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Table 3

IN VIVO RELATIVE INCORPORATION OF ^{14}C-Phe INTO ^{14}C-Phe A.I.F. OF FETUSES FROM STARVED SHAM- AND X-IRRADIATED RATS

Treatment	(a) Litter	(b) Number per litter	(c) $\bar{X}$ fetal weight, mg	10^{-3} dis/min/g fetus (d) ^{14}C-Phe A.I.F.	(e) ^{14}C-Phe A.S.F.	(f) (d) + (e)	(g) Relative incorporation of ^{14}C-Phe into ^{14}C-Phe A.I.F. (d)/(f)
Starved and sham irradiated	R	12	158	28.8	55.6	84.4	0.341
	S	12	137	26.8	51.4	78.2	0.343
	T	12	144	30.8	57.6	88.4	0.348
	U	10	131	27.3	54.9	82.2	0.332
	V	14	135	19.9	36.7	56.6	0.352
	W	11	135	23.0	40.1	63.1	0.365
	X	11	154	30.3	44.2	74.5	0.407
	Y	14	121	20.7	39.6	60.3	0.343
						$\bar{X}$	0.354
						S.E.	0.010
Starved and irradiated	Z	11	125	21.1	49.5	70.6	0.299
	AA	13	104	24.0	51.5	75.5	0.317
	AB	14	116	22.9	46.5	69.4	0.330
	AC	15	118	24.7	49.5	74.2	0.333
	AD	12	103	18.3	38.0	56.3	0.325
	AE	12	120	21.6	45.8	67.4	0.320
	AF	11	120	11.4	27.6	39.0	0.292
	AG	13	136	26.4	46.5	72.9	0.362
						$\bar{X}$	0.322
						S.E.	0.010

analysis of variance of the data in Tables 2 and 3 showed statistically significant effects due to both X radiation ($p < 0.05$) and food and water deprivation ($p < 0.05$). No indication of interaction between these two factors was detected; thus the effects are additive.

The multiple-range test[18] indicated that incorporation in the starved and sham-irradiated and starved and X irradiated groups was significantly less than incorporation in the respective fed groups ($p < 0.05$). Further, it indicated that ^{14}C-Phe incorporation in the starved irradiated group was significantly lower than the incorporation observed in any of the other experimental groups ($p < 0.05$). Thus, these data indicate that, in the fetus present in its intrauterine environment, X radiation causes a small but significant decrease in the incorporation of ^{14}C-Phe into protein per unit weight of fetal tissue.

Irradiated fetuses increased in weight from day 13 to day 14 to the extent of only 72% of the increase observed in the unirradiated fetuses. Thus, expressed in terms of relative incorporation per whole fetus ($g \times c$, see Tables 2 and 3), the differences were greater than the differences in relative incorporation per unit weight of fetal tissue.

Table 4 presents data on the incorporation of ^{14}C-Phe into the A.I.F. in fetal cell-free systems. Since precursor ^{14}C-Phe was main-

Table 4

INCORPORATION OF ^{14}C-Phe INTO THE ^{14}C-Phe A.L.F. OF A CELL-FREE SYSTEM FROM FETUSES OBTAINED FROM FED AND STARVED SHAM- AND X-IRRADIATED RATS

	Fed				Starved			
Treatment	Litter	Number per litter	$\bar{X}$ fetal weight, mg	Incorporation, 10^{-3} dis/min/g fetus	Litter	Number per litter	$\bar{X}$ fetal weight, mg	Incorporation, 10^{-3} dis/min/g fetus
Sham irradiated	AH	11	216	33.1	AT	10	214	23.4
	AJ	8	229	34.3	AU	11	253	26.5
	AK	9	140	32.2	AV	9	138	28.6
	AL	12	146	34.1	AW	11	132	34.6
	AM	12	143	44.3	AX	8	143	27.0
				$\bar{X}$ 35.7				$\bar{X}$ 28.0
				S.E. 2.1				S.E. 2.1
Irradiated	AN	14	196	20.8	AY	9	219	13.6
	AO	10	201	22.2	AZ	9	194	22.0
	AP	12	111	30.1	BA	12	130	20.4
	AQ	12	110	15.8	BB	7	109	26.9
	AR	10	102	29.1	BC	12	106	19.8
	AS	8	116	23.0				
				$\bar{X}$ 23.5				$\bar{X}$ 20.5
				S.E. 2.1				S.E. 2.1

tained constant in the cell-free systems employed in these studies, incorporation is expressed as ^{14}C-Phe incorporated into the A.I.F.

Incorporation of ^{14}C-Phe into the A.I.F. in fetuses of the fed group was reduced by irradiation ($p < 0.05$). Similarly, irradiation decreased ^{14}C-Phe incorporation in the starved group ($p < 0.05$). Thus, the data indicate that in all cases fetal incorporation of ^{14}C-Phe into the A.I.F., as measured by the cell-free system, was reduced by fetal exposure to X rays. Incorporation in sham-irradiated animals was significantly reduced by food and water deprivation ($p < 0.05$). However, incorporation in irradiated animals was not affected by food and water deprivation. It was not determined whether fetuses derived from starved irradiated animals differed nutritionally from fetuses derived from fed irradiated animals. Thus the results indicate that, while maternal food and water deprivation decreases incorporation of ^{14}C-Phe in the fetus, irradiation causes a still further decrease in the incorporation of ^{14}C-Phe into fetal protein.

Irradiation was accompanied not only by a depression in in vitro incorporation but also by a decrease in the fetal growth rate. Thus, expressed in terms of incorporation per whole fetus (incorporation per gram × fetal weight), the differences were greater than those based on incorporation per unit weight of fetal tissue.

It should be noted that the data presented in this study measure incorporation of a labeled precursor into protein. An effort has been made to estimate (in vivo studies) or control (in vitro studies) the amount of label available for incorporation. However, no information was obtained on precursor pool size or precursor specific activity. Thus, it is not possible at this time to equate decreased incorporation of amino acid into the A.I.F. with decreased protein synthesis. The decreased growth of the irradiated fetuses suggests that the total pool of protein in the irradiated fetuses did not increase to the same degree that it did in the sham-irradiated fetuses during the 24-hr period following irradiation. Such a finding would be consistent with either decreased synthesis of protein or an increased rate of degradation of protein with no change in the normal pattern of protein synthesis as a consequence of irradiation.

The difference between incorporation in sham- and X-irradiated fetuses was small though significant in the in vivo studies with starved animals. In the studies with the cell-free system derived from sham- and X-irradiated fetuses, the differences were much greater. The latter studies examined incorporation in the post mitochondrial supernatant and thus measure cytoplasmic incorporation to the exclusion of mitochondrial and nuclear incorporation of labeled precursor into the A.I.F. Studies currently in progress are investigating the effect of fetal irradiation on cytoplasmic, nuclear, and mitochondrial protein synthesis.

CONCLUSIONS

This study investigated the effect of fetal irradiation on fetal growth and fetal incorporation of labeled phenylalanine into the acid-insoluble fraction of the fetus, both in vivo and in a fetal cell-free system. The following observations were made relative to the 24-hr period following fetal irradiation:

1. The increase in fetal weight observed in the irradiated fetuses was significantly less than that observed in unirradiated fetuses.
2. The in vivo incorporation of labeled phenylalanine into the acid-insoluble fraction of the fetus shows a small but significant decrease following irradiation.
3. The incorporation of labeled phenylalanine into the acid-insoluble fraction of a fetal cell-free system was reduced following irradiation to a greater extent than that observed in the in vivo studies.

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OPEN DISCUSSION

GLASSER: Dr. Shore, do your irradiated mothers experience an anorexic period?

SHORE: I really cannot tell you. We did not make such a measurement.

GLASSER: What is the incorporation of ^{14}C phenylalanine into the dam?

SHORE: We concentrated strictly on the fetus. We have done studies on the effects of radiation on liver enzyme synthesis in adult and adolescent rats. We found effects that parallel the slope we found here.

MAHLUM: Have you looked at incorporation at earlier times after radiation to account for the difference in the weight that you see in the 24-hr period?

SHORE: Yes, we have, and this is what has led us to these interesting lines of thought.

HOW X RAYS BLOCK LIMB REGENERATION IN SALAMANDERS AND A METHOD FOR REINSTATING REGENERATION

S. MERYL ROSE
Tulane University, New Orleans, Louisiana

ABSTRACT

Evidence that limb regeneration fails in salamanders after X irradiation because nerves fail to make proper contacts with the epidermis is reviewed. This failure to make proper contacts results in a failure of the flow of information between cells. X-rayed cells are capable of regeneration and take an active part in it if the communication system is restored by grafts of normal tissue.

It has long been known that X irradiation in the dosage range from 2000 to 7000 R prevents limb regeneration in salamanders.[1] We are just beginning to understand how this is accomplished.

Salamanders are the highest forms in the vertebrate series that can regenerate perfect limbs. If a distal part is removed, for example, the forearm and hand, the distal part of the stump transforms to the missing part. Any part of the limb can form the missing distal structures.

When a part is missing, the distal part of the stump loses structure, i.e., fibers and skeletal matrix dissolve and muscle fibers break up. The remaining cells become embryonic in type. The blastema that they compose resembles an embryonic limb bud and like a limb bud grows rapidly.[2] Within 12 days to a month after amputation, the new pattern of the missing part of the limb can be seen. Clearly there must be some kind of communication between cells if they can respond to something missing and cooperate to regenerate the missing structures.

Two observations indicate that X rays block regeneration by decreasing communication between cells. First, if a piece of limb skin is grafted to the tail of a salamander and the tail amputated through the graft, the regenerate is a tail. If the tail is X-rayed before receiving

normal limb skin, the X-rayed tail does not regenerate. In addition, the tail does not suppress the regeneration of the limb skin, which can then transform to the distal part of a limb.[3,4]

The second observation is that X-rayed tissues do not lose structure and dedifferentiate appreciably. They behave as though nothing were missing.

We are beginning to understand how X rays block regeneration. First of all it is not by blocking cell division.[5] As many epidermal cells divide in X-rayed limbs as in normal limbs.[6] The first clue is that limbs fail to regenerate after either X-irradiation or denervation, and they fail in the same way.[7] The limbs do not undergo the series of changes leading to new structures.

The nerves by their presence and position determine whether and in what direction morphogenetic information will travel. If one grafts or deviates nerve bundles in worms or salamanders, a new axis of morphogenesis is established.[8] For example, a piece of ventral nerve cord of the annelid, *Clymenella*, grafted under and at right angles to the dorsal skin causes an outgrowth. The nature of the outgrowth, whether it be head or tail, depends primarily upon the region in which the outgrowth occurs. A piece of anterior cord grafted to the anterior part of the body causes an outgrowth that becomes a head. Anterior cord grafted to the posterior part of the body also causes an outgrowth, but the posterior region causes it to become a tail.[9] The nerves set up axes along which information can travel. The region where the new axis is located is no longer controlled in the original pattern but is free to transform to the most distal structure not present along its new axis.

Axes of control were demonstrated in the salamander limb by the interference resulting when two axes interacted when placed at an angle to each other.[10] If two limbs were sewed together after the skin between them had been removed, both regenerated whole hands after amputation when the limbs lay parallel. If the angle between them was 30°, some of the fingers in the medial position did not regenerate. At 60° only the most lateral fingers regenerated, and at 90° all or almost all regeneration was suppressed. Monroy[10] reasoned that the interference was linear in nature and that wherever lines crossed morphogenesis could not occur.

Figure 1 shows regeneration of control doubled stumps (neither limb irradiated) and of experimental doubled stumps (right stump irradiated). Part a of Fig. 1 shows that, when the control limbs were in a parallel position, there was no interference from the longitudinal lines of control and both stumps regenerated normally. When the stumps were at an angle, the more central lines intersected, central structures were lost, and only the lateralmost regions, where there was no interference, were able to regenerate. At very wide angles even

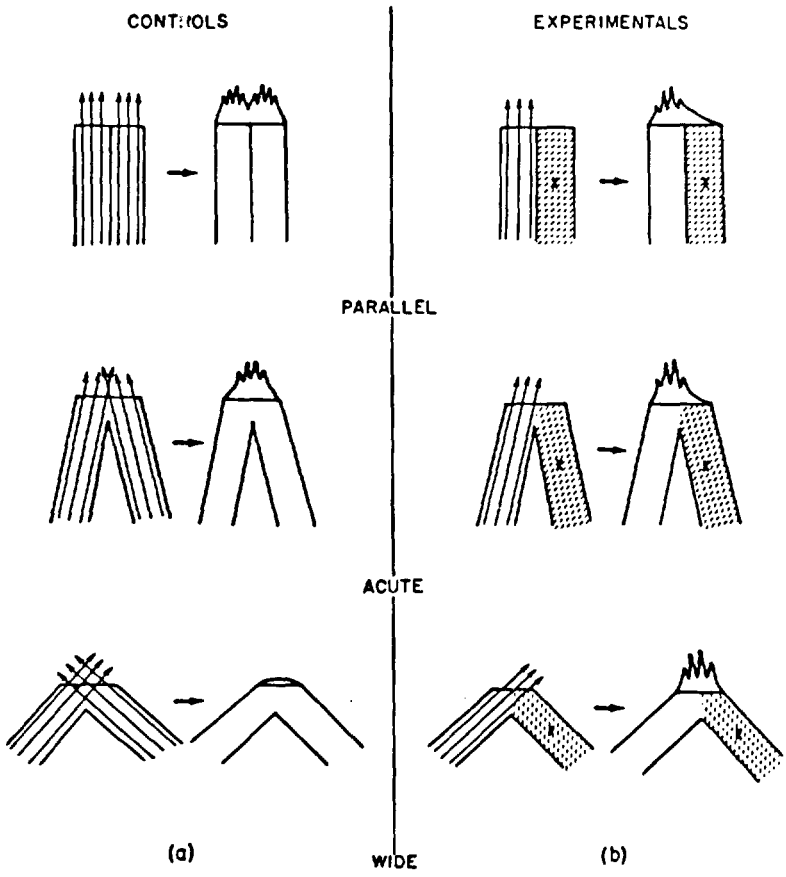

Fig. 1—Regeneration of control doubled stumps (neither limb irradiated) and experimental doubled stumps (right stump irradiated). [From J. C. Oberpriller, J. Exp. Zool., 168:*421 (1968).]*

the most lateral lines intersected, and no regenerate was produced. Part b shows that, when the experimental stumps were in a parallel position, a single hand was produced, apparently from the unirradiated limb, which was still exerting its longitudinal control. When the stumps were at an angle, the same phenomenon is seen. The regenerate that forms is produced by the unirradiated stump; the irradiated limb neither regenerates nor exerts any interference on the regenerating limb.

Jean Oberpriller[11] reasoned that, if X rays do obliterate lines of control, an X-rayed limb sewn to a normal limb at an angle would not interfere with the regeneration of the normal limb. This proved to be true (Fig. 2). This tells us that X rays destroy the operation of linear

Fig. 2—Regeneration when two limbs were grafted together at a wide angle and both hands were amputated. The right limb, which had received an X-ray dose of 2000 R, failed to regenerate a hand and did not interfere with the regeneration of a hand on the unirradiated left limb. [From J. C. Oberpriller, J. Exp. Zool., 168: *419 (1968).]*

morphogenetic controls. How this is done is being studied. The first results have been obtained.

The studies are based on the fact that X-rayed limbs can recover regenerative ability if normal limb tissues are grafted to them.[12,13] Skin that has not been X-rayed or just the epidermis[14] covering an X-rayed limb can cause the regeneration of a perfect limb. The question has been whether the graft of normal tissue provides all the cells for regeneration or whether the graft in some manner enables the X-rayed tissues to participate.

One can study an early step in the failure of X-rayed tissues to regenerate by providing them and normal limbs with tritiated thymidine. In both the normal and the X-rayed limbs, the epidermis picks up tritiated thymidine and epidermal cells continue to divide, as can be seen on autoradiographs. The X-rayed limbs can function for many years, but regenerative ability does not return. It can return, as noted above, if the limb is provided with a normal epidermis. Then, as part of the series of steps in regeneration, the internal X-rayed cells of muscle, connective tissues, and nerve sheath do make new DNA and proceed to regenerate.

The last question is what the normal graft does to reinstate regenerative ability. A comparison of normal stumps, X-rayed stumps,

and X-rayed stumps covered with normal epidermis indicates that in the normal stumps nerve fibers grow into the epidermal wound epithelium covering the distal end of the stump.[15,16] There the nerve endings make a synaptic junction with the epidermal cells.[17] In limbs with a normal nerve supply, this intimacy of nerves and epidermis is a prerequisite for regeneration.[18] The nerves making this contact are afferent nerves. In the event that all afferent nerves are removed and the limb regenerates with an increased efferent supply,[19] the contact between nerves and epidermis is not necessary for limb regeneration.[20,21] Presumably in this case contact between efferent nerves and other tissues performs the function necessary for limb regeneration.

In X-rayed limbs after amputation, the nerves fail to enter the epidermis and regeneration fails. When the X-rayed limb stump has normal epidermis at its tip, nerves do enter the epidermis, and the internal changes necessary for regeneration occur. A reciprocal experiment has been performed. If the limb nerve still attached centrally is dissected from the limb, put to the side, and shielded during irradiation of the limb and then is replaced in the limb, such a nerve will make contact with the irradiated epidermis, and limb regeneration will occur.[22] If either the epidermis or the nerve is not irradiated, it can make contact, and the trophic nerve function is performed. This trophic function apparently permits the flow of morphogenetic information between cells. Without that flow after denervation or X-irradiation, a limb does not begin to regenerate. Further research is necessary to determine whether in other cases where trophic function has been decreased by irradiation it could be restored by normal grafts.

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EFFECTS OF X RAYS ON THE DIFFERENTIATION OF GASTRULAS OF AMPHIBIANS AND ON THE DIFFERENTIATION OF THE FEATHER GERMS IN THE SKIN OF CHICKEN EMBRYOS

M. REYSS-BRION
Laboratoire d'Embryologie et de Tératologie du C.N.R.S. et du Collège de France

ABSTRACT

X-ray irradiations of the ectoderm of young gastrulas of amphibians and of the skin of chick embryos show that the tissues that are differentiating are very radiosensitive but that the inductor tissues are able to support very strong doses of X-rays.

Autoradiographic and biochemical studies point out a fall in the DNA synthesis in the tissues after irradiation.

I have irradiated in toto young gastrulas of an amphibian urodele, *Pleurodeles waltlii* (Michah.), with various doses of X-rays. After a dose of 1170 R, the embryos are deprived of a nervous system in 95% of the cases. Higher X-ray doses kill all embryos.

To find which anlage is affected by X-rays, I have irradiated either the reacting ectodermal field or the inductive chordomesodermal field with 1170 R (Fig. 1). Following irradiation of the ectoderm, the differentiation of the ectoderm into nervous organs is inhibited in 86% of the cases and remains incomplete in the others. Following irradiation of the chordomesoderm, the inductive potentialities of the dorsal blastoporal lip are active in 100% of the cases. This property is retained even after very strong doses of X-rays, e.g., 150,000 R (Fig. 2).

These results indicate that at the gastrula stage even very strong doses of X-rays have no important effect on the inductor, while very low doses are able to suppress the differentiation of the competent field.

In order to study this point further, I irradiated the ectodermal cap of young gastrulas with lower doses of X-rays (1050, 800, 300, and 150 R).

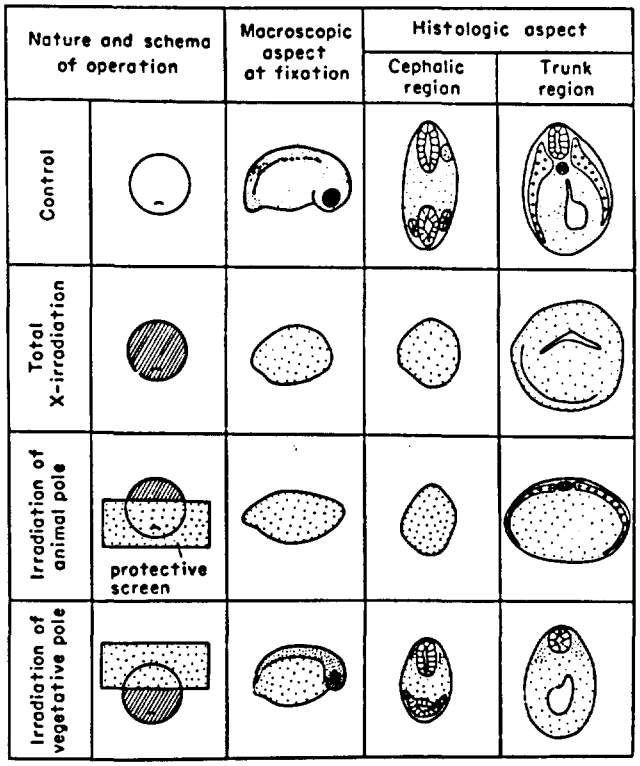


Fig. 1—Result of irradiation with 1170 R on the young Pleurodele *gastrula.*

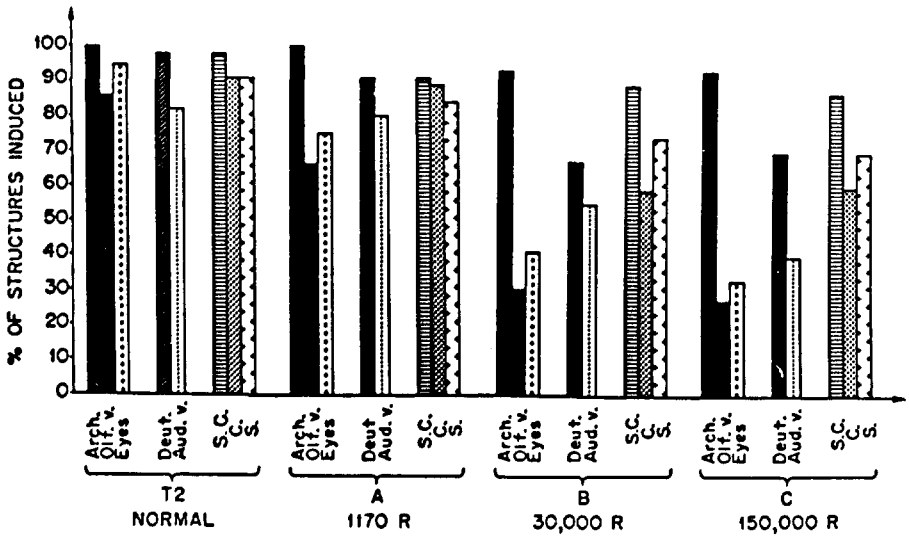


Fig. 2—Percentage of structure induced in a fragment of ectoderm by a dorsal blastoporal lip, either normal or X-rayed.

RESULTS

Measurements of the treated embryos 3 days after irradiation, show that the size of the treated embryos decreases when the intensity of the irradiation increases (Fig. 3). The findings from histological

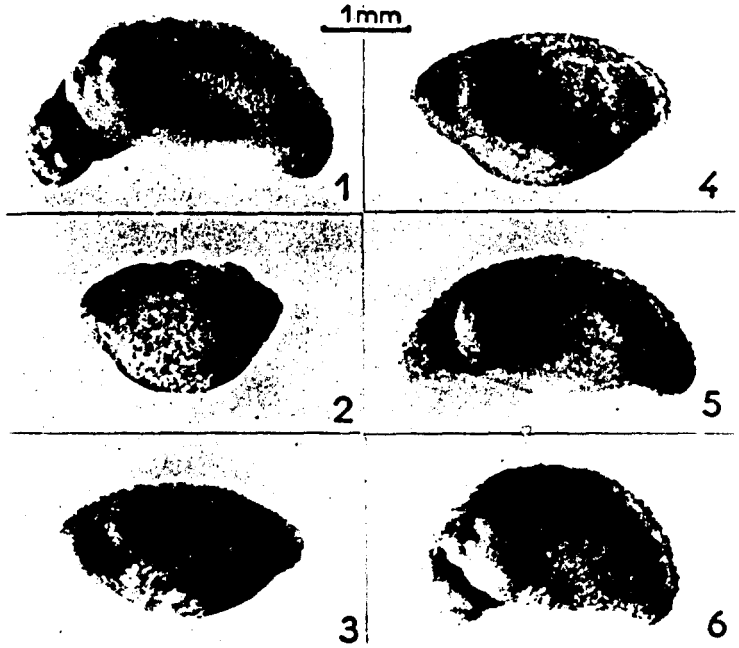


Fig. 3—Effects of irradiation of the ectodermal cap (3 days after irradiation). (1) Control; (2) 1050 R; (3) 800 R; (4) 550 R; (5) 300 R; and (6) 150 R.

sections of the anterior brain, posterior brain, and trunk of these embryos are summarized in Fig. 4.

After doses of 150, 300, 550, and 800 R, spinocaudal structures remain normal though a little reduced. They begin to disappear after 1050 R in 50% of the cases.

In contrast, the archencephalic and deuterencephalic structures begin to disappear very early. After 550 R, they are very rudimental and never develop sense organs; after 800 R, in 82% of the cases they are not differentiated; after 1050 R, they are always absent (Fig. 5). So, the loss of competence of irradiated ectodermal cells toward inductors occurs according to a determined sequence: the deuterencephalic and archencephalic structures are the first to disappear, then the spinal cord.[1]

I have checked these findings using another model system for tissue interaction, a system less complex than the whole egg and one in

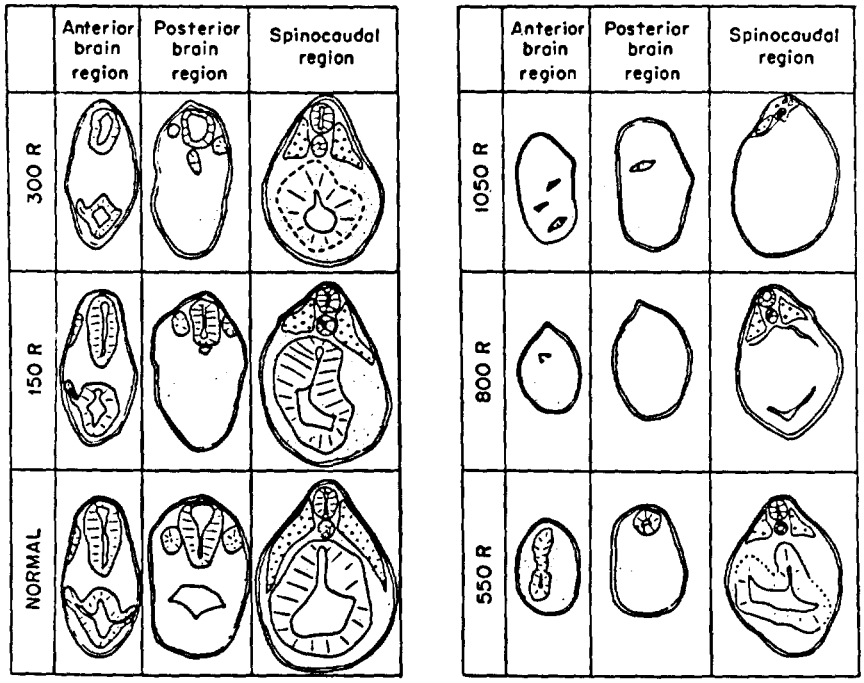


Fig. 4—Results of localized irradiation of the ectoderm of young gastrula.

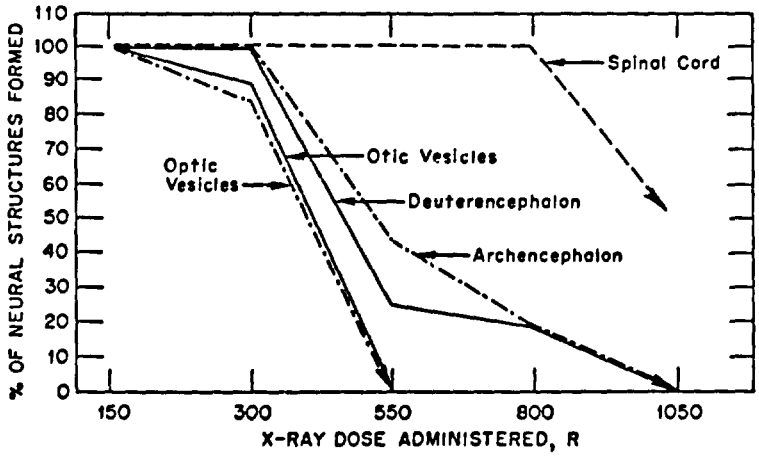
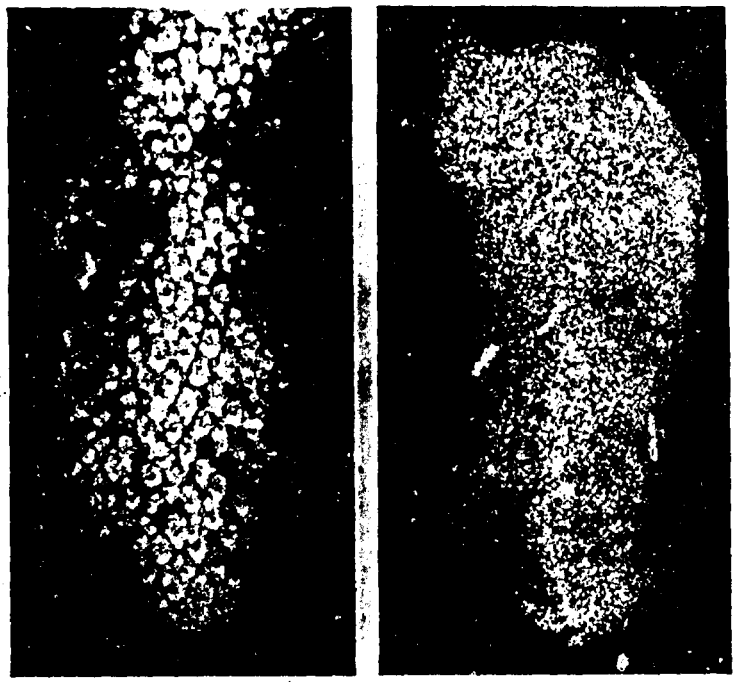


Fig. 5—Progressive loss of neural competence after irradiation of the gastrula ectoderm.



(a) (b)

Fig. 6—Seven-day-old chick embryo skin cultivated for 5 days. (a) Control. (b) Irradiated with 1000 R (no feather germs).

Fig. 7—Results of the reassociation of normal dermis and normal epidermis. Magnification, 240×.

which the inducing and responsive tissues can be easily separated and can be obtained in quantities sufficient for biochemical studies. I chose the chick embryo skin.

METHODS

The dorsal skin of chick embryos at the stage of feather-germ differentiation, that is, around 7 days of incubation, is spread out on the medium culture following the method of Wolff and Haffen,[2] then it is irradiated and cultured for 5 or 6 days. Under these conditions, the dose of X-rays (60 kv and 8 ma) which suppresses the differentiation of the feather germs in 90% of the cases is 1000 R (Fig. 6).

The two components (dermis and epidermis) were separated by trypsin, irradiated separately, and finally recombined.

RESULTS

In the control, after recombination of a normal epidermis with a normal dermis, normal feather germs are obtained in 100% of the cases (Fig. 7). The same result is obtained with recombination of irradiated epidermis with normal dermis, but only in 95% of the cases (Fig. 8). After recombination of a normal epidermis with an irradiated dermis, the explant shows no signs of feather germs in 61% of the cases (Fig. 9). In 39% of the cases some are present, but they are very small.

Consequently it can be concluded that in this interactive system irradiation of the dermis is responsible for the inhibited differentiation. On the other hand, the epidermis is very little affected by X-rays.

The next step was to try to relate these observations with changes in the metabolism of skin cells. Hence, the possible changes in the nucleic acids and protein synthesis were explored by autoradiographic and biochemical techniques in collaboration with Y. Kong and J. M. Kirrmann.[1]

Autoradiographic Studies

Incorporation (10 to 100 μCi/culture medium) of thymidine (CH_3-T). In the controls, there is a heavy incorporation of thymidine in dermal cells at the site of feather germs. In the irradiated explants, 2 to 3 hr after irradiation, cells are less heavily labeled than in controls and are randomly distributed. After 4 hr some recovery seems to take place. In both cases, only traces of the label were found in the epidermis. (Fig. 10).

Incorporation (10 to 100 μCi/culture medium) of uridine (5-T). Uridine is uniformly taken up by the epidermal and dermal cells, and

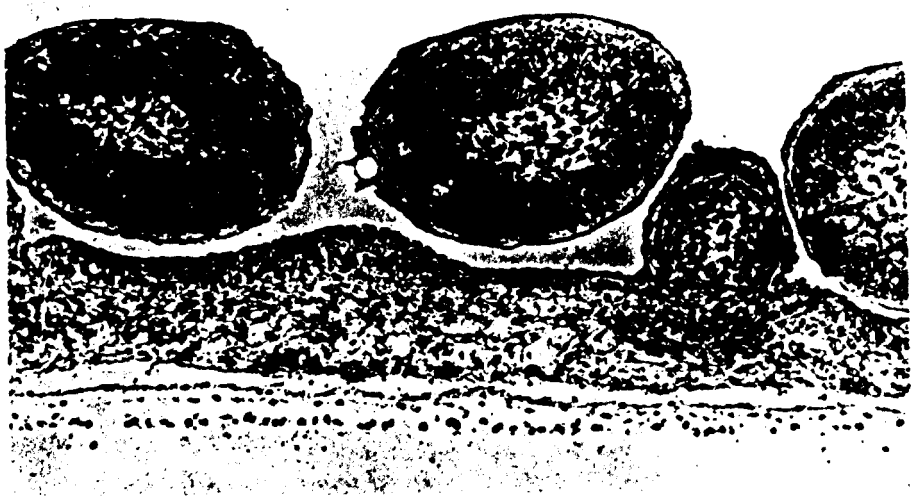
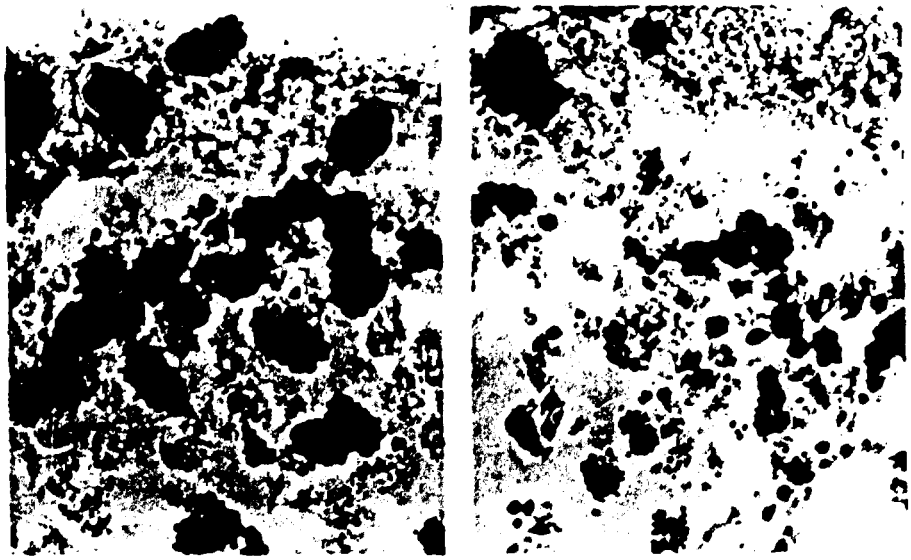


Fig. 8—Results of the reassociation of normal dermis with irradiated (51,000 R) epidermis. Magnification, 180×.

Fig. 9—Results of the reassociation of irradiated (1000 R) dermis with normal epidermis. No feather germs. Magnification, 180×.



(a) (b)

Fig. 10— (a) Incorporation of tritiated thymidine in a control (after 2 hr). (b) Incorporation of tritiated thymidine in an irradiated explant (after 2 hr).

there are no differences between controls and irradiated explants. In spite of the fall of the DNA synthesis, the RNA synthesis does not stop.

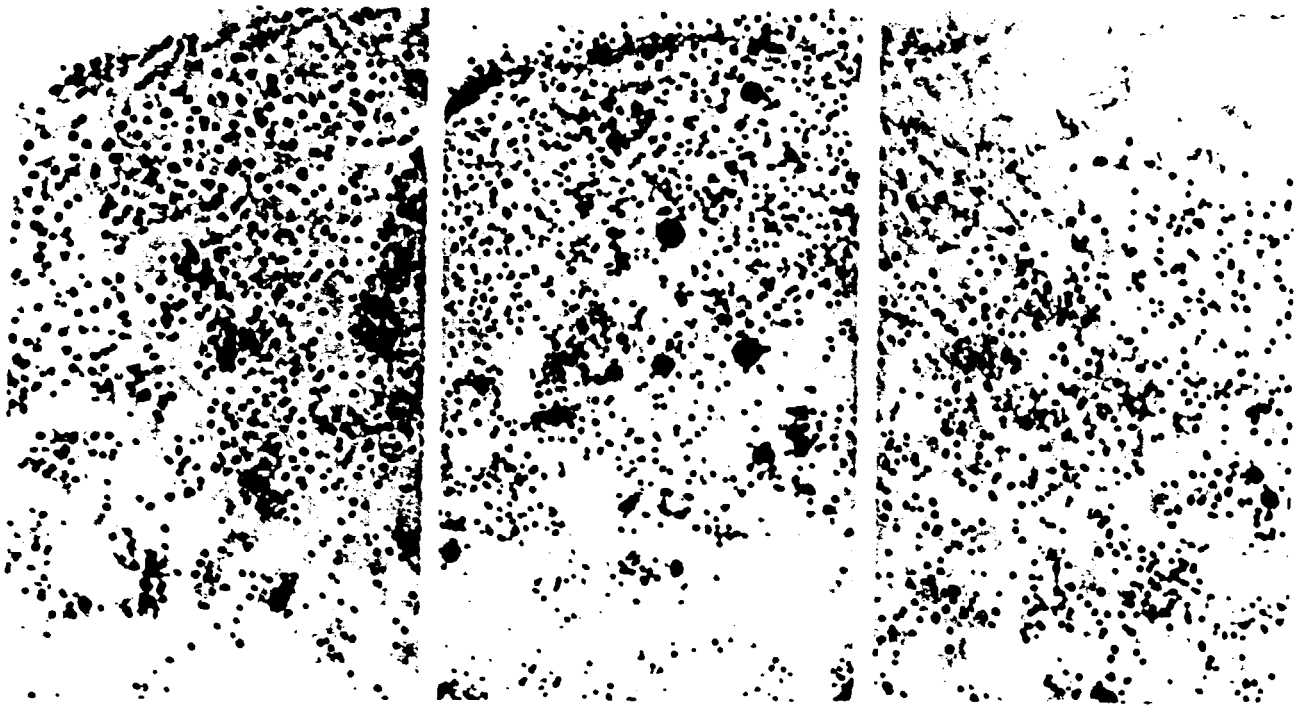
Incorporation (10 to 100 μCi/culture medium) of D-L leucine (4-5-T). The uptake of leucine very much resembled that of uridine, but the cells are more heavily labeled. In some explants (controls or irradiated), however, the epidermis remained unlabeled in these conditions, suggesting periods of low protein synthesis (Fig. 11).

Quantitative Determinations

The first quantitative determinations seem to confirm the observations. In the controls, thymidine is actively incorporated during the first 4 hr after administration. After 20 hr the total quantity of thymidine incorporated decreases. The pattern of incorporation is the same in the irradiated explants as in the controls, but there is an inhibition of 20%, which ceases after about 20 hr.

The incorporation of uridine and of leucine increases in the controls as in the irradiated.

When we measured the same incorporation with higher doses of X-rays, we found that: incorporation of thymidine decreases strongly between 0 and 1600 R; incorporation of uridine increases till 1200 R, and decreases between 1200 and 1600 R; incorporation of leucine decreases very slightly when the X-ray doses are greater than 800 R.



(a) (b) (c)

Fig. 11—Incorporation of D-L Leucine H_3. (a) Control, 2 hr on the radioactive medium. (b) Irradiated with 1000 R, cultivated 4 hr. (c) Control cultivated 4 hr.

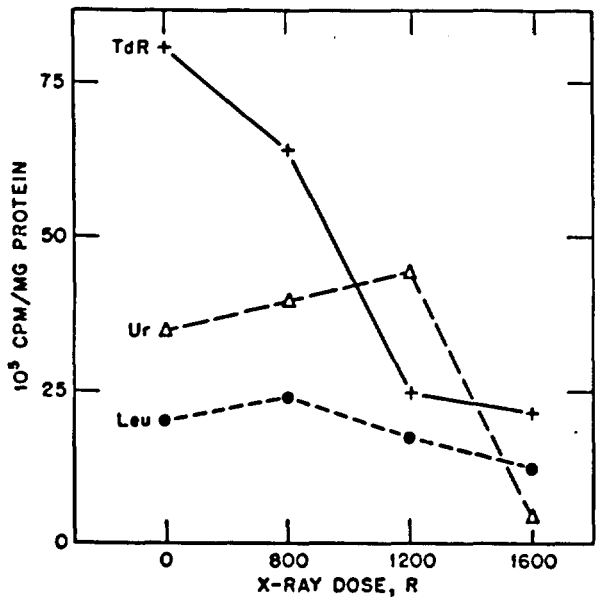


Fig. 12—Incorporation of thymidine (TdR), uridine (Ur), and leucine (Leu) at different doses of irradiation.

Between 800 and 1200 R, which are doses acting upon differentiation, only the DNA synthesis is notably affected (Fig. 12).

DISCUSSION

The fact that irradiation of the inductors does not affect their inductive capacity suggests either that the active factors have already been synthesized and stored in the cells or that synthesis goes on despite irradiation.

If we assume that the inductive substance is a protein, this could also mean that the phase of protein synthesis is not very radiosensitive. However, irradiation of the responding cells prevents their differentiation, which, in normal tissues, is always accompanied by a definite increase of the DNA synthesis. This may suggest that the primary target of the irradiation is the DNA synthesis of the differentiating tissues.

Far reaching conclusions should not be drawn from these fragmentary results. We know too little about genetic information in the cells of Metazoans to make further hypotheses on this subject.

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OPEN DISCUSSION

SCHJEIDE: Let me make a remark. It has been found that the bone matrix which has been completely decalcified can serve as an inducing agent for formation of bone by surrounding mesenchymal cells. Nothing is being secreted by the dead bone matrix, and the present thought is that it merely presents a surface which the mesenchymal cell recognizes and that this is enough of a message. In your case do you see any secretion from the inducing tissue of chondroitin sulfate or collagen?

REYSS-BRION: No secretion.

LINDENBAUM: I cannot help reacting to the suggestion of some esoteric inducing factors for collagen. The idea of a dead matrix inducing the later formation of bone apatite I think should not be thought of as having anything to do with biological systems.

SCHJEIDE: Well, if you irradiate the bone very heavily to cause derangement of the matrix proteins, you do not get induction. And on the other hand, a sponge will not induce bone. The information contained in a dead bone matrix may not be the true message, but it is a message that results in the production of normal calcification.

LINDENBAUM: I think its a semantic difference, but I'm objecting to the use of the word "message,"—unless you want to call a chemical reaction that produces something out of something else "a message."

SCHJEIDE: I take the opposite view.

BEAUMONT: Is there any evidence of a transfer of labeled RNA or protein from the donors dermis to the epidermis?

REYSS-BRION: We do not have any evidence of transfer of material because the two tissues were not separated at the final incubation.

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EPILOGUE

MELVIN R. SIKOV
Battelle Memorial Institute, Pacific Northwest Laboratory,
Richland, Washington

In the prologue, Dr. Noonan pointed out many of the differences between the format and scope of this symposium and the one held in Oak Ridge in 1953. The interest in this field is indicated by the impressive number of reports published in the intervening years; there are over 1000 literature citations in the papers of this symposium. Of greater importance, however, has been the increased sophistication in approach and in interpretation evident in the studies reported upon in the past 4 days. These studies clearly reflect the conceptual advances that have been made in the past several years. The roles of improved instrumentation and more-definitive approaches to problems of microdosimetry are particularly evident.

As we have seen, the measurement of the cross-placental transfer of radionuclides has become highly quantitative, and the available data extend over a wide variety of species, including man. In addition to the determination of radiation doses in experimental animals, sufficient information was available, for a few nuclides, to allow estimation of the radiation dose to the human fetus following exposure of pregnant women. Our progress beyond the descriptive stage is illustrated by the studies on the dynamics of transfer as well as those in which it has been possible to separate the role of the fetus from that of the placenta.

The metabolism of inorganic elements by the neonatal organism and the changes which occur during maturation have long been neglected areas of research. The amount of quantitative data obtained recently in a variety of species is most impressive. The mechanisms for these age-related differences are also being elucidated through studies on the role of diet and on the binding of radioactive nuclides at the tissue level.

Studies of factors that influence the response of the perinatal animal to radiation include studies to evaluate the role of genetic factors and of immunologic competence in the response of the neonatal animal. Other studies have indicated the relevance of usually ignored factors, such as season of the year.

Relatively few investigators have been interested in the long-term effects of radiation on the intrauterine or neonatal animal. Many of the groups involved in this area of research are represented at this symposium. We have seen that the differences between the late effects of irradiation of the perinatal animal and of the adult provide clues to the unique nature of the response of the immature animal to radiation. Studies with radionuclides have also pointed out differences and have raised several, yet unanswered, questions about the mechanisms of effect.

Renewed interest in the effects of continuous or fractionated irradiation was evident. Although many of the studies reported were descriptive, this interest may signal coming advances since comparable studies in the adult led to some of our more useful working hypotheses on the mechanism of radiation effects. As an indication of the understanding that may result, studies combining split-dose techniques for irradiation of the cerebrum with quantitative end points have suggested that partial recovery occurs in the fetal brain. Many morphological studies are still being conducted, but there is a distinct trend toward elegance of approach. The increasing use of the electron microscope to investigate the finer details of many of these morphologic changes has been evident. We also saw the improved understanding that has resulted from the use of fine beams of X rays or protons delivered to narrowly defined areas of the brain. Much of the current work is involved with elucidating the mechanisms responsible for morphologic changes described in earlier studies.

The increasingly quantitative approach contrasts markedly with earlier work. For example, growth is being measured as an increase in DNA rather than in total weight. The effects of radiation on cell proliferation are being subjected to definitive scrutiny through the use of tritiated thymidine labeling. At least one group has carefully measured the size of important functional compartments, which has allowed them to propose quantitative explanations for age-dependent differences in radiosensitivity.

The emphasis on quantitation also encompasses the physiological and biochemical changes that result from irradiation during the perinatal period. Reports included measurements of enzyme activities as well as protein, DNA, and RNA content. A few laboratories are carrying this a step further and are attempting to use such data to define the effects at the molecular level.

I have spent the last several minutes pointing out contrasts between what transpired at Oak Ridge 16 years ago and at the present meeting, and I am afraid that these differences may have obscured one very important similarity. It is apparent that underlying the many experiments with radiation and radioactive materials there is a basic desire to understand the mechanisms and processes of normal development. It is clear that we have made substantial progress in this direction and that the recent advances in technique and concept should accelerate this trend.

LIST OF PARTICIPANTS

[Participants who presented papers are not included in this list. They are listed in the Contents]

ALPEN, Edward L.
U. S. Naval Radiological Defense Laboratory
San Francisco, Calif.

ANSPAUGH, Lynn R.
Lawrence Radiation Laboratory
University of California
Livermore, Calif.

*BIBB, W. R.
Division of Biology and Medicine
U. S. Atomic Energy Commission
Washington, D. C.

BOEGLER, F.
Institute of Biology
Munich, Germany

BRAMLET, Roland C.
The Long Island Jewish Hospital
Queens Hospital Center Affiliation
Jamaica, N. Y.

BRAZLEY, L. C.
U. S. Atomic Energy Commission
Richland, Wash.

BROOKS, Frank T.
U. S. Atomic Energy Commission
Division of Biology and Medicine
Washington, D. C.

BUKO, Lorant
University of Connecticut
Storrs, Conn.

CASARETT, Alison P.
New York State Veterinary College
Cornell University
Ithaca, N. Y.

*CASARETT, George W.
Department of Radiation Biology and Biophysics
University of Rochester
Rochester, N. Y.

CURNEW, R. D.
Colorado State University
Colorado Cooperative Wildlife Research Unit
Fort Collins, Colo.

DATTA, Surinder P.
University of Wisconsin-Parkside
Kenosha, Wis.

FOX, Marian C.
Division of Technical Information Extension
U. S. Atomic Energy Commission
Oak Ridge, Tenn.

FUQUA, P. A.
Hanford Environmental Health Foundation
Richland, Wash.

HAMPTON, James C.
Department of Anatomy
Northwestern University Medical and Dental School
Chicago, Ill.

HOWLAND, Joe W.
University of Rochester
Strong Memorial Hospital
Rochester, N. Y.

HUNTER, Jehu C.
National Institute of Child Health and Human Development

*Session chairman.

National Institutes of Health
Bethesda, Md.

LINDENBAUM, Arthur
Division of Biological and Medical Research
Argonne National Laboratory
Argonne, Ill.

MAWHINNEY, B. S.
Radiation Research Laboratory
University of Iowa
Iowa City, Iowa

MAY, Harold
Argonne National Laboratory
Argonne, Ill.

MAYES, Mary G.
Southwestern Radiological Health Laboratory
Public Health Service
Las Vegas, Nev.

MINAMISAWA, Takeru
National Institute of Radiological Sciences
Chiba-Shi, Japan

NOAH, R. F.
U. S. Department of Agriculture
Riverton, Wyo.

PALMITER, C. C.
Federal Radiation Council
Washington, D. C.

ROBERTS, Joan M.
University of Detroit
Detroit, Mich.

ROBISON, William L.
Lawrence Radiation Laboratory
University of California
Livermore, Calif.

ROSARIO, Ben
Northwestern University Medical School
Chicago, Ill.

*RUGH, Roberts
Radiological Research Laboratory
Columbia University
New York, N. Y.

SHARP, Joseph C.
Division of Laboratories and Research
New York State Department of Health
Albany, N. Y.

SNYDER, Walter
Health Physics Division
Oak Ridge National Laboratory
Oak Ridge, Tenn.

STANNARD, J. N.
School of Medicine and Dentistry
University of Rochester
Rochester, N. Y.

TAMPLIN, A. R.
Lawrence Radiation Laboratory
University of California
Livermore, Calif.

van der SMISSEN, E.
Southwestern Radiological Health Laboratory
U. S. Public Health Service
Las Vegas, Nev.

WILSON, William
U. S. Air Force
Kirtland Air Force Base
Albuquerque, N. Mex.

*YAMAZAKI, J. N.
University of California—Los Angeles Medical Center
Los Angeles, Calif.

Battelle-Northwest Laboratory

AMSTER, Robert L.
BAIR, W. J.
CATT, D. L.
CLARKE, W. J.
CRAIG, D. K.
CROSS, F. T.
DILLEY, J. V.
FOSTER, R. F.
FRAZIER, M. E.
HESS, J. O.
HONSTEAD, J. F.
HORSTMAN, V. G.
HOWARD, E. B.
HUNGATE, F. P.
KILLINGSTAD, N. Kay
KORNBERG, H. A.
MANZEL, D. B.
PARK, J. F.

*Session chairman.

PARKER, H. M.
PETERSON, Gary R.
SMITH, V. H.
SOLDAT, J. K.
SULLIVAN, M. F.
TEMPLETON, W. L.
THOMAS, J. M.
*THOMPSON, R. C.
TOMBROPOULOS, E. G.
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