation of a group of pellets in a sufficiently localized area to produce large (coalescent) sites of destruction.
- Group 3. Implantation of pellets alongside the anterior descending branch of the left coronary artery with the intention of producing thrombosis in a short segment of the artery and possibly distal infarction.

Pre-operative and post-operative serial electrocardiograms were obtained from dogs that had coronary and coalescent site implantation.

The experimental data are presented in the accompanying tables. Table 1 shows the nature and size of local destruction of the myocardium produced by the implantation of the single pellet (A) and multiple, approximated pellets (B). The measurements represent the diameter of the lesion as measured in the fresh, gross, sliced myocardium. The electrocardiographic and postmortem data on the pericoronary implantation animals are given in Table 2.

Isolated pellets of approximately 1 mc produce a 13- to 14-mm diameter area of necrosis in the myocardium (and adjacent pericardium and fat) (Figures 1A and B), which eventually contracts to a fibrotic area 6- to 8-mm in diameter (Figure 2). If the pellets are placed 5 to 8 mm apart, a coalescent infarct is obtained.

The production of coronary thrombosis by implantation of Y^{90} pellets along the anterior descending branch of the left coronary artery was obtained with varying results in the present study, but with this background, it is probable that a more uniform result is obtainable. The variability of the electrocardiographic and pathologic data should also be less, but these findings are sufficient to emphasize the importance of producing the coronary obstruction in the intact, unanesthetized animal. The clinical counterpart certainly manifests considerable variation, which should be evaluated and controlled.

Complete thrombosis in the coronary artery at the level of pellet implantation is shown in Figure 3. There has been necrosis and medial hemorrhage, with complete intraluminal thrombosis. The artery was patent a short distance beyond this site. At a level approximately 1 cm below the thrombotic site in this animal, there was a plaque in the subintimal-media level.

The infarct was in the pre-apical and apical area of the left ventricle, which was supplied by the triangular major division of the anterior descending branch of the left coro-

Table 1
EFFECT OF IMPLANTATION OF YTTRIUM-90 PELLETS INTO LEFT
VENTRICULAR MYOCARDIUM

Dog (no.)	Yttrium-90 pellets (no.)	Pellet strength (mc)	Time	Electrocardiographic findings	Major diam. of single infarct (mm)
A. Single Sites					
265	2	1.0	S* 14 days		13 - 14
292	1	1.0	S 18 days		14
951	2	1.0	S 20 days		12
670	2	1.0	S 21 days		11
667	2	1.0	S 32 days		10 - 11.5
63	4	1.5	S 5 mos.		6 - 8
	4	1.0	4 mos.		
40	4	1.5	S 13 mos.		6 - 8
	5	1.0	12 mos.		
107	2	1.0	D [†] 1 day		Inaccurate data
222	2	1.0	D 2 days		" "
B. Coalescent Groups					
292	7	1.0	S 18 days	Early Infarct pattern	3 sites
	2				17 x 12 x 11
	4				25 x 10 x 10
124	6	1.0	S 3 mos.	Minimal chest lead changes	21 x 17 x 2 to 6
653	5	0.5	S 4 mos.	No changes	16 x 11 x 5
613	4	1.0	S 4 mos.	Minimal chest lead changes	Inaccurate data

* Sacrificed.

† Died.

nary artery. The site showed fibrosis and marked thinning of the wall over an area 3.8 x 3.5 cm, with approaching diverticulation of the apical portion of the left ventricle.

A non-thrombosed coronary artery (Figure 4) shows that one portion had been affected. The irradiated area is thickened and shows minimal encroachment on the lumen of the artery.

Table 2

EFFECTS OF IMPLANTATION OF YTTRIUM-90 PELLETS ALONG ANTERIOR
DESCENDING LEFT CORONARY ARTERY

Dog (no.)	Yttrium-90 pellets (no.)	Pellet strength (mc)	Time	Electrocardiographic findings	Postmortem findings
673	2	1.0	D* 4 days	1. Incomplete data	1. Coronary thrombosis 2. No infarct
831	2	0.5	D 1 mo.	1. Infarction pattern 2. Episodic ventricu- lar tachycardia	1. Thrombosis 2. No gross infarct
665	2	1.0	S [†] 4 mos.	1. Infarction pattern with resolution	1. No thrombosis 2. No gross infarct
757	2	0.5	S 5 mos.	1. In and out of coronary insuffi- ciency pattern	1. Thrombosis 2. No gross infarct
528	2	0.5	S 5 mos.	1. Minimal changes	1. No thrombosis 2. No infarct
227	4	0.8	S 6 mos.	1. No changes	1. No thrombosis 2. No infarct
594	2	1.0	S 6 mos.	1. Infarction pattern with residual 2. Episodic ventricu- lar tachycardia	1. Thrombosis 2. Coronary sclerosis 3. Large, thin- walled infarct

* Died

† Sacrificed

SUMMARY

Implantation of radioactive yttrium pellets (Y^{90}) into the ventricular myocardium will produce reasonably standard areas of necrosis.

Implantation of these pellets along the anterior descending branch of the left coronary artery can produce thrombosis, electrocardiographic indications of coronary insufficiency (and infarction), and myocardial infarction.

Figure 1A. The anterior surface of the ventricular myocardium of dog 265 showing 2 infarct sites 14 days after implantation of 1.0-mc Y^{90} pellets.

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Figure 1B. Microscopic central cross section (8 X) of one of the 14-day infarcts in dog 265. The area of necrosis and hemorrhage is surrounded by a layer of edema and cellulitis. In the fresh specimen the cellulitis, although not visible here, was distinct.

Figure 2. Microscopic central cross section (8 X) of 5-month-old infarct in dog 63. This is the full thickness of the myocardium, and the retraction is clearly visible.

Figure 3. The anterior descending branch of the left coronary artery of dog 594 is occluded by thrombosis. This is at the level of Y^{90} pellet implantation (6 months before). The wall is necrotized with medial hemorrhage. The surrounding area is fibrotic and hypocellular. Mag., 13 X.

Figure 4. A patent coronary artery obtained in dog 665 4 months after implantation of the Y⁹⁰ pellets. One wall of the vessel shows necrosis and medial hemorrhage with only partial interference with the lumen. A small branch below this is thrombosed and is being organized and vascularized. Well to the other side, the vein shows only surrounding fibrosis. Mag., 25 X.

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PLACENTAL TRANSFER OF RADIOACTIVE DIGITOXIN IN PREGNANT WOMEN AND ITS FETAL DISTRIBUTION^{*†}

By

G. T. Okita, E. J. Plotz,[‡] and M. E. Davis^{‡**}

The purposes of this study were to investigate the passage of labeled digitoxin across the placental barrier of pregnant women and to determine the relative concentration of both the unchanged drug and its metabolic products in the various fetal organs.

Four pregnant women who were hospitalized in the Chicago Lying-in Hospital were selected for this investigation. Three underwent therapeutic abortion of the fetus and one carried an anencephalic monster to delivery.

Uniformly labeled digitoxin⁽¹⁾ with a specific activity of 0.25 to 0.5 μ c per mg was administered intravenously to the four subjects. Three to 5 hours before hysterotomy, the 3 women were given the radioactive drug in doses ranging from 0.25 to 0.5 mg. A dose of 0.5 mg was administered to the fourth woman at 2 to 3 hours before the expected time of delivery. In this case, the fetal heart beat stopped during delivery, 2 hours after administration of the drug.

During hysterotomy, the amniotic sac was left intact. The sac was then rinsed with saline, and the fluid aspirated. Cord blood and various organs were taken from the aborted fetuses. Maternal blood samples were obtained immediately before administration of the labeled digitoxin and after removal of the fetus. Samples of uterine muscle were also obtained. Only maternal blood samples were taken from the patient whose pregnancy was almost full-term. Fetal blood was withdrawn by cardiac puncture. All tissue samples were removed as quickly as possible after expulsion of the fetus, rinsed thoroughly with saline, frozen at once with dry ice, and stored at -17°C . The extraction procedure for isolation of the unchanged drug from blood has been described previously.⁽²⁾ Isolation of the labeled drug from solid tissues was accomplished by means of a slightly modified procedure.⁽³⁾

All radioactive fractions other than unchanged digitoxin were combined and considered to represent the radioactivity from the metabolic products of the parent compound. An internal gas-flow Geiger counter was used to determine the radioactivity of the extracted fractions, with the exception of the original tissue residue from the homogenate fraction. All counts were corrected for background, self-absorption, and counter efficiency. The tis-

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** With the technical assistance of J. H. Curry, Jr., and F. D. Smith, Jr.

sue residue was combusted to carbon dioxide in a vacuum combustion line and then measured for radioactivity in an ionization chamber by means of a vibrating reed electrometer according to the method of Brownell and Lockhart.⁽⁴⁾ Recoveries of 96 ± 5 per cent were obtained.

The data in Table 1 indicate that the cardiac glycoside crossed the placental barrier as evidenced by its presence in the fetus between 1.7 to 5 hours after injection of the labeled drug to the mother. Less than 0.1 per cent of the injected dose was found as unchanged digitoxin and less than 0.33 per cent was found as its metabolites in the aborted fetuses. In the near-term fetus, 0.84 per cent appeared as the unchanged drug and 3.49 per cent as metabolic products. This difference may be attributable to the increase in size since there was no significant increase in the concentration on a tissue-weight basis.

Table 1
AMOUNT OF C¹⁴-DIGITOXIN AND ITS METABOLITES IN HUMAN FETUSES

Experiment	Dose (mg)	Approx. length of gestation (weeks)	Time after injection of drug (hrs)	Per cent of injected dose in fetus	
				Digitoxin	Metabolites
1	0.25	11	5	0.05	0.18
2	0.50	12	3.5	0.10	0.33
3	0.50	12	2.8	0.08	0.28
4	0.50	34	1.7	0.84	3.49

When the total radioactivity in the maternal and fetal body was calculated on an equivalent body weight basis, it was found that the near-term fetus had almost twice the concentration of the maternal body; whereas the 11- to 12-week fetuses had from 3 to 6 times that amount.

The distribution of labeled digitoxin and its metabolic products in the various organs of 11- to 12-week fetuses is shown in Figure 1. The fetal heart and kidney had relatively higher concentrations of the labeled drug and its metabolites than the other organs that were studied. In all organs there was a higher concentration of the metabolic products than there was of the parent compound. The presence of metabolites in the fetal tissues suggests either a metabolic conversion of the cardiac glycoside by the fetal tissue or passage of the metabolites across the placental barrier or perhaps both. The high metabolite to digitoxin ratio in the fetal liver may be an indication that the drug is detoxified in this organ. The passage of digitoxin across the blood-brain barrier correlates well with the known effects of digitalis on the central nervous system.

The concentration of digitoxin and its metabolites in the organs of the near-term fetus is shown in Figure 2. The concentration of the drug and its metabolites in the organs that were selected for study is lower in general than that found in the organs of the 11- to 12-week fetuses. Part of this difference may be accounted for if the concentration of the drug

Figure 1. Concentration of radioactive digitoxin and its metabolites in human fetal organs at 11 to 12 weeks of gestation. One-half mg of labeled digitoxin administered intravenously 2.8 to 5 hours before therapeutic abortion. Fetal tissues obtained from the patient receiving 0.25 mg were corrected for 0.5 mg dose. All figures represent the average value from tissues of three fetuses.

Figure 2. Concentration of radioactive digitoxin and its metabolites in human fetal organs at 34 weeks of gestation. One-half mg of labeled C^{14} -digitoxin administered intravenously 2 hours before death that occurred during delivery.

is expressed in terms of its distribution per cell since fetal cells are smaller in size during the 11th to 12th week of gestation than during the terminal stages of pregnancy.

It is of interest that the biliary excretion of digitoxin and its metabolites can be demonstrated in the unborn fetus by the presence of these compounds in the liver, gall bladder, and intestine.

It was not possible to make a direct comparison of the concentration of digitoxin in the maternal heart with that in the fetal heart. However, in a separate experiment, a sample of auricular appendage was obtained from a patient undergoing cardiac surgery who had been injected 3 hours previously with 0.5 mg of C¹⁴-digitoxin. A comparison of the concentration of the labeled drug and its metabolites in the fetal heart with that in the adult myocardial tissue is given in Table 2. The heart of the near-term fetus has approximately twice as much of the unchanged drug as the adult auricular appendage. The concentration in the hearts of the 11- to 12-week fetuses is approximately 10 times greater. Here

Table 2

AMOUNT OF C¹⁴-DIGITOXIN AND ITS METABOLITES IN HUMAN FETAL
AND ADULT AURICULAR APPENDAGE

Sample	Dose (mg)	Time after injection (hrs)	Digitoxin (μ g per 100 g tissue)	Metabolites (μ g eq. per 100 g tissue)
Fetal heart (12-week gestation)	0.5	2.8 - 5	11.6	42.5
Fetal heart (34-week gestation)	0.5	1.7	2.0	8.2
Adult auricular appendage	0.5	3	1.1	2.5

again this difference may possibly be explained on the basis of differences in cell size. Wollenberger *et al.*⁽⁵⁾ have postulated that mature and immature cells may have roughly the same number of drug molecules per cell, but differ on a tissue-weight basis. Therefore, Wollenberger believes that this may account for the findings that the heart of immature experimental animals is able to tolerate a larger dose of ouabain than that of mature animals.

If Wollenberger's findings can be extrapolated to human beings, it seems likely that the minute amount (less than 1 per cent) of digitoxin that crosses the human placenta may be considered to be nontoxic to the unborn child.

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PROTEINS IN MULTIPLE MYELOMA. V. SYNTHESIS AND EXCRETION OF BENCE-JONES PROTEIN^{*†}

By

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Data from previous studies^(1,2) in which the stable isotope N^{15} or C^{13} was given orally to patients with multiple myeloma revealed that Bence-Jones protein is not derived by cleavage of circulating serum proteins nor by degradation of tissue proteins and suggested that the abnormal urinary protein is formed rapidly *de novo* from free amino acids and is excreted rapidly. The rate of synthesis and excretion of the Bence-Jones protein could not be determined precisely with the stable isotopes, and, hence, lysine, uniformly labeled with C^{14} , was chosen for the rate study that is described in this paper.

A patient who excreted a large amount of Bence-Jones protein and who did not have an abnormal serum globulin was selected for this investigation. She was injected intravenously with the labeled lysine. The urine was collected by urethral catheter.

The serum and urinary proteins were characterized by electrophoresis and by ultracentrifugation.^(3,4) The Bence-Jones protein was precipitated at $4^{\circ}C$ by dialysis against 3 M ammonium sulfate at pH 5.2. The heavy precipitate was removed by centrifugation, washed twice with salt solution, dissolved in water, and then centrifuged until clear. The solution was then dialyzed against 0.9 per cent NaCl until free of sulfate ion. Aliquots that had been freed of chloride ion were lyophilized. The Bence-Jones protein crystallized after several changes with distilled water and dialysis in the cold. The crystals dissolved when brought to room temperature.

Prior to injection, the uniformly labeled L-lysine was tested for toxicity and sterility.

For determination of radioactivity, the lyophilized protein was dissolved in 0.05 N NaOH, deposited on oxidized copper planchets, and dried. A flow-gas counter was then used to determine the radioactivity on each planchet. Corrections were made for self-absorption. Determinations were also made of the specific and cumulative activities of exhaled CO_2 by Dr. G. T. Okita by means of a vibrating reed electrometer.

The amino acids in 5-ml samples of urine were decarboxylated by reaction with ninhydrin at $100^{\circ}C$ with 15 mg of glutamic acid as carrier to obtain an estimate of the excretion of the lysine that had been administered. The CO_2 was trapped with $Ba(OH)_2$ and counted as $BaCO_3$ on an aluminum planchet.

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The patient (MB), a severely anemic 64-year-old female with multiple myeloma, was admitted to the Argonne Cancer Research Hospital for study and terminal care. Roentgenographs revealed marked degenerative changes in the thoracic region of the spine. Transfusions were necessary to maintain the initial hemoglobin level of 7.4 g per cent. The serum proteins, which were normal at first, fell to a total protein of 4.8 g per cent with 1.7 g per cent globulins. Renal insufficiency was evident, and blood urea nitrogen and uric acid were both found to be elevated. The patient died of uremia 2 months after admission. Severe amyloidosis involving the entire gastro-intestinal tract, amyloid nephrosis, and diffuse marrow changes were observed at autopsy.*

Although the patient's protein intake varied considerably because of anorexia, she excreted daily an almost constant amount of Bence-Jones protein (about 10 g per day).

On the day that the experiment was begun, the patient was given sedation, and a Foley catheter was inserted. At this time 5 per cent glucose was given intravenously.** Then, over a 7-minute period she was injected intravenously with 300 μ c of L-lysine in 100 ml of pyrogen-free solution. A complete urine collection by catheter was made during the ensuing 13 hours. The catheter was then removed, but collections of urine were continued at intervals of 4, 6, or 12 hours until 90 hours had elapsed and 13 specimens had been obtained. A final specimen was taken at 13 days. An aliquot of each sample was frozen for later study and the rest was used immediately for isolation of the Bence-Jones protein.

Respiratory CO₂ samples were collected over the first 24-hour period.

Figure 1A demonstrates the absence in patient MB of a predominant globulin component of a type that has been found to be present in about two-thirds of the cases of multiple myeloma.⁽³⁾ Area analysis revealed that the serum proteins in this patient were distributed as follows: albumin 53.8 per cent; α_1 -globulin 5.3 per cent; α_2 -globulin 17.0 per cent; β -globulin 19.4 per cent; and γ -globulin 4.6 per cent. On the basis of these figures, the α_2 - and β -globulin were significantly elevated.

The Bence-Jones protein appeared to be quite homogeneous and moved with a single sharp boundary in electrophoresis and ultracentrifugation (Figures 1B and C). The protein formed regular crystals (Figure 2) in distilled water at 4°. These dissolved at room temperature.

Application of the dinitrophenyl (DNP) method for analysis of the amino end-group revealed only trace amounts of ether soluble DNP-amino acids. Hence, the protein is one of a group of crystallizable Bence-Jones proteins that are either devoid of end-groups that are detectable by the DNP method or contain N-terminal amino acids that yield water soluble DNP-derivatives.⁽⁵⁾

At pH 8.6 in veronal buffer the Bence-Jones protein had a mobility of -2.6×10^{-5}

* We are indebted to Drs. Robert Wissler and Benjamin Spargo of the Dept. of Pathology, The University of Chicago, for the autopsy report.

** Dr. Milton Weiner aided in devising the protocol and in medical management of the patient, under the direction of Drs. Robert Hästerlik and George V. LeRoy.

Figure 1. Electrophoretic diagrams of the serum proteins (A) of subject MB and of a specimen of the radioactive Bence-Jones protein (B), and ultracentrifugal diagrams of the latter protein (C). All experiments were performed in veronal buffer pH 8.6, ionic strength 0.1. The arrows in A and B indicate the origin and direction of migration. Since the photographs of A and B were taken after 200 minutes at the same current (16 ma), the mobilities can be compared by visual inspection. In C, sedimentation was from right to left, with photographs taken at 32-minute intervals at a rotor speed of 59,780 rpm,

Figure 2. Photomicrograph of crystals of Bence-Jones protein from patient MB. Mag. 53X.

$\text{cm}^2 \text{volt}^{-1} \text{sec}^{-1}$ and a sedimentation constant of 3.4 Svedberg units.

The specific activity curve for the protein is shown in Figure 3. The first specimen, obtained at 30 minutes after injection of the labeled glycine, was devoid of radioactivity. Thereafter, however, there was a sharp rise in radioactivity which reached a peak at 9.5 hours. After that time, there was a steep drop and then a gradual tapering off. The declining curve appeared to have two logarithmic segments. The first, terminating at 36 hours, corresponds to a half-time of 15 hours; and the second, terminating at 90 hours, corresponds to one of about 60 hours.

Figure 3. Specific activity-time curves of the Bence-Jones protein isolated from the urine of subject MB and of the BaCO_3 obtained by reaction of the urine with ninhydrin. The initial sample of urine, collected by catheter for the first 30 minutes after injection, proved to be inactive and is not represented on the curve.

The rate of excretion of the labeled protein is a function of the urinary volume and protein concentration as well as the specific activity. For this patient, the balance is incomplete because collection of urine was terminated on the 4th day. The hourly rate was however calculated during the first 90 hours from the total protein recovered in each specimen and from its radioactivity.

Of the total activity eliminated during the 90-hour period, 19 per cent was excreted between 9 and 13 hours after injection, and 45 per cent at 5 to 17 hours afterward. It is thus estimated that the latter amount is as great as that excreted in the 12-day period following the time that is shown in Figure 3.

Two dialysis experiments were performed to test whether the adsorption of lysine by proteins might give spurious results in this tracer study of protein synthesis.⁽⁶⁾ The data shown in Table 1 for experiment I indicate that dialysis, without lysine outside the sac, effectively reduces the adsorbed radioactivity to a negligible quantity. In experiment II (Ta-

Table 1
TEST FOR ADSORPTION OF LYSINE-C¹⁴ BY BENCE-JONES PROTEIN

Experiment number	Dialysate	No. of changes	Cpm per mg. protein
I. Unlabeled protein and lysine-C ¹⁴ change dialysis sac.	0.15 M NaCl	10	11
Control	0.15 M NaCl	4	6.6
	No dialysis	--	21,500
II. Labeled protein	10 mg per cent lysine in 0.15 M NaCl	3	
	0.15 M NaCl	2	518
Control	No dialysis		525

ble 1), there was no significant change in the specific activity of the labeled protein upon dialysis against free, inactive lysine.

In addition, no activity above background was found in BaCO₃ obtained from the reaction of ninhydrin in a solution containing labeled protein and inactive carrier amino acid, whereas the specific activity of BaCO₃ obtained from ninhydrin decarboxylation of the same specimen of protein (and carrier) after hydrolysis was 696 cpm at infinite sample thickness. From these findings it was concluded that the Bence-Jones protein was labeled only by incorporation of the isotopic lysine through the peptide bond.⁽⁵⁾

As shown in Figure 4, the expiration of C¹⁴O₂ was rather rapid initially and reached a maximum at 2.5 hours after injection of the C¹⁴-labeled lysine. The decline in radioactivity was almost exponential for the first 8 hours thereafter, and then there was a slower nonlogarithmic decrease in specific activity. The cumulative activity of the expiratory C¹⁴O₂ continued to rise for 24 hours, when about 3.9 per cent of the injected radioactivity had been exhaled.

It appears that the first steps in the metabolism of the carbon chain of lysine involve deamination with subsequent oxidative decarboxylation yielding CO₂,⁽⁷⁾ which would become evident fairly soon in the expired CO₂. This series of reactions results in the formation of glutaric acid, of which the further metabolism is unknown.

In a patient that had been injected with glutamic acid-1-C¹⁴ instead of labeled lysine to learn whether this might be a better compound to use to study protein synthesis, the specific activity of the expiratory C¹⁴O₂ was maximal within the first half hour, two hours earlier than was the case with lysine. In addition, the maximal specific activity of the C¹⁴ was about four times greater in the glutamic acid-treated subject, although both patients had received equivalent activity of the natural isomer (Figure 4). However, the maximum specific activity of the Bence-Jones protein from the patient given lysine was seven times greater than that of the one given glutamic acid.

Figure 4. Comparison of the specific activity-time curves for the expiratory CO_2 in two subjects, the one (MB) given $300 \mu\text{c}$ of L-lysine C^{14} , the other, injected with ca. $450 \mu\text{c}$ of DL-glutamic acid-1- C^{14} .

The blood level of radioactivity is maintained longer when lysine is used so that the utilization of this compound leads to a more efficient incorporation into the protein than does glutamic acid. On the other hand, glutamic acid-1- C^{14} gives a more immediate picture of protein synthesis.

The data from this study of the lysine-injected patient give a more complete picture of the rate of formation and excretion of the Bence-Jones protein than do the data from studies of two patients who were injected respectively with N^{15} or C^{13} labeled glycine^(1,2) but are not complete enough to permit an accurate calculation of the kinetics of these processes. The rate of change in the radioactivity of the Bence-Jones protein is a function of at least four factors: 1) the rate of decline in the specific activity of the free lysine; 2) the rate of synthesis of the protein; 3) the rate of its release into the circulation; and 4) the rate of renal excretion. Because the first urine sample was nonradioactive, the sum of the last processes requires only about 30 minutes. Therefore, the length of time for the synthesis and excretion of Bence-Jones protein is about as long as the rate of synthesis of the serum proteins, which have a total turnover time of from 5 to 15 days in the human being. The rapid decline in the radioactivity (Figure 3) of the Bence-Jones protein indicates that it cannot be derived from the serum or other preformed tissue proteins.

Since the specific radioactivities of the urinary amino acid, the Bence-Jones protein (Figure 3), and the expired CO_2 (Figure 4) do not decline exponentially and the declining portions of the curves are quite similar, it can be concluded that the Bence-Jones protein was formed de novo from the administered amino acid. The data in Figure 3 also suggest

that the Bence-Jones protein is not withheld selectively by the kidney, a finding that is compatible with the fact that N^{15} is excreted as rapidly by way of incorporation into Bence-Jones protein as by way of the urine.⁽²⁾ A comparison of Figures 3 and 4 reveals that lysine is metabolized to CO_2 6 hours sooner than it is incorporated into the protein.

Fifty per cent of the radioactivity excreted by way of the labeled protein within the first 90 hours and about 25 per cent of that so excreted during the first 2 weeks was voided within the 12-hour interval that began at 5 hours after injection. Hence, the bulk of the Bence-Jones protein synthesized and released into the circulation within any given interval is excreted in the ensuing 12 hours so that the circulating protein does not participate actively in the metabolic pool of nitrogen. The sharp and rapid decline in the curve of Figure 3 precludes the presence of a large pool of Bence-Jones protein.

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AN INTERPRETATION OF ORGAN WEIGHT CHANGE INDUCED BY TOTAL-BODY IRRADIATION*

By

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INTRODUCTION

Preliminary to the therapeutic application of the high energy proton beam from the University of Chicago synchrocyclotron, we measured the RBE of these protons as a function of their penetration in water. The physical properties of such radiation, and a description of the instrumentation used, will be reported at a later time.⁽¹⁾ Organ weight change in mice was chosen as the indicator of radiation effect; in particular, weight loss by the thymus and spleen⁽²⁾ 5 days after total-body exposure. The purpose of this paper is to discuss the principles of this type of experiment with some attempt at generality.

THE IDEAL INDICATOR

An indicator, or indicating system, is necessary to show a relation between the quantity of radiation absorbed and the magnitude of the radiation effect. An "ideal indicator" for radiological end-points is defined as a biological system that is subject to radiation damage and in which the damage can be assayed in an unambiguous and easily reproducible way. Further, it is one in which the damage is such that the term "universality" can be applied: that is, an interpretation of the damage can be made in terms of parameters that are common to a large class of systems, even though the magnitudes of these parameters may vary from one system to another. The number of such parameters should be minimal (since given enough arbitrary constants any experiment can be "explained"), preferably subject to individual variation, and quantitatively measurable. This is a rather large order for biology, and such an ideal indicator for the biologic effects of radiation probably does not exist. But if it did, it would probably take the form of a homogeneous aggregate of cells subject to injury by radiation and connected to a reservoir of foodstuffs and information (stimuli) which is not affected by the radiation. A bacterial suspension with cells transferred after exposure to a nonirradiated nutrient medium possibly satisfies the requirements, but certainly lacks universality if organized mammalian tissues are classed in the same universe as the suspension. The suspension is not an aggregate in the sense of tissue as an organized collection: the effect of radiation on tissue could be an impairment of cellular ability to adhere to surrounding cells and of the ability to func-

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tion as a unit rather than any great damage to the individual cells as entities. The damage of interest is therefore to a property that single-celled suspensions do not possess.

Another possible approximation to the above requirements might be found in an experiment in which a part of the tissue (say a functioning organ) of an animal is exposed separately, and observation is made of the loss of function of that organ. The animal minus this organ is then supposed to represent the infinite, uninjured reservoir, and the organ, the stressed tissue. Again, the results are ambiguous so far as interpretation of the radiation effect on that organ is concerned: the rest of the animal body uses, if the organ was indeed functioning, information from the organ and in turn feeds back information to it.* But of course, the information fed back will be a function of the signal received, so that the reservoir is affected by the radiation. This effect can be minimized presumably by using organs that are weakly interacting with the rest of the body—"weakly interacting" being intuitively clear but operationally undefined. The choice of thymus and spleen, for example, reflects only a hope that the interaction is weak, since the function of these organs is incompletely understood. (The apparent extreme susceptibility to radiation injury of thymus and spleen remains the most attractive feature for the choice of these as indicators.) Further, the use of weight change may not necessarily show function change, only contributing to the confusion. A carcinoma might represent just such a weakly interacting aggregate; it draws material from the rest of the body without—in a short enough time so that the reservoir is not essentially depleted—furnishing information itself. However, even in this case, there is evidence that the interaction is two-way and not necessarily weak. For example, there is a rapid increase in liver catalase when a tumor is implanted in a mouse tail.

In this sense, total-body exposure with special organ weight change as end-point is probably the poorest choice of approximation. The initial effect of the exposure is obscured by recovery factors operating not only in the tissue cells of interest, which we would want to observe and which are present even in the bacterial cells mentioned above, but operating throughout the body of the animal. These factors are, no doubt, complicated enough so that without a detailed understanding of the total-body function, they cannot be described with any coherency. (Moreover, if such a detailed knowledge were at hand, it would not be necessary to use such a gross probe as radiation to gather information if the viewpoint is that of a worker who wishes to use the radiation as a tool to study biologic phenomena.) We would expect that, in this case, the end-point parameter, organ weight change after exposure, would be a rather complicated function of the time after exposure. Also, after the initial injury to the total body, the time dependence of the recovery pattern should be a function of all of the parameters that govern the normal operating characteristic of the

* The word "information," analogous to "stimuli" is used here in much the same sense, although not so mathematically precise, as in the by now well-known Shannon-Weaver communication theory. It is a generalized signal, here, of uncertain origin, and of unknown chemical description, which is "sent" to the organ; the organ responds to the signal by growing, say, and sends out information of its own.

body. Therefore, there should be different patterns for differences in strains or sex, for example. These are all elementary remarks; the point, that radiation injury is a symptom complex involving the entire organism, even when the injury route is localized; is belabored possibly more than it is worth. It is stressed because of the ubiquitous appearance in the literature of empirically fit curves, containing rather accurately determined "constants" to data taken under conditions that may sensitively affect the data but in a manner that is not understood. The precision (and lack of reproducibility within the limits of that precision from one laboratory to the next) of these empirical constants may serve only to obscure the basic mechanisms, rather than to enhance our understanding.

THE RECOVERY PATTERN: EXPERIMENTAL

At least two carefully done experiments published recently are pertinent to this discussion.^(3,4)

Kohn and Kallman found that mouse thymic weight, measured 5 days after X irradiation, decreased with dose in a fashion similar to earlier results.⁽²⁾ However, when they measured the weight at different times after total-body exposure they found a maximum effect that appeared at a variable time after the initial irradiation depending on the dose: about 1 day for maximum loss for exposures below 100 r to 5 days for those above 400 r. They propose, indeed, that a more rational end-point than the "five-day weight" (for the thymus) would be the minimum weight of the organ at any time after exposure. This has in point of fact, the virtue of making the results considerably less sensitive to extraneous environmental changes. The result, in this case, is a rather complicated analytical expression for the dose-effect relation, e.g., not an exponential, but which the authors claim to represent the physiologic response more sensibly. Some of their results are shown in Figure 1A.

Grahn finds highly significant data that indicate pronounced differences between the weight response of a given organ when sex differences in any strain, or strains, are compared. His data show, generally, an effect that increases with the time of observation after the initial exposure until a maximum effect is reached at a time that is roughly the same for each strain, and of the same relative value, and then diminishes at a rate that is dependent upon the strain being treated. This finding is not inconsistent with Kohn and Kallman's result that the dose needed to produce the effect, if maximum effect is used as end-point, was the same for the two strains they tested.

In other words, the initial effect (on thymus) appears to be not inconsistent with the assumption that it is insensitive to strain. After a period of time, depending on the properties of the organ itself and on—we speculate—which recovery mechanisms have been affected by the radiation, the effects diverge when such differences as age, sex, and strain of the animals are compared. It thus appears that the least ambiguous end-point, if organ weight changes resulting from total-body exposure are to be used, should be the weight at a time early enough so that recovery processes have not had time to take over significantly. In the case of thymus, where some recovery appears even after the first day, this

Figure 1. (A) Thymus weight as a function of time after exposure to 200 or 400 r of 250 kv X rays, from the data of Kohn and Kallman. Each point on the 200-r curve has been given a constant 15 per cent standard deviation, consistent with the rest of their paper. The smooth curve is calculated according to the model described in the text. (B) Thymus and spleen weight as in (A). The standard deviations are not shown but are about 20 per cent at each point. The smooth curves are fit by eye.

may not be practical.

The data of these experiments⁽³⁾ are nearly in agreement with similar, although less elaborately designed, studies of our own. A population of 160 CF No. 1 male mice, 7 to 8 weeks old, was randomized by selection of body weight within one standard deviation from the mean weight. The animals were then divided into three groups of 50, 50, and 60 mem-

bers each. The two groups of 50 were exposed to 250 kv X radiation, using conventional techniques for the exposure; group 1 receiving 200 r and group 2, 400 r. The relative weight loss of spleen and thymus was then followed for 5 days after exposure, and the data were recorded as organ weight on a given day divided by organ weight of the unexposed control animal on the same day. (Actually, at this age, there is little change in the weight of the unexposed organ over a 5-day period; variance analysis of the control weights showed that there was no significant difference between the different age control groups, and they were therefore lumped together as a single population.) A group of 50 was housed in 5 cages with 10 animals per cage. On each day, animals were taken at random from each of the 5 cages. On each day, excision of the organs was done, as nearly as possible, at the same time. The results are given in Figure 1B, which shows that the greatest weight loss occurs within the first day after exposure, the maximum effect is reached in 2 to 3 days, and thereafter recovery sets in. The maximum effect occurs in spleen at 2 days after exposure to 200 r and roughly 4 days after exposure to 400 r. There is some indication that the time for maximum effect is greater at 400 than at 200 r for thymus, but the statistical uncertainties do not permit a firm conclusion. The initial slopes of the curves are too great to measure with even reasonable accuracy; they increase with exposure. The weight recovery pattern for thymus is significantly different in shape from that for spleen.

A SIMPLE MODEL FOR RECOVERY

Properties of the Model

The simplest physical model available will be chosen and used until a better one appears. Although it is non-unique, such a model may prove useful to collate diverse experimental phenomena.

Consider a gland like the thymus and its growth pattern from the time of conception of the animal. In the early stages of the fetal growth, the organ has not yet been formed to a degree where it can properly be called an organ. At some time after conception the organ tissue, which may still consist of a relatively small number of cells, is differentiated enough so that it may, perhaps, be viewed as a homogeneous, localized aggregate that grows in an infinite medium containing nutrient and furnishing information for growth, but which is—in the concept of the model—itself not affected by the organ growth. In this view—which is possibly superficial—the growth of the aggregate will be a linear function of the surface area available for the diffusion of nutrient into the interior, i.e., in this growth phase, the rate of change of weight at a given time is proportional roughly to the $2/3$ -power of the weight at that time. (This ignores, for example, any vascular development in the organ.) This would give the result that the weight itself is proportional to the cube of the time, measured from some ill-defined zero time but which is, no doubt, some time between conception and birth. Actually, not all of the organ material remains viable (as organ tissue) after formation, and the growth rate is reduced by a term that is probably roughly proportional to the weight. Furthermore, as the organ increases in size, the inner cells do not get their full share of materials diffusing through the surface, and therefore such cells in-

crease in number (in the case of a tumor implant they actually become necrotic) at a smaller rate than $w^{2/3}$. Also, the total rate may be increased by a constant source term whose magnitude and origin lie in the nature of the embryonic growth. However, in this initial phase it is plausible to put the weight of the organ as proportional to t^n where t is the time after the organ is first recognizable as an entity and n is of the order of 3, but may be more or less than 3 depending on the magnitudes of the other-than-surface-terms.

Some careful observations on growth by Reinhardt⁽⁵⁾ may be cited in this connection. Using the Long-Evans strain of rat, he measured organ weights (thymus, mesenteric lymph nodes, spleen, and cervical nodes) from birth to old age. He found that the thymus reached maximum size at an age of about 7 weeks (about when sexual maturity is attained) and then decreased to eventual extreme involution with a great weight loss. The other tissues approached their maximum weight with somewhat slower rates (in the case of spleen to about 90 per cent of maximum in 14 weeks) and stayed relatively constant thereafter. The weight of thymus 1 day after birth, for example, was about 3 per cent of the maximum weight observed, as was also the case for splenic weight. Since the weight at birth of these organs is so very small, it probably will not be a very drastic approximation in what follows to take the time of birth as corresponding to zero weight and to ignore completely what happens pre-natally. The point of the above discussion is simply to make clear that at least two distinctly different growth phases may be realized; while the second takes over smoothly where the first leaves off, it must be understood that an expression that describes the second phase must not be extrapolated back to times earlier than that for which it is defined.

Evidently what is required of the model for growth in the "regulated" phase, i.e., after birth, is a single expression that will give a period of rapid growth up to a maximum weight after which the weight decreases. It should be possible for the model to yield either a time for maximum weight so late that the point is never reached and the organ appears to approach slowly an equilibrium size, or so early that the organ declines in size soon after it reaches its maximum. Presumably, the size is correlated in some way with function, but a functional description will undoubtedly be formidably complicated even when the function is clearly understood, and we want to describe the growth only in terms of phenomenological parameters.

A simple model satisfying these requirements could be the following: After birth of the whole system, the organ may be considered to be the analog of an electrical capacitor, with a small amount of charge on its plates; the capacitor is shunted by a resistor, and if nothing is done to prevent it, the charge will leak off through the resistor. Biologically, this is equivalent to saying that the organ cells ("charge") slough off in a random fashion, and therefore at a rate proportional to their number. The constant of proportionality is a combination of a property of the organ itself ("capacitance") and a property of the external medium, including perhaps any wall or membrane surrounding the organ ("resistance").

If w is the organ weight at any time t , then the differential equation governing the decay will be

$$\frac{dw}{dt} + \frac{w}{\tau} = 0 \quad (1)$$

where τ is the time constant of the electrical analog and must be determined empirically. Now suppose that a part of the whole system, the body of the animal, is considered to be a signal generator (reservoir) that furnishes information (stimuli, vital material) to the organ. At some time t_0 , which may coincide with birth, this signal is turned on. Depending on which organ is considered, and just what use the body finds for it, the signal may be a pulse of finite duration, or a step function that is turned on and stays on. For simplicity, the signal may be taken as an exponential voltage pulse with a characteristic time that may be either short or infinitely long. With this signal applied, equation (1) becomes

$$\frac{dw}{dt} + \frac{w}{\tau} = \frac{w_0}{\tau} - t/\tau_s \quad (2)$$

where w/τ is proportional to the signal strength, and τ_s is its duration. It should be pointed out here that the model, equation (2), is not unique in interpretation, except in a very general way. In general, it has the form

$$(\text{rate of growth}) = (\text{rate of flow in}) - (\text{rate of flow out})$$

that is, of course, characteristic of a large class of biologic and thermodynamic phenomena. The "in" term, or source, has been specified as an exponential pulse (which includes in the limit as $\tau_s \rightarrow \infty$ a constant source) but it could also be, for example, a constant plus a variable source. The exponential source may be interpreted as, instead of a pulse of "information," rather a source of material that diminishes in strength as the animal grows older because of the failure of some other regulatory mechanism. The constant source could be an infinite supply of chemicals: infinite meaning that it is either independent of the well-being of the rest of the animal or that it is so intimately associated with the rest of the animal that it is itself replenished at the same rate as it is being used. (In the first instance the organ could, perhaps, continue to grow even in the extreme case that the animal dies. In the second case, the supply would be turned off immediately upon death, or perhaps even at the onset of disease.) An obvious addition to equation (2) might be a feed-back term, which could introduce higher derivatives of the weight.

It should also be noted that the time t_0 , after which equation (2) is supposed to be valid, is taken to be a point in time. More realistically, one should consider a time interval during which a chain of reactions takes place, presumably, and the system approaches more and more closely to a point where a new "information center" can be conceived unambiguously.

To return to equation (2); let w_0 be the weight of the organ at time t_0 and let time be measured from $t_0 = 0$. For easier notation, let $\lambda_s = 1/\tau_s$, $\lambda = 1/\tau$. Integrating, we get

$$w = \frac{W}{1 - \frac{\lambda_s}{\lambda}} (e^{-\lambda_s t} - e^{-\lambda t}) + w_0 \quad (3)$$

As stated above, it is consistent with Reinhardt's data to take zero time at birth and $w_0 = 0$ as a simplification; t in equation (3) is then the age of the animal. The disquieting feature of equation (3), that it predicts negative weight before birth can be comfortably ignored, since it is not defined before birth anyway and the mathematical approximation will not very much affect our conclusions.

At best it is possible to make only a rough estimate of the parameters in this expression. A guessed fit to the Kohn and Kallman thymus data for normal growth is shown in Figure 2. The numbers will serve only to give a useful orientation on the magnitudes and

Figure 2. Growth of thymus in CAF_1 female mice, from Kohn and Kallman. The vertical dashes are a measure of standard deviation, with the point at 45 days arbitrarily set equal to unity. The smooth curve is given by $w = 2.6 (\exp(-t/75) - \exp(-t/25))$ and is not a best fit.

are not to be taken as accurately determined constants. However the time $\tau = 25$ days is probably not too far from reality and will be used later. It is clear from Figure 2 that the growth expression represents the data after a fashion. The model does indeed have qualitative predictive value, but any statement other than this general one is not warranted because of both the paucity of data and the cursory nature of the concepts used. However, it will be shown below that the model can be used to collate certain results in radiological experiments.

Now suppose that at time T the total body is irradiated for a short time interval. Let the material in the organ that has been damaged be in the amount x and the undamaged ma-

terial be y so that at any subsequent time $x + y = w(t)$. In particular, let

$$\begin{aligned} x(T) &= (1 - a)w(T) \\ y(T) &= a w(T) \end{aligned} \quad (4)$$

The function $a = a(D)$, where D is the delivered dose, is the undamaged fraction of organ material; one might have, for example, $a = \exp(-pD)$ where p is a constant. We expect that the damaged material should be lost at a different rate than undamaged material, probably at a greater rate but not necessarily so. (If this were not the case, in a dynamic system such as the one we are discussing, then there would be no effect at all.) Then the loss rate for damaged material may be written as

$$\frac{dx}{dt} = -\lambda_D x \quad (5)$$

where the rate of loss for damaged material, λ_D , we will call the "sloughing-off rate." For the undamaged material we have the same form as equation (2)

$$\frac{dy}{dt} = -\frac{y - w_e}{\tau} - \lambda_s t \quad (5')$$

Let $w_c(t)$ be the weight of an identical but unexposed control organ given by equation (3). We make the assumption that the values of the parameters (λ , λ_s , W) are not affected by the radiation; this is already implicit in (5'). Then

$$\frac{dw_c}{dt} + \lambda w_c = \frac{dw_c}{dt} = \lambda w_c \quad (6)$$

After integration of equations (5) and (6), and substitution of the condition of equation (4), we get for $t \geq T$ the observed surviving fraction of organ weight S as

$$S = \frac{w(t)}{w_c(t)} = 1 - (1 - a)f(\delta, T) \quad (7)$$

where $\delta = t - T$ is the time of observation after the exposure, and

$$f = \frac{w_c(T)}{w_c(T+\delta)} (e^{-\lambda \delta} - e^{-\lambda_D \delta}) \quad (8)$$

Note that equation (7) shows that unless the times T , δ , can be chosen to give $f = 1$, one might conclude⁽⁴⁾ incorrectly, or at any rate inconsistently with this model as an alternative, that the organ consisted of an "insensitive fraction" and a remainder that is sensitive to the radiation.

In this model, the surviving fraction depends not only on δ , the time after exposure, but also on the age at exposure and on recovery factors normally present as growth parameters. More important, in a total-body exposure, the characteristic parameters (λ , λ_s , W) will almost certainly change; indeed, characterizing the change by only three parameters is rather shabby treatment of the formidable array of both subtle and gross radiation-induced biochemical changes described in the review literature.⁽⁶⁾ Nevertheless,

for the moment, we may proceed as if these long range ("abscopal" in Mole's descriptive word)⁽⁷⁾ effects did not exist, in order to get a qualitative idea of what the time dependence of the survival might look like.

Let us, then, investigate briefly the properties of the time dependence. In a typical experiment⁽¹⁻⁴⁾ the exposure might be made at a time T when the organ is at or near its maximum size; and therefore when the weight is slowly changing. The value of the maximum is such that for the times δ , of interest—say less than 10 days for thymus—only a few per cent error will be made (see Figure 2) by putting $w_c(T + \delta) = w_c(T)$. The error introduced will be to slightly over-estimate the recovery effect. Then, from equation (8), the time dependent factor becomes

$$f = e^{-\lambda \delta} - e^{-\lambda_D \delta} \quad (9)$$

and is therefore independent of the age of the animal at the time of exposure so long as the conditions of the approximation are not violated. This has an extreme value (S has a minimum if $\lambda_D > \lambda$) at the time

$$\Delta = \tau \frac{r}{r-1} \log r \quad (10)$$

where $r = \tau_D/\tau$ and the value of f at this time is

$$f_e = (1 - r) r^{\left(\frac{r}{1-r}\right)} \quad (11)$$

For times such that $\lambda_D \delta$ and $\lambda \delta$ are small compared with unity

$$f = +(\lambda_D - \lambda) \delta = \frac{\delta}{\tau} \frac{1-r}{r} \quad (12)$$

so that the initial time dependence is linear. In the case that $r \ll 1$ (the rate of growth is much smaller than the sloughing rate), the maximum appears at a time nearly equal to the relaxation time for sloughing, τ_D , and in this case also f_e approaches unity. Therefore, in this case only $S_e = a(D)$. That is, if $\tau_D \ll \tau$, the minimum value in the time survival approximates the dose response of the organ. (Of course $f_e = 1$ exactly only when $\tau_D = 0$, all the damaged material is lost immediately after exposure.)

In another extreme case, the age at exposure and also the age when the weight observation is made may be taken when the organ is still growing in a rapid and linear phase. (Take $\lambda_g = 0$.) Under this approximation $\lambda \delta$, $\lambda_D \delta$, and λT are all much less than unity. Then after some simple algebra, $w_c(T + \delta)/w_c(T) \approx 1 + \delta/T$ and $f = (\lambda_D - \lambda) \delta \cdot T/(T + \delta)$. In this case there should be only a small observed effect, since $f \ll 1$ by these assumptions; at high doses, the surviving weight for a given δ will level off to a fraction near unity (i.e., to $1 - f$). More generally, if exposure is made on very young animals, but the mathematical approximation is not good, then at high doses there may be less effect observed than if the exposure were made when the organ is mature, even though the initial dose sensitivity may be greater for the young organ.

Application and Discussion

The result of the first approximate case above, that the minimum of the survival versus time curves tends to measure directly the dose response of the organ, is consistent with Kohn and Kallman's⁽³⁾ suggestion (see The Recovery Pattern: Experimental). But it is consistent only in a very restricted sense, even apart from the mathematical approximation. The parameters τ_s , W and to a lesser extent τ are to be viewed as characterizing the whole animal, and although this effect was ignored in the above analysis, we have no way of knowing, at present, just how dose dependent these parameters are. If they change significantly on irradiation, then equation (7) will no longer be valid; w_c in (7) should be replaced with a w_D , which has the same form as w_c but with different values for the parameters. The function f will have a much more complicated form than (8) and it will no longer, in general, be true that even for $\tau_D \ll \tau$ is the extreme value of f nearly equal to unity. Then we will no longer be measuring the direct effect on the organ, which we choose to call the function $a(D)$. In fact, the Kallman and Kohn result (Figure 1) that the time when the minimum survival appears is dose dependent shows immediately that the assumption that the critical parameters are independent of dose is not strictly true for thymus, although in this particular case the approximation is evidently not very drastic. Far from being simple, in this situation, the minimum survival fraction involves at least three growth parameters, the dose dependency of these parameters, the sloughing rate, the age at exposure and, of course, the desired dose parameter p (under the pleasant but unlikely assumption that the direct response is a simple exponential) a total of at least nine. This is already too large a number to be determined in a single experiment with any confidence in the result, even if we grant the essential correctness of the model.

The smooth curve through the 200 r data of Figure 1 is calculated using $S = 1 - 0.93 [\exp(-\delta/25) - \exp(-\delta/0.83)]$. Here the number $0.93 = 1 - a$ is obtained by normalizing at the minimum point and the sloughing time $\tau_D = 0.83$ days is obtained by observing that the minimum in S is fairly well-defined and occurs at 3 days after exposure. Unfortunately, since $\tau_D \ll \tau$ the curve is rather insensitive to the precise value of τ , and therefore these data represent only a fair check on the model. However, the general features of the data are rather well represented; in fact, the deviations from the model calculation are surprisingly small if it is considered that the condition (τ, τ_s, W) be independent of dose is not well satisfied. The 400 r curve shows a minimum at 4 days instead of at 3 days. With the parameter values used, the maximum value $f_e = 0.87$ (i.e., at 3 days). This means that survival at 3 days versus dose should level off at high doses to give a constant fraction of 13 per cent of the organ weight, which apparently is not sensitive to radiation. Kohn and Kallman's data do not show a constant fraction; they do show two distinctly different sensitivities, and the least sensitive fraction indeed appears to be of the order of 10 to 20 per cent abundant. The fact that there is no completely insensitive part can be explained away by the fact that the growth parameters are evidently themselves slowly varying functions of dose.

Another example of data analysis by means of the model is given by the results of

Storer and Langham⁽⁸⁾ on the regression of testis measured at 4 weeks after exposure. They used CF No. 1 mice, 4 to 5 months of age. (Their published data utilized the regression of testis weight on total-body weight, which is not included, in any obvious way, in the present theory; however this should have only a second order effect on the shape of the resulting curve.) Their data are plotted in Figure 3, and a fair (not "best") fit to the data* is given by $S = 0.38 + 0.62 \exp(-D/192)$ with D the dose in roentgens.

Figure 3. Testes surviving fraction versus dose, from Storer and Langham. Observation made on 4- to 5-month animals, 28 days after exposure. The smooth curve is $S = 0.38 + 0.62 \exp(-D/192)$.

Some data are given in Figure 4 that show the growth of testis in CF No. 1 mice. Points for ages greater than an average of 5-1/2 weeks represent animals taken from a common pool that were initially between 5 and 6 weeks old. They were housed in groups of 10 in a cage and were selected randomly for sacrifice from each cage. The earlier

* These authors fit a logarithmic dependence to their data, of the form $S = a - b \log pD$ where a, b, p are constants, two of them independent. This is a rather unsatisfactory procedure, esthetically at least, since the form implies that the organ weight for zero dose was infinite, while at dose larger than some constant the weight goes negative. It turns out that the solution given in the text for this case, $S = 1 - f + f \exp(-pD)$, looks like a straight line when plotted on $\log(\text{dose})$ paper for values of dose near $1/p$. For if $|pD - 1| < 1$ then

$$S = 1 - f(1 - e) - fe(pD - 1)$$

while if $S = a - b \log pD$ then

$$S = a - b(pD - 1).$$

Hence if $a = 1 - f(1 - e)$ and $fe = b$ then near $D = 1/p$ the two relations are closely the same.

Figure 4. The weight of testes in CF No. 1 mice versus age of the mouse. The vertical dashes are the standard error of estimate and the horizontal dashes indicate that the age at each point is uncertain by at least one-half week. The smooth curve is $W = 200 (1 - \exp(-t/9))$.

points were made from fewer mice, taken from the offspring of a group of pregnant females purchased for this purpose; randomization for these was probably not very good since only 3 litters are represented. (Some replicate points [shown as x's] were taken from controls for other experiments in the laboratory.) Furthermore, even for the animals that were all started at 5 to 6 weeks, the age distribution at each point is critical. This curve is presented as a qualitative picture, rather than as good data. We take, as promised in the preceding sections, the zero of time at birth, and it would appear from the data that this can lead to no large error. Then at least squares fit for the time τ , assuming that the variation in the exponential source is negligible ($\lambda_s = 0$) results in $\tau = 9 \pm 2$ weeks. (It should be made clear that the intention is only to show that observation is not inconsistent with the model, rather than to make accurate predictions.) The variation of $w(T)/W(T+\delta)$ for the mature animals used by Storer and Langham is also negligible; then the value of τ_D , the sloughing time can be estimated. The regression on dose levels off to a constant fraction of about 0.38 at large dose. The value of f at 4 weeks (the time of observation) should then be given by $1 - 0.38 = 0.62$. Then substituting in the expression for f (equation 8) with $\tau = 9$ weeks and solving for τ_D we get $\tau_D = 1.3$ weeks. These numbers in turn substituted into equation (10) give a time for maximum testis atrophy at 3.2 weeks; the observed time for maximum effect has been stated^(3,8) to be about 4 weeks.

While this is hardly better than fair agreement, it should be noted that a 3 per cent error in the measured "constant insensitive fraction" produces about 15 per cent error in the predicted value of the time of maximum.

The evidence that the data for testis are at least potentially fittable by using dose independent growth parameters is a possible argument in favor of using testis as a standard radiological indicator, as suggested by at least one worker.⁽⁹⁾ The weight regression in this case appears to be a function of the dose delivered to the organ only.

CONCLUSIONS

It should be emphasized that the two examples discussed above are to be taken only as provocative of thought along the lines of data interpretation by means of normally present quantitative growth relations. The data that now exist have not been taken with a view toward verifying any such theory, and, indeed, the variable nature of the biologic material and the sensitivity of the model predictions to quantitative errors will make this difficult. The existence of the case (thymus) where the parameters are dose dependent means also that the detailed theory (equation (4) onward) will have to be modified to include such dependencies. Thus, in most cases, although the basic attitude of accounting for growth patterns remains constant, the detailed application must be different from one problem to another.

One conclusion is inescapable. That is, any radiological phenomenon should be treated as a systemic disturbance, rather than as a purely local situation. To the worker interested in determining the effect of radiation in a more or less universal sense, this is of considerable annoyance. If a quantitative theory of effects extraneous to the direct effects of the radiation were available—and the model discussed in this article may be adequate—then the radiation effects could perhaps be unraveled.

The model discussed was designed, not unsuccessfully, to separate into different factors, the effects of radiation on the material of an organ, and the weight response of the organ to a sudden damage to a part of it caused by any means. From the standpoint of the radiologist, only the radiation effect on the tissue is of direct interest; the physiologist is interested in function. But observations on organ weight regression on dose alone (or on total-body weight, very likely) will give a set of numbers that, without detailed analysis, bears very little relation to either of these desires. This leaves many experiments of the kind discussed here in the class of determination of clinical parameters, without much claim to universality. Mole's statement⁽⁷⁾ that total-body radiations, properly should be classed as "medicine" rather than radiobiology, is supportable in this sense.

However, it is still true that the relative effect of two kinds of radiation can be investigated without reference to the dynamic properties of the response. For example, an RBE for highly ionizing radiation with respect to minimum ionizing radiation can be measured if it is clear that the time factors are not dose dependent. Note that this is not the case for thymus, except very approximately. Define the RBE = R such that if $S_A(D)$ is the effect as surviving weight using radiation A and $S_B(D)$ is the effect at the same dose D using

radiation B then $S_A(D) = S_B(RD)$; R is a measure of the effectiveness of radiation A (say protons) with respect to radiation B (X rays, and then $R > 1$). From above

$$S_A(D) = 1 - [1 - a(D)]f$$

$$S_B(D) = 1 - [1 - a(RD)]f$$

it is clear that f must not be dose dependent if the observations on A, B are to be made at the same time after exposure. It does not help much if f were to be replaced by f_e (measuring at the time of maximum effect) since f_e may change with dose in a different manner than just by a scale factor. A possible simplification for determining RBE is given in the Appendix.

SUMMARY

A brief discussion of the rationale behind the use of organ weight changes (in total-body exposure to radiation) is given. A simple mathematical model is introduced that attempts, with some success, to correlate the normal growth pattern of the organ with the time dependence of its weight after the exposure is made. One simple result of the model is that it predicts an apparent "insensitive fraction" of organ weight: apparent in the observation even though it is not truly present in the radiation response of the organ. The model is applied to two conventional experiments, and is found to be quite consistent with these if gross qualitative features are emphasized, and even semi-quantitative in detail. However, it is concluded that this model may or may not be adequate to cover any but a small class of problems involving the response of a large organism where the response of only a small part of it is observed. Because of this, the use of organ weight changes probably cannot furnish very much basic information. For the determination of clinically important quantities like the RBE, the technique of observing organ weight changes is probably a valid one.

APPENDIX

If $a(D)$ is, for example, exponential, then S will in general not be exponential but only approximate to one for small values of the dose. Nevertheless it can be a fair approximation to fit an exponential to S for purposes of measuring RBE. What is the error in R produced by doing this? Let the true functions for radiations A, B, be S_A, S_B and let s_A, s_B be the exponential approximations: $s_A = e^{-AD}$. Choose A, B to be good fits by least squares (the integration over the region restricted to not too large D).

$$\delta \int (\log s_B - \log S_B)^2 = 0$$

Therefore

$$B = - \frac{\int D \log S_B dD}{\int D^2 dD}$$

$$A = - \frac{\int D \log S_A dD}{\int D^2 dD}$$

The RBE using the exponential approximations call $R_o = B/A$; we want $R - R_o$. Write $S = s(1 + \phi)$ where $\phi(D)$ is unknown but assumed small. Then on substitution

$$AR_o = AR - \frac{\int D\phi \, dD}{\int D^2 \, dD}$$

For example let $\phi \triangleq qD$. If the S from the model is used, then $q \triangleq (1-f)B$ is obtained by putting $a(D) = \exp(-BD)$. Then

$$\frac{R-R_o}{R_o} \triangleq 1 - f$$

For example, in the testis weight changes cited in the text, a 40 per cent error in RBE will be made by measuring with both radiations up to 250 r (say at a single dose) and fitting with an exponential. However, this is probably less than the error made by obtaining the ED50 for each case and taking the ratios of these since if f is indeed so large a small error in S (at 50 per cent) will produce a large error in the D corresponding to this S .

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PRETHERAPEUTIC STUDIES WITH THE CHICAGO SYNCHROCYCLOTRON*

By

S. D. Warshaw† and D. G. Oldfield

INTRODUCTION

Some studies made of the properties of the high energy proton radiation generated by the University of Chicago synchrocyclotron are reported in this paper.

The synchrocyclotron accelerates protons to an energy of 460 Mev with a nominal duty cycle of about 1 per cent. Until now, the machine has had a serious defect, from the point of view of radiology, i.e., the lack of a high intensity external beam. The beam that was available for these studies, which are of a largely preliminary nature, consisted of that small fraction of the internal cyclotron beam that evades the well-known resonance blow-up at $n = 0.2$ and escapes by symmetric emission around the perimeter of the "impact parameter" circle. The properties of this beam and the operation of the cyclotron will be discussed elsewhere.⁽¹⁾ Since this investigation was completed, some improvements have been made⁽²⁾ including a novel experimental shield, a strong-focusing condensing lens system, and a large steering magnet; the present beam intensity now produces of the order of 100 rep/minute or more depending on the beam optics selected. A description of the instrumentation and techniques used for the less intense beam is given below; more detailed measurements using the more intense beam will be made in the future.

DOSIMETRY AND INSTRUMENTATION

Instrumentation: The primary physical problem in the use of any kind of radiation is the determination of the dose and the spatial distribution of the dose. High energy protons can be formed into a rather well-defined beam and have a well-defined range in materials. The general properties and advantages of such radiation, from the viewpoint of the radiologist, have been discussed in detail by Tobias *et al.*⁽³⁾ Some of the instrumentation in use at Chicago and some of the preliminary results on measurements of dose distribution for the low intensity beam are the subject of this section. The instrumentation bears no claim to originality, except in detail, but is a relatively simple and convenient setup for cyclotron radiology.

The basic dose meter was a modified thimble chamber (Figure 1). Except for the met-

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Figure 1. Schematic diagram of thimble chamber.

al parts; the entire cylindrical body of the chamber is cast out of Araldite 502* an epoxy resin of low electrical conductivity. The ionization cavity is spherical, 1/4 inch in diameter, with the inside surface coated with Aquadag. The collecting electrode is an aluminum wire that runs the length of the main tube and is insulated from the cylindrical guard electrode by teflon bushings; the remainder of the space inside the guard tube is filled with Superlawax. For this critical leakage region it was believed that the advantage of possible disassembly outweighed that of the easier fabrication obtained by also filling this space with Araldite. The cavity is formed by two hemispheres, one machined into the upper main casting, and one into the lower casting. Electrical contacts are made within an aluminum fixture at the top of the main tube and brought out through a standard "Type HN" teflon-insulated connector for the signal, and "Type BNC" connector for the polarizing voltage. The measured leakage resistance from collector to guard was better than 10^{13} ohms. Since the density of the Araldite is nearly unity (about 1.1 g/cm^3) and it is an organic material mostly carbon and hydrogen, there is no first order correction for the wall material when this chamber is embedded in a tissue-like phantom.

* Ciba Chemical Co., Kimberton, Pa.

Parallel plate chambers were used for general monitoring of the beam. These were constructed conventionally, of thin lucite plates made conducting with Aquadag, and a guard ring formed on the collector plate by scratching out the Aquadag with a sharp pair of dividers. They are 3-1/2 inches in diameter with a 1-1/2 inch air space at atmospheric pressure.

External circuitry consisted of either a vibrating reed electrometer or a 100 per cent inverse feedback d-c amplifier with a CK5886 electrometer tube as pre-amplifier. The amplifier, designed originally for use as the "Betatron Health Monitor"* is quite stable, showing less than 0.5 mv per hour zero drift rate after a two-hour warm-up. A necessary modification was made in the mechanical construction of the pre-amplifier. It was found that the CK5886 circuit that was used responded to stray radiation, so that even in the absence of a chamber, any small fraction of the beam scattering into the pre-amp would give a signal. Therefore, the pre-amp was enclosed in a steel can to shield against the stray magnetic fields present in the experimental area and filled with Superlawax. Input impedances were changed by means of plug-in units, each consisting of a modified HN-connector joined to a brass shielding tube containing the (potted) impedance. A switch was provided to short the grid to the feedback point. (Before this unit was built, a remote control system using subminiature, low-leakage thermal relays was used to switch impedances in and out; this turned out to be more trouble than the advantage of remote control was worth.) A sketch of the pre-amp, showing the connections to the chamber and the plug-in impedance, is given in Figure 2. When connection of the pre-amp to the chamber was not direct, it was made with a double-shielded, graphite-coated coaxial cable (Amphenol No. 21-406) that reduced pick-up and microphonic effects in the cable to a tolerable level. The high-level signal from the amplifier in the experimental area was then brought up through a long cable to the control area, where the signal was metered or recorded; the remote control box also had a "zero" adjustment for the d-c amplifier.

Auxiliary equipment included a Leeds and Northrup Speedomax recorder arranged to be used as a ratio meter: here, the battery used normally for balance was replaced by the signal from the d-c amplifier that represented the denominator of the ratio desired, and minor changes in the slide-wire circuit were made in the recorder. This was useful to locate beam axes by scanning with the thimble while using a flat chamber to monitor, or to quickly record Bragg curves. For accurate work—the transient response of the recorder not being adequate to accommodate the sometimes very rapidly fluctuating cyclotron beam—it was found preferable to measure the total charge collected, rather than the instantaneous ionization current. Having only a single vibrating reed electrometer, an electromechanical voltage-integrator circuit** was built. This device operates as an analog computer, as is seen from Figure 3. The presence of the auxiliary feedback potentiometer makes it possible to use this circuit as an analog for radioactive decay also, and there-

* By Mr. D. Connors.

** We wish to thank Mr. C. H. Wood for the construction of this device.

Figure 2. Schematic diagram of pre-amp and plug-in impedance unit.

Figure 3. Schematic diagram of integrator circuit.

fore as an "activity computer."

In Figure 3, let N be the number of counts registered on the "units" dial (10 counts per shaft revolution). Let r be the fraction used of the sensitivity-adjust helipot R ; the switch functions s_1 , s_2 , and s_3 are given in the inset to Figure 3 for any position of the (ganged) switches. Let V_{in} be the input voltage, k the coefficient of proportionality between the tachometer output voltage and the velocity of the output shaft, $V_{tach} = k dN/dt$, and let E be the full voltage applied across the feedback helipot. This potentiometer can be connected to any one of the shafts "units," "tens," "hundreds" . . . for which $n = 1, 2, 3, \dots$. Then it can be shown very simply that as the overall system gain becomes large

$$\frac{dN}{dt} + \lambda N = \beta V_{in}$$

where $\lambda = s_2 r E / s_3 k 10^{n+1}$ and $\beta = s_1 r / k s_3$. Thus, the device is, to a good approximation, the analog of a target being activated, the activity decaying with a mean life $\tau = \frac{1}{\lambda}$ and the activating beam current proportional to V_{in} . While the beam is on, the register dials will turn at an ever decreasing rate; when the beam is turned off, the register will unwind at an exponential rate, the dial reading at any time being proportional to the activity of the target at that time. In switch position 3, 4, 5, $s_2 = 0$, $s_1 = 1$ so that $N = \frac{r}{k s_3} \int_0^t V_{in} dt$. The constant k has to be determined empirically by integrating a known constant voltage for a definite period of time. The device is limited in the range of input signals: for very small inputs it becomes nonlinear due to frictional drag in the tachometer, and for large signals the amplifier saturates. (Hence, the presence of the switch s_3 , which in practice is set so that the ratio of output shaft speed to signal voltage is the same over a factor of 100 change in signal strength.) However this limitation permits enough flexibility for most of our purposes.

For dosimetry, the thimble chamber plus integrator were used to establish the absolute dose, and the ratio of charge collected by the thimble (expressed as integrator counts) to the charge collected by the large flat chamber placed in the beam was determined; for actual exposures subsequently, the flat chamber was used to monitor the dose. Charge from the monitor chamber was measured on a low-loss capacitor* tied into the feedback loop of an Applied Physics Corp. vibrating reed electrometer. The output of this instrument is recorded on a Brown extended-range recorder; the electrometer pre-amplifier, located near the chamber, was fitted with auxiliary relays so that the reed could be zeroed, or a resistor put in place of the capacitor, remotely from the control area. In addition, an extrapolar switch wafer installed on the automatic range-switching shaft of the recorder permitted the use of a simple relay circuit: this was arranged in such a way that when the recorder pen carriage arrived at any pre-set dose, it tripped a microswitch, thus actuating the relay circuit that turned off the cyclotron pulser. At the same time, on-off signals could be furnished to other circuits, such as a clock or the voltage integrator.

* Obtainable from the John E. Fast Co., Chicago. These capacitors have better than 10^{14} ohms leakage resistance and a negligible amount of charge hysteresis effects.

The thimble chamber charge was converted to delivered dose by a simple calculation. Let $\dot{D}$ be the dose rate in rep per second; let n be the proton flux (protons/cm²/sec), $(dE/dx)_w$ the stopping power of water in ergs/gm/cm², and w the energy needed to make one ion pair in air, and $q = 93$ ergs/gm/rep. The ionization current is given by $i = \dot{D}V / 3 \times 10^9$ where V is the chamber volume. This numerical relationship will be restated. In terms of the proton flux, $\dot{D} = \frac{n}{q} \left(\frac{dE}{dx} \right)_w$. Alternatively, the ionization current is $i = \frac{n}{w} \left(\frac{dE}{dx} \right)_{V\rho_{\text{air}}}$ where ρ is the density of the air (at NTP) in the chamber. Therefore $\dot{D}/i = Sw/Vq\rho_{\text{air}}$ where S is the stopping power of water relative to air. Using $w = 32.2$ e.v. and the measured volume of the thimble, assuming that it is all effective, this relation gives $\dot{D}/i = 3 \times 10^9/V$ also, if $S = 1.04$ which is close to measurements at these proton energies.^(3,4) The actual dose is then read off the integrator dials as described above; errors in measurement (from the integrator and from the Victoreen resistor used in the input of the d-c amplifier) are estimated to be less than 2 per cent. The chamber calibration was checked by measuring the flux of protons that passed through it for a measured count. This was done by placing a foil (53 mg/cm²) of polyethylene (CH₂) in front of it and activating to get the reaction $C^{12}(p,pn)C^{11}$; C^{11} is a 20.5-minute positron emitter, and at the energy used for calibration, the reaction cross-section is 31 millibarns.⁽⁵⁾ Since the activation times had to be about 30 minutes with the beam intensity available, it was necessary to make a correction for radioactive decay during the run and to account for fluctuations in the beam intensity. This was done automatically by the integrator now connected as an activity computer, by setting in the known mean life for C^{11} . However, it was realized that the many secondary neutrons produced in the chamber walls would in turn produce secondary protons of a lower energy than the primary beam. Since the reaction cross-section at lower energies is three times the high energy cross-section, these would contribute to the foil activity (if the foil were mounted just in front of the thimble chamber) much more than their population warrants. Therefore in the measurement, the ratio of counts from the thimble chamber to the charge collected from the flat monitor, considerably upstream of the thimble, was first obtained. Before the activation, the thimble was removed and replaced with the foil, while the flat monitor remained; a secondary monitor, which was always in position, provided the signal to the activity computer. The resulting proton flux, when substituted in the above expression for ionization current as a function of flux, gave a thimble calibration within 2.5 per cent of the expected value, well within the errors of measurement of the carbon activity.

Dosimetry: It was desired to establish an RBE for proton exposure. Therefore the dosimetry measurements were done with this particular goal in mind. The high energy beam at Chicago is, at 460 Mev, nearly minimum ionization radiation: it should therefore have an RBE, with respect to medium hard X rays, of nearly unity. Hence the measurement was to be made for protons of a lower energy, in fact about 4 times minimum ionization was chosen. It should be noted here that Tobias *et al.*⁽³⁾ have already reported that the RBE (using a 30-day acute lethal dose in total-body exposure of mice) for 340-Mev protons and for 180-Mev deuterons is approximately unity. Since the stopping power for

180-Mev deuterons is approximately the same as it is for 90-Mev protons, we should expect that the RBE for such protons, or protons of higher energy, would also be unity, at least if measured by acute lethal dose. However, since the use of organ weight changes, for certain organs, is a more sensitive criterion of radiation effects than acute radiation death, we could in fact hope that a measurable effect would be apparent at even 100 Mev.

With the beam intensity low, at the time of these measurements, cutting down the energy to the required value by placing an absorber near the cyclotron (about 25 feet from the experimental space) was out of the question. Scattering in the absorber would have reduced the available intensity to too small a value for use. Therefore the entire high energy beam was allowed to enter a long tank of water through a lucite window. (Note that the calculated range in water of 460 Mev protons⁽⁶⁾ is 102 cm.) Since the beam is emitted from a localized "source" which has a rather complicated distribution in space, it was expected—and found—that the beam intensity profile was less uniform than might be desired. The beam, after "spilling out" from the cyclotron, first passed through the 0.01-inch thick aluminum port window of the vacuum tank, then through about 5 feet of air, then through a 5/8-inch collimator followed by a 9-foot long cylinder containing helium at atmospheric pressure and sealed by 0.008-inch Mylar windows. The function of the helium was to minimize the gas scattering between the exit and entrance collimators; even for these high energy protons, the multiple scattering angle in air over such distances becomes large enough so that a considerable fraction (about 45 per cent, for this geometry) of the beam could strike the sides of the exit collimator thereby producing many neutrons⁽⁷⁾ and energy-degraded protons that would contaminate the experimental results. The exit collimator was about 2 inches in diameter, with the relative sizes of entrance and exit slits calculated from the known⁽¹⁾ beam divergence. Following the exit collimator, the beam passed through another 7 feet of air before entering the tank.

Under these conditions, as mentioned above, the dose distribution cannot be expected to be very flat in transverse section or sharply defined in range. In particular, the energy spread in the beam due to even the small amount of radial and phase oscillations, increased by straggling in the air path and windows, depresses the peak of the Bragg curve relative to the initial part and smears the width of the peak considerably. Figure 4 shows the calculated effect* of an arbitrarily chosen 10-Mev spread given to Tobias' 180-Mev deuteron beam. The beam he studied, by reason of an excellent focussing and deflection system, was quite clean, and the peak width appears to be due mainly to natural straggling. The small additional energy spread clearly produces a very pronounced effect on the range curve. At any rate, the net effect of a rather divergent beam, not very uniform in cross-section, with (by estimate) at least 1 per cent energy spread as it leaves the cyclotron, would be to yield an isodose distribution that lacks a clear boundary at the edge and does

* This was calculated by folding in a Gaussian spread to Tobias's sample curve. Let $i(x)$ be the ionization curve for zero energy spread and $g(R-R_0)$ be the distribution function in range of the initial beam with a mean range R_0 . Then the ionization curve with a finite energy spread is $I = \int_0^\infty i(x-R)g(R-R_0)dR$. Here x is the residual range.

Figure 4. Calculated effect of 10-Mev initial spread on Tobias' Bragg curves.

not have the well-defined, uniform "hot-spot" that is characteristic of heavy, charged radiation near the end of its range. Hence, it was considered inadvisable to make total-body exposures with the animals at the Bragg peak because the dose distribution over the animal would be nonuniform. Since it was also desirable to expose the animals at as low an energy as possible, they would have to be placed in the beam just before the rise in ionization toward the end of the range.

Measurements of the isodose distribution were made with the 1/4-inch cavity chamber. The chamber was mounted to the moving stage of a scanning unit; this consisted of a 20-inch long lead screw, which moved a carriage in the x-direction, and on the carriage another lead screw which moved the stage (and chamber) in the y-direction. Both motions were effected remotely by means of motors. No z-motion was available on this unit, and therefore the center of the chamber was positioned precisely (using a telescope directed along the known beam direction) so as to be in the center of the beam along the entire length of travel. The signal from the cavity was integrated, and integrator counts were recorded for a fixed amount of charge recorded on the electrometer that was connected to the (non-moving) monitor. A series of beam profiles, dose versus y at different values of x then yielded by graphical construction the isodose distribution given in Figure 5. Note that the desired hot-spot is clearly visible but it is not nearly so striking as that given in reference 3. Only the last one-third of the range of the 460-Mev protons is shown. This

Figure 5. Isodose distribution and Bragg curve for the last one-third of the range of 460-Mev protons in water.

figure is reproduced without comment, since it is only an approximation to the desired distribution. The ultimate requirement is, of course, a very intense Bragg peak that can be exploited in the treatment of deep tumors. A Bragg peak-to-valley ratio of, say, 10 to 1, and an RBE for tumor damage of only 2 means that a small tumor could be irradiated with 20 times the effective dose given to the skin. Work is now in progress with the new installation at Chicago to improve this distribution.

Finally, the mice for the experiment to be described in the next section were enclosed in lucite tubes (with air intake and exhaust) and placed in the water such that the residual range of the primary beam at the center of the tube was 7 g/cm^2 ; this corresponds to a proton energy of about 90 Mev, and at this energy $(dE/dx)_w = 8 \text{ Mev/g/cm}^2$ while minimum, ionization in water is 2.07 Mev/g/cm^2 .

RBE MEASUREMENTS

A discussion of an interpretation of organ weight changes after radiation exposure has been given in another paper.⁽⁸⁾ Here some measurements of spleen and thymus weight loss will be given and analyzed with a view toward getting a measure of an RBE for the proton beam. It is to be emphasized that the final number for RBE has an uncertain biologic significance if it is taken at quantitative face value. However, it is also true that within an uncertainty that is exclusive of random errors of measurement, the RBE indicates a difference between the effects of different kinds of radiation, and this difference may very likely apply to a wide class of biologic material. (The reasons for the uncertainty are discussed in reference 8.)

The physical dosimetry has been discussed at length in an earlier part of this paper. This section reports the RBE for protons slowed from 460 Mev to a mean of 90 Mev, with total-body exposure, the reference radiation being 250 kv X rays (target distance = 82 cm, current = 30 ma, nominal dose rate = 16 r per minute, filtration = 0.5 mm Cu + 1 mm Al) from a General Electric "Maxitron." The animals were irradiated in plastic tubes on a thick lucite tray. The exposure was measured by a Victoreen thimble chamber inserted into one of the animal tubes during irradiation; roentgens were converted to rep by multiplying by 93/83 as usual.

Biological: The mice used were strain LAF₁^{*} males, averaging 7 weeks of age. For the animals bred in the Argonne Cancer Research Hospital animal farm, it was not possible to ensure effective randomization across litters because since relatively few mice are weaned each week, no record was kept of the litter-origin of each mouse. In a grand average of many separate runs, over many months, however, any discrepancies from this cause should have averaged out. In any experimental run, selection of mice was made by total-body weight; the animals were housed 10 to a cage, with each cage group scheduled for a specified dose. The mean body weight, across cages, was adjusted to be equal—with in one standard deviation—and animals whose body weights differed from the population

* More specifically (C47L/He x A/He)F₁.

grand average before selection by more than one standard deviation were rejected. One large group of these animals was obtained from a commercial supply firm, and used to establish the X-ray regression. For these animals, while the thymus weights were not significantly different from the mean weight of thymus controls for the Argonne animals, the spleen weight was nearly twice as great and the total-body weight considerably different. The coefficient of variability for thymus and spleen for the commercial animals was larger than for the home grown variety. (The abnormal spleens were not found to be diseased but may be symptomatic of a diseased condition elsewhere in the body.) Since the reason for using this strain was the hope that its highly inbred character would lead to a homogeneous population, this result was rather disappointing. Table 1 shows a comparison of spleen and thymus weights for the Argonne, the commercial, and 7- to 8-week-old CF No. 1 males purchased from Carworth Farms. The population samples for the LAF₁ commercials were chosen from among the best, in terms of variability, of those available; the samples of LAF₁ Argonne were about average. Evidently there is no great advantage in using the rather expensive LAF₁ strain.

Table 1

COMPARISON OF HOMOGENEITY OF SEVERAL MOUSE POPULATIONS

Strain	Spleen		Thymus	
	Mean of 10 (mg)	σ (per cent) ^(b)	Mean of 10 (mg)	σ (per cent)
LAF ₁ Argonne	98.1	19	43.7	24
	94.9	23	35.8	29
LAF ₁ Commercial ^(a)	173.3	23	43.7	39
	181.0	42	38.0	35
CF No. 1	118.0	33	39.2	23
	101.9	30	35.1	33

(a) Variance analysis between the commercial and Argonne spleen groups shows that they are not of the same population to better than the 1 per cent confidence level. The same analysis for thymus shows that they are randomly selected to the 1 per cent level.

(b) Each pair of means were of the same randomly selected population, to the 5 per cent level.

After exposure, the animals were replaced in their cages and stored in the same room as the control animals. At 5 days post irradiation, controls and exposed animals were sacrificed by etherization, and the spleen and thymus excised. The spleen was weighed immediately after excision; the thymus was fixed in 10 per cent formalin solution for 24 hours, the adherent fatty tissue was removed, and the gland weighed.⁽⁹⁾ An auxiliary experiment

gave confirmation (Table 2) that the ratio of wet to dry weight for thymus remained constant for both exposed and control animals.

Table 2
WET AND DRY THYMUS WEIGHTS

X irradiation (r)	Mice (No.)	Mean weight (mg)				Ratio dry/wet
		Wet	Coefficient of variability (%)	Dry	Coefficient of variability (%)	
0	20	45.2	17	10.0	22	.221 $\pm$.058
100	20	25.3	19	5.1	24	.202 $\pm$.062
200	25	18.0	20	4.0	16	.222 $\pm$.056

The dry weight was obtained by vacuum desiccation of the fixed organs for 24 hours. The water loss after 24 hours became negligible; desiccation of unfixed organs gave essentially the same result but with a greater variance, since it was considerably more difficult to remove the adherent fat from the fresh organ.

Tests for statistical consistency in the wet versus dry weights, and in all other data of this paper that were in doubt were made by a Fisher variance analysis,⁽¹⁰⁾ and population homogeneity checked to better than the 5 per cent confidence level. Occasionally, single animals were rejected from a sample if the isolated weight differed from the mean by more than 3 standard deviations.

Results: The results are expressed as the ratio of exposed organ weight to control organ weight and designated S, for surviving fraction. The standard error of estimate for weights was used to convert to an estimated error in S. Figures 6 and 7 show the measured S for thymus and spleen versus the dose expressed in rep. In the case of spleen (X radiation, commercial animals), while the spleen weight was greatly different from that of the Argonne animals, the ratio of exposed organ weight to control weight (S) turned out to be not significantly different. The S-values taken with the commercial animals are marked as solid points on Figure 7. The data were fitted by least squares with curves of the form $S = \exp(-D/D_0)$ in accordance with the Appendix to reference 8. Table 3 gives the values of D_0 for each of the four cases.

DISCUSSION OF RBE MEASUREMENT

From Table 3 it can be seen that although the experimental error in the spleen determination was larger than that for thymus, the two results are substantially the same, i.e., the RBE with respect to 250 kv X rays is about 2. It was stated above that the RBE obtained by Tobias *et al.*⁽³⁾ was approximately unity, for roughly corresponding radiation, as measured by acute lethal dose in total-body exposure. It seems not unreasonable that the two results do not agree since death is a drastic and arbitrary response that involves

Figure 6. Surviving fraction of thymus; ED 50 = effective dose for 50 per cent survival.

Figure 7. Surviving fraction of spleen; ED 50 = effective dose for 50 per cent survival.

Table 3

DOSE SENSITIVITIES FOR SPLEEN AND THYMUS WEIGHT
CHANGES WITH EXPONENTIAL REGRESSION

ORGAN	D_0 (X radiation) (rep)	D_0 (protons) (rep)	R = RBE
Spleen	515 ± 32	295 ± 33	$1.75 \pm .23$
Thymus	250 ± 5.8	120 ± 4.1	$2.08 \pm .07$

a complex of prior responses including, for example, the loss of function of certain vital organs. However, the results are not in satisfactory agreement with those of Carter, Bond, and Seymour.⁽⁹⁾ These authors report that the RBE for neutrons ranging in energy from 3 Mev to 20 Mev was 1.6 using mortality, 1.7 using spleen weight loss, and 1.5 using gut weight loss. It is true, of course, that some differences in the two experimental results can be accounted for by strain and sex differences, and in particular, by normal growth differences (see reference 8). However their result that mortality as indicator yields the same answer that the organs do, is rather surprising. The neutrons used in their experiment produced a recoil spectrum of protons that varied from about 10 times minimum ionization to several times this figure, and we should have expected that the RBE measured for these neutrons would be considerably larger, by any end-point, than the roughly 4 times minimum ionization (in the middle of the mouse) protons used in the present experiment. Carter *et al.*⁽⁹⁾ state that there is probably no marked variation in RBE over the neutron energy range from 3 to 20 Mev since their results agree with those of other workers using 14-Mev monoenergetic neutrons. However, the recoil proton spectrum from a 3- to 20-Mev spectrum of neutrons will be somewhat like that from a 14-Mev monoenergetic beam, although more peaked at higher specific ionization. Therefore those two results should have been rather close.

Assuming that Carter's statement is correct, then the results of the present experiment imply that its validity might extend down to a mean specific ionization of about 4 times minimum. Since the dosimetry for the neutrons is somewhat uncertain, such a conclusion is speculative.

The findings of Vogel *et al.*⁽¹¹⁾ are more consistent with our own results. Using fission neutrons, these authors found an RBE of 4.5 or more with respect to Co⁶⁰ γ radiation. Since the RBE for γ radiation is 0.5 with respect to 250 kv X rays, Vogel's result is RBE = 2.3 on the same basis, and therefore larger than the slightly less than 2 we have found.

SUMMARY

Instrumentation and dosimetry for the high energy proton beam of the University of Chicago synchrocyclotron is described. Since the beam is of usable but low intensity, and equipment for increasing its intensity and for focusing it to get a good isodose distribution has only recently become available, these studies are to be considered of a preliminary nature. The beam was slowed down to a residual range of 7 g/cm² of water (from an initial 107 g/cm²) in a long water tank. The RBE at this point was measured with respect to 250 kv X rays using the technique of mouse spleen and thymus weight change observed 5 days after exposure. The result was RBE = 1.75 $\pm$.23 (spleen). RBE = 2.08 $\pm$.07 (thymus).

ACKNOWLEDGMENT

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NEUTRON FLUX DEPRESSION AND SELF-PROTECTION
CONSIDERATIONS IN ACTIVATION OF COBALT-60
FOR USE IN MOVING BEAM THERAPY*

By

L. H. Lanzl and L. S. Skaggs

In the kilocurie cobalt-60 radiation therapy unit of the Argonne Cancer Research Hospital, the shield containing the source can revolve in a vertical circle about a recumbent patient. It is necessary to place the collimator rather close to the source in order to provide suitable clearance between the revolving source shield and the patient. A design limit of 1 cm was chosen for the penumbra at the center of revolution. (The penumbra is defined as the width of that portion of the beam lying between 10 and 90 per cent of the central beam intensity.) These geometrical restrictions placed a limit of 0.75 cm on the diameter of the source. It was decided that the cobalt source should be in the shape of a right cylinder, of such a length that the self-absorption of its γ rays could reduce the radiation, emanating from the end of the cylinder, to about 65 per cent of that due to the total activity.

Based on these considerations, the source weighs 11.7 g and is 0.75 cm in diameter and 3 cm in length. A total cobalt activity of 1800 curies, or a specific activity of 154 curies per g, was planned to provide dose rates that were comparable to those obtained with conventional therapy apparatus.

For the activation of cobalt-60 in a reactor, neutron flux depression and self-protection are important considerations. The "flux depression" results from the net flow of neutrons into an absorber immersed in a diffusing medium, and the "self-protection" results from a neutron flux reduction toward the central portion of an absorber caused by the high peripheral absorption. Absolute measurements of these quantities were made for us at the Brookhaven National Laboratory⁽¹⁾ on a single piece of cobalt of the above-mentioned dimensions. The results gave a factor of 0.77 for self-protection and 0.58 for flux depression. The combined effect is that a source of the size and shape of the one that we use will only reach 45 per cent of the calculated activity on the basis of a uniform, undepressed thermal neutron flux.

We can make an estimate of the effect of flux depression and self-protection from our experience in producing a 658-curie, 56 curies per g, cobalt source in the Materials Testing Reactor in Idaho. The dimensions of this source were identical to those of the source tested at Brookhaven, i.e., 3 cm long and 0.75 cm in diameter. Its activity was determined by means of a calibrated Bureau of Standards thick-walled Victoreen ionization chamber in the following way: The activity of a 10-curie source of identical dimensions was deter-

* A summary of this paper appears in *Nucleonics*, 14: No. 1, p. 66 (1956).

mined by open-air measurement with the Victoreen ionization chamber. Then the activity of the new source was compared with that of the 10-curie source by ionization measurements in identical collimated γ -ray beams.

The undepressed thermal neutron flux at the position of this source in the reactor was 2×10^{14} neutrons/cm²/sec.⁽²⁾ Using a thermal neutron cross-section of 34 barns, a half-life of 5.3 years, assuming no burn-up of cobalt-60, and using the actual operating time of the reactor for the activation of this source, we calculate that the specific activity of a source of infinitesimal size would be 156 curies per g. From the observed value of 56 curies per g, we find the combined effect of self-absorption and flux depression to be 0.36. This figure may have more relation to reality than the Brookhaven figure, but it is, of course, not as accurate because of the uncertainties in the value of the undepressed neutron flux of a large-scale operating reactor.

The effect of self-protection can be decreased by dividing the source into a number of smaller pieces and dispersing these pieces throughout a greater volume of the reactor during activation. This also decreases the flux depression since, in effect, the target area of the source for thermal neutrons is increased. In order to study these effects, the authors compared the induced activity of a single piece of cobalt with that of three different assemblies of small pieces made by dividing and distributing the source in different ways. The activations were accomplished in the CP-5 reactor of the Argonne National Laboratory in a region of a flat neutron flux. In the first experiment (Figure 1), the assemblies and their arrangement during activation were as follows: (a) Fifteen 0.75-cm diameter by 0.2-cm thick disks of cobalt embedded in graphite over a length of 21 cm, with the disks equally spaced; (b) seven 0.25-cm diameter by 3-cm long rods embedded in graphite over a length of 21 cm with the rods placed end-to-end; and (c) a single, solid right cylinder 0.75 cm in diameter and 3 cm long, embedded at the center of a 21-cm piece of graphite. The diameter of the graphite rod was 2 cm in all three cases. The neutron flux was approximately 7.5×10^{11} neutrons/cm²/sec, and the time of irradiation for each of the samples was two minutes.

In the second experiment (Figure 2), fifteen disks of cobalt of the same dimensions as activation (a) above were distributed peripherally about a 2.34-cm diameter cylinder of aluminum of 3.8-cm length. This cylinder was placed within a cylinder of 2.98-cm outside diameter, and the central portion was filled with aluminum. A second single piece of the dimensions of activation (c) above was embedded at the center of an aluminum cylinder of 3.8 cm in length and 2.98 cm in diameter.

For reasons of convenience, the samples were allowed to decay for several days before measurement; this also served to eliminate the 10.4-minute isomeric activity. The sources were made from very high purity cobalt, and no other activity was expected, or was found. The combined effect of neutron flux depression and self-protection was evaluated by counting the γ activity of the sources with an end-window Geiger counter. During the measurement, the disks in both experiments were stacked one above the other to form a volume equivalent in size and shape to that of the single piece. The rods were arranged

Figure 1. Arrangement of assemblies and results of cobalt during activation and measurement of first experiment.

IRRADIATION
CONFIGURATION COUNTING
CONFIGURATION RELATIVE
ACTIVITY

1.00

1.55 ± 0.02

Figure 2. Arrangement of assemblies and results of cobalt during activation and measurement of second experiment.

with one in the center and the other six stacked around the first. This formed a bundle with overall dimensions equal to the single piece, but because of the interstitial air space, the weight was only 78 per cent of either the single piece or the disks.

The radiation was measured along the axis of the cylindrical assembly in each of the five cases and was so collimated that only the radiation from the end of the source, i.e., from a circular area having a 0.75-cm diameter, was detected. An aluminum filter, sufficiently thick to stop all β particles, was placed between the source and the counter.

The activities in the first experiment normalized to unity for the single piece were 1.37 ± 0.01 for the rods and 1.55 ± 0.01 for the disks. For the second experiment, the activity of the disks was 1.55 ± 0.02 normalized to unity for the single piece. These results would appear to favor the division of the single large source into a number of disks for either distribution; but if account is taken of the 22 per cent reduction in weight of the rods, and if the calculations are made on the basis of specific activity, then the result for the rods is 1.77. Thus, if the rods were made so that there were no interstitial air spaces when stacked together, the improvement in activity would be 77 per cent above that for the single source. However, there would be some loss in this case due to a greater γ -ray absorption factor.

The advantage of one of the disk arrangements over the other depends on available reactor space and variation of neutron flux over the assembly.

If the Brookhaven result for self-absorption and flux depression of 45 per cent for the single large cylinder is used, we see that the figure for dividing the source into seven cylindrical rods would be 62 per cent; and for dividing the source into fifteen disks of 2 mm thickness, 70 per cent. And again, if we had used close-packed rods and neglected any difference in γ -ray absorption, we would find that flux depression and self-absorption would reduce the activity to 80 per cent of that calculated using the undepressed neutron flux.

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INFRARED MICROANALYSIS OF SOLIDS USING PRESSED POTASSIUM BROMIDE PELLETS*

By

R. Pickering

In the ordinary infrared analyses of solid samples, it is our practice to run the samples in half-inch pressed KBr disks on a Perkin-Elmer Model 21 spectrophotometer. Our standardized procedure is to weigh out 150 ± 5 mg lots of dry KBr re-drying them after weighing in a vacuum oven set at 105°C for at least 1 hour to eliminate spurious water vapor absorption. One to 2 mg of sample is weighed out (± 0.1 mg for ordinary work), with the preferred weight varying with the nature of the sample, e.g., 1.7 ± 0.2 mg for steroids. The KBr and sample are ground together in an agate mortar, and the mixture is pressed in vacuo (at about 13,000 pounds per square inch for at least 1 minute after preliminary evacuation) in a half-inch disk press[†] to give a disk 0.45 ± 0.01 mm thick.

Thus, for a satisfactory spectrum, a sample of at least 1 mg was usually required, the same limit also being obtained with solution studies, running the material as a 1 per cent solution in a Perkin-Elmer microcell. Reduction of this minimum sample requirement was desirable since fractional mg quantities of materials are sometimes of interest. Reflecting microscope systems have been developed, but, as well as being expensive, they are not well adapted for use with the double beam instruments, particularly the Model 21 that uses the optical null principle.

The possible use of micro-KBr-disk pressing as a solution was suggested by the descriptions of an infrared beam condensing optical system and associated micro-KBr-pellet technique developed by Anderson and his co-workers.⁽¹⁻³⁾ In applying the method to our instrument, it was found that the silver chloride optical lens system is not needed, in point of fact, not even desirable. The condensing system might be justified for the ultra-micro range of a few μg , but that range is beyond the scope of our present interest. For the fractional mg range, defined here as from 0.9 to about 0.05 mg, the Model 21 instrument permits access to a sufficiently small beam cross section without optical manipulation. The intrinsic energy absorption of the AgCl lens (in the 40 to 50 per cent range) would seem to offset any moderate reduction in usable beam image that is only 3×12 mm in our instrument at the microcell adaptor window.^{**}

Although easily constructed micro dies have been described by Anderson,^(2,3) and one

* Published in part in Analytical Chemistry, 78:280 (1956).

† Made by the Research and Industrial Instruments Company, 30 Langton Road, Brixton, London, S.W. 9, England.

** The optical system was developed for a Baird instrument which has larger slits and requires a larger beam cross section than the more refined Model 21 instrument.

of them is to be made commercially available by the Beckman Company, Fullerton, California,⁽⁴⁾ no mention has been found of the adaptation of existing commercial half-inch presses to do microdisk pressing with comparable evacuation. The simplicity of an adaptation devised in our laboratory for pressing micro disks permits the convenient conversion of our half-inch disk press so that it serves a double purpose. The method utilizes the existing die shell with comparable evacuation by means of a sleeve-holder suggested by the Anderson-Smith micro die.⁽²⁾ In this method, the micropellets are pressed directly into a steel sleeve that serves as a convenient ring holder for mounting and handling.

The 6-mm diameter disk die is illustrated in Figure 1. The same principles have been used to construct a die used for pressing rectangular pellets 3 x 10 mm. The rectangular sleeve cuts off so little beam energy in the microcell position that the 100 per cent adjustment is well within the range of the adjustment control at normal operating conditions. The

Figure 1. Micro-die adaptation for pressing 6-mm potassium bromide disks in vacuo. From the left: cross-sectional view of press utilized, showing positioning of the microdie; lower die; sleeve; upper die. Scale as indicated.

energy loss with the 6-mm diameter sleeve in the microcell position is roughly comparable to that with the ordinary half-inch disk holder in the normal cell location. Because it is somewhat more convenient to use in practice, most of our microsample work has been with the 6-mm die. We have run 0.3- to 0.5-mg samples (in 50 mg KBr) and have obtained results comparable with those of ordinary spectra. The lower limits, however, have not been established.

All the die parts are made of stainless steel tooled to fit the press, with slight allowances for evacuation. The die faces are hardened and highly polished to optical flatness. The sleeves are beveled on the lower edge to facilitate removal after pressing. The best

procedure for filling the die is to use loading tubes and tampers, which are shown in Figure 2. The tubes and tampers were shaped from brass.

Figure 2. Loading tubes and tampers for micro dies. From the left: Tamper and loading tube for 6-mm die; tamper and loading tube for 3- x 10-mm die. Scale: diameter of the loading tubes is 0.5 in.

The lower die is assembled in the press in the same manner as the half-inch dies, the sleeve usually being dropped down the bored cylinder onto the die. The loading tube is then placed in position, the powder mixture added through the tube, and the tamper used. Removing the tamper and tube, the upper die is allowed to slide into place. Then the assembly is completed, and the micro-pellet pressed like the half-inch disk, except that lower pressure is applied (7000 pounds per square inch).

The aluminum holder devised for mounting the 6-mm sleeve in position in the micro-cell adaptor of the spectrophotometer is illustrated in Figure 3. An identical holder, except for a wider window tapering to half-inch diameter, is used for the rectangular micro-pellet. This holder is also used for holding half-inch disks in the microcell position as an alternative to the regular holder that requires a compensatory holder in the reference beam.

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Figure 3. Holder for sleeved 6-mm microdisk. From the left: exploded view showing retaining screws, retaining ring, holder, and handle; front (or top) view. Scale: diameter of holder is 1 in.

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STUDIES ON PLASMA FACTOR(S) AFFECTING RED CELL PRODUCTION

By

L. O. Jacobson, L. Plzak, W. Fried, and E. Goldwasser

There is evidence that a humoral substance(s), which mediates erythropoiesis, is present in the plasma of subjects with certain experimentally-induced or clinical anemias.^(1,2) This substance has been designated as erythropoietin.^(3,4)

The method that we have used to study this phenomenon in normal rats and the data we obtained are described elsewhere.⁽⁵⁾ Our procedure may be summarized as follows: Male Sprague-Dawley rats are made anemic by a standardized schedule of bleeding. Blood is then collected from these animals into heparinized tubes, and the plasma is separated by centrifugation at 3000 rpm and stored at -10°C . (Such plasma is termed "anemic plasma.") Normal rats are injected intravenously with 2 ml of the anemic plasma on each of 3 successive days, and control animals are injected with normal plasma in the same way. One ml of Fe^{59} citrate solution is given via the tail vein 2 hrs after the last injection of plasma. Twelve or 16 hrs after the injection of Fe^{59} , a 1-ml sample of blood is withdrawn from each rat, and the radioactivity of the sample is counted in a Nancy Wood well-type scintillation counter. The average uptake of Fe^{59} (expressed as per cent of injected dose) of the red cells from rats that received normal plasma is 37 ± 4 , whereas the average value for normal rats injected with anemic plasma was 48 ± 3 . This difference in incorporation of Fe^{59} is evidence of an increased rate of erythropoiesis in rats injected with anemic plasma. As pointed out by others,⁽⁶⁾ the uptake of Fe^{59} by the newly-formed red cells correlates closely with the number of reticulocytes and with the erythroblastic activity in the marrow. However, the difference in erythropoiesis between the rats that received anemic plasma and those that were given normal plasma is rather small even when iron uptake, reticulocyte values, and erythroblastic activity are all taken into consideration. Thus the results are difficult to interpret except under ideal experimental conditions.

To circumvent this we found that when young adult Sprague-Dawley rats are hypophysectomized by a standard technic,[†] erythropoiesis is reduced to a minimum by 10 to 21 days after the operation. This reduction is shown by a tenfold decrease in the incorporation of Fe^{59} and a fivefold decrease in the number of reticulocytes. The administration of anemic plasma (2 ml daily for 3 days) at the time when the minimal level of erythropoiesis is reached results in a three- to sevenfold increase in Fe^{59} incorporation (Table 1) and from a three- to eightfold increase in reticulocyte values in the recipients. No signifi-

[†] The hypophysectomized rats were obtained from the Hormone Assay Laboratories, Inc., Chicago, Illinois.

Table 1

THE UPTAKE OF Fe^{59} BY ERYTHROCYTES OF HYPOPHYSECTOMIZED RATS
AFTER ADMINISTRATION OF NORMAL OR ANEMIC PLASMA

Ave. % Fe^{59} uptake into RBC of rats *		
Plasma injected	Prior to hypophysectomy	10 Days post hypophysectomy
Normal	37 ± 4	4 ± 2
Anemic	48 ± 3	18 ± 3

* 16-hr sample.

cant change is observed at this same interval in the control hypophysectomized rats that were given normal plasma or saline. The blood volume and red cell mass, as determined by Cr^{51} studies, remains within normal limits during this initial 3-week period. A single intravenous or intraperitoneal injection of 2 ml of anemic plasma given 24 hours before the injection of Fe^{59} brings about a significant increase in the incorporation of Fe^{59} in hypophysectomized rats. Plasma from rats exposed to 1000 r of X radiation and then made anemic by bleeding (hematocrit < 25%) within the first 3 days after exposure produces an appreciable uptake of Fe^{59} in hypophysectomized rats.

The plasma from hypophysectomized rats subjected to hemorrhage (hematocrit < 25%) exerts an effect on the uptake of Fe^{59} in hypophysectomized rats that is comparable with that observed after the administration of anemic plasma. If a total of 6 or more injections of 2 ml each of normal plasma is given instead of 2 ml of anemic plasma on a 1- to 3-day schedule, a significant effect can be demonstrated on the uptake of Fe^{59} in the hypophysectomized recipient. Anemic plasma from other species (e.g., rabbit, dog, or human being) can be assayed readily in the hypophysectomized rat.

At this time we do not need to enter into the problem raised by others⁽⁷⁾ of whether there is a specific pituitary factor that has a direct control on erythropoiesis. It is an accepted fact that the red cell mass eventually establishes a new equilibrium level after hypophysectomy.⁽⁷⁾

The simplest explanation for our findings may be summarized as follows: Within a few days after hypophysectomy, an overall reduction occurs in the metabolic processes of the animal. Coincident with the reduced metabolic requirement and before the red cell mass has fallen to its new level of equilibrium, the hypophysectomized animal is more or less comparable to an animal that has been made polycythemic by red cell transfusion. Hence, it may be postulated that the production of the plasma factor(s) responsible for maintenance of the steady state of circulating erythrocytes is reduced to a minimum because there is already an abundance of red cells. The situation is thus the reverse of that which holds when a normal animal is bled. Under these circumstances, red cell production is reduced to a minimum. When anemic plasma is administered to an animal in this

state, there is an exaggerated response in terms of the production of new red cells. As final and complete equilibration of red cell production and the new reduced red cell mass slowly become established in the hypophysectomized animal (circa 3 mo.), the number of reticulocytes and the uptake of Fe^{59} have risen from the minimal values found shortly after hypophysectomy to those that are consistent with the new steady state. We have found that producing a polycythemia in a normal animal by repeated red cell transfusion reduces erythrocyte production, which confirms the work of others.⁽⁸⁾ We have studied the uptake of Fe^{59} in these polycythemic animals and have found that the response to anemic plasma is comparable with that of hypophysectomized animals. That irradiated animals produce the factor in abundance is evidence that the factor is formed in an organ or tissue that is relatively radioresistant.

At this stage we assume that the factor(s) in normal plasma and in the plasma of normal or hypophysectomized animals subjected to phlebotomy that stimulates erythropoiesis in hypophysectomized and in polycythemic assay preparations is one and the same. However, the isolation and identification studies that are now in progress may reveal a somewhat more complicated picture. We are investigating many aspects of the problem of the control of erythropoiesis, which has been made possible by these sensitive assay procedures. Details of these studies will be forthcoming in a series of papers.^(9,10)

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