Time-Dependence of the Effect of X-Irradiation on the Formation of Glycerol Phosphate Dehydrogenase and Other Dehydrogenases in the Developing Rat Brain

BY J. DE VELLIS AND O. A. SCHJEIDE

Laboratory of Nuclear Medicine and Radiation Biology, Department of Biophysics, School of Medicine, University of California, Los Angeles, Calif. 90024, U.S.A.

(Received 10 October 1967)

X-irradiation (100–1500 r) administered to the heads of rats 8–30 days of age inhibited the development of glycerol phosphate dehydrogenase (L-glycerol 3-phosphate–NAD oxidoreductase, EC 1.1.1.8) in the brain stem and cerebral hemispheres. At 40 days of age and older no effect was observed. This inhibition was a delayed phenomenon, dose-dependent and with no recovery. It is proposed that the inhibition of enzyme formation is related to radiation damage caused to DNA. Actinomycin D inhibited the development of glycerol phosphate dehydrogenase in a manner similar to ionizing radiation. Four other dehydrogenases also showed age-dependent radiosensitivities. ‘Malic enzyme’ (EC 1.1.1.40), lactate dehydrogenase (EC 1.1.1.27) and malate dehydrogenase (EC 1.1.1.37) ceased to be radiosensitive at about 8 days of age and isocitrate dehydrogenase (NADP) (EC 1.1.1.42) at 16 days. The correlation between developmental increase in enzyme activity and radiosensitivity held closely for glycerol phosphate dehydrogenase and isocitrate dehydrogenase and to a smaller extent for the others.

Ionizing radiation has been shown to prevent the synthesis of induced enzymes in micro-organisms by Clayton & Adler (1962) and by Pollard (1964). In the regenerating rat liver, X-irradiation inhibited the formation of thymidine kinase (EC 2.7.1.21) and DNA polymerase (EC 2.7.7.7), but not that of other enzymes known to increase during regeneration (Van Lancker, 1962; Myers, 1962). Dietary induction of two liver enzymes has been shown to be sensitive to irradiation, whereas hydrocortisone induction of tyrosine transaminase (EC 2.6.1.5) was radioresistant (Pitot, Peraino & Lamar, 1965). Greengard, Baker, Horowitz & Knox (1966) found that radiation impaired the induction of hepatic tryptophan pyrrolase (EC 1.13.1.12) by tryptophan but not by hydrocortisone; however, the induction of tryptophan pyrrolase by hydrocortisone was reported to be radiosensitive by Mishkin, Shore & Davis (1967). Hietbrink & DuBois (1966) and Nair & Zeitlin (1967) observed that irradiation to the head alone inhibited the development of two enzymes of the rat liver. This varied effect of ionizing radiation on enzyme formation justifies further studies, particularly with respect to radiosensitivity of constitutive enzyme synthesis, a subject that has thus far received little attention.

Several reports have dealt with the effect of radiation on the activity of enzymes in developing and mature mammalian organs. However, few have attempted to correlate the time of irradiation with the time of enzyme formation. Further, consistent omission of such parameters as cell-population changes and DNA, RNA and protein contents makes the interpretation of changes in enzyme activities difficult. de Vellis, Schjeide & Clemente (1967) observed that the administration of 750 r of X-rays to the heads of 2-day-old rats resulted in a differential inhibition of the post-irradiation development of five brain dehydrogenases as well as in a decrease in protein synthesis. It was proposed that radiation affected their developmental activities in the post-irradiation period by inhibiting their synthesis. In the present work irradiation was administered at various times during the development of these enzymes to ascertain if this inhibition was time-dependent. Most emphasis has been put on glycerol phosphate dehydrogenase (L-glycerol 3-phosphate–NAD oxidoreductase, EC 1.1.1.8), since its development in the brain regions studied occurs after the cessation of cell division, which is known to be one of the most radiosensitive processes. These

conditions therefore provide a unique system in which to study the effects of X-irradiation on the synthesis of a constitutive enzyme.

MATERIALS AND METHODS

Irradiation. For each sex, litter-mate rats (Wistar strain) were matched by weight and divided into two equal groups, one treated with 750 r of X-rays to the head only, unless otherwise stated, and the other receiving no treatment. The irradiation factors were: 250 kv, 15mm., 36.0 cm. target-animal distance, 0.21 mm. Cu inherent, 0.48 mm. Cu filter, 1.0 mm. Al filter, HVL 1.35 mm. Cu, centre of parabolic filter. Each animal was secure (unanaesthetized) in a plastic box and the body shielded by 6 mm. lead plates. The heads were exposed to single doses at a rate of 150 r/min.

Chemicals and enzyme assays. The actinomycin D used was a gift from Merck and Co., Rahway, N.J., U.S.A. All other chemicals were of the highest available purity and were obtained from the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.

Cerebellum and olfactory lobes were dissected from fresh brains and discarded. The remainder was divided into two parts: the cerebral hemispheres and the brain stem (boundaries: anterior, optic chiasma including the rostral pole of the thalamus and hypothalamus; posterior, up to and including the medulla oblongata). These tissues were homogenized and dehydrogenase enzymes were assayed as described by de Vellis *et al.* (1967). One unit of any enzyme was defined as the amount that catalyses the transformation of 1 μ mole of substrate/min. at 30°. Enzyme activity was expressed as units of enzyme/brain region as defined above.

To facilitate proper interpretation of enzyme-activity changes in irradiated tissues, total activities/brain region rather than specific activities/mg. of protein were used for each enzyme. Expressing results in terms of specific activities can only be valid if there is no difference in protein content between control and treated tissues. Further, in this instance, since the number of cells in the brain regions studied ceased to increase a few days after birth, an increase in total activity represented a net increase per cell.

RESULTS

Time-dependence of radiation effect. To test if the developmental increases in activities of the five dehydrogenases studied were equally radiosensitive during their development, rats were irradiated at various ages after birth. X-irradiation (750 r) administered to the heads of rats at 8, 11, 16, 20 and 30 days after birth resulted in an inhibition of the post-irradiation development of glycerol phosphate dehydrogenase activity in the brain stem and the cerebral hemispheres. Irradiation at 40 days and later resulted in no discernable effect on post-irradiation enzyme activity even up to 40 days after irradiation (Table 1). Thus the radiosensitive period corresponded to the time of maximum enzyme-activity increase (Fig. 1). Inhibition was consistently observed after a delay; the duration of this delay could not be determined exactly for all

Fig. 1. Effect of irradiation at 20 days of age on the developmental increase in activity of glycerol phosphate dehydrogenase (a) in the cerebral hemispheres and (b) in the brain stem of male rats. Each point represents the mean of enzyme-activity measurements in controls (●) or in litter mates irradiated at 20 days of age (○). For other details see Table 1, where the same data are presented in percentages.

irradiation times because of an insufficiency of measurements. However, for animals irradiated at 20 days, a significant difference between control and irradiated rats was observed by 29 days of age. A graphic presentation of the development of enzyme activity in normal animals and in animals irradiated at 20 days of age is shown in Fig. 1. Increases in enzyme activity seemed to slow down and come to a stop sooner in irradiated than in control animals. There was no recovery in irradiated brain regions to normal values.

Variations in the protein content of the two brain regions studied and in body weight after irradiation are shown in Table 1. Irradiation at 16 days and later produced no significant effect on the protein contents of brain stem or cerebral hemispheres. However, irradiation at earlier ages did affect the developmental increase of protein, but not as markedly as after irradiation at 2 days of age (de Vellis *et al.* 1967). Body weight was also less affected with increasing age. It is noteworthy that body weights of animals irradiated at 20 days were

Table 1. *Glycerol phosphate dehydrogenase activity in the developing rat brain after irradiation at various ages*

Litter mates of control animals were irradiated to the head (750 r) at various ages as described in the Materials and Methods section. Enzyme activity and protein content per pair of irradiated cerebral hemispheres (XC) and per irradiated brain stem (XBS) were assayed at various times after irradiation and were expressed as a percentage of values obtained with control litter mates. Results are given as mean $\pm$ S.D.; S.D. is not indicated for animals irradiated at 8 days of age, for which brain samples were pooled and assayed in a single experiment. The numbers of irradiated rats are given in parentheses. Results from male rats only are presented.

Age (days)		Glycerol phosphate dehydrogenase activity (% of control value)		Protein (% of control value)		Body wt. (% of control value)
Irradiation	Death	XC	XBS	XC	XBS	
8 (3)	26	54.7	57.1	79	80	75
8 (3)	30	57.6	42.4	91	88	83
8 (4)	42	59.7	56.9	90	87	79
11 (6)	20	73.2 $\pm$ 6.5	82.9 $\pm$ 7.1	95 $\pm$ 6	93 $\pm$ 4	92 $\pm$ 3
11 (6)	26	60.0 $\pm$ 3.5	59.3 $\pm$ 4.5	93 $\pm$ 3	91 $\pm$ 6	80 $\pm$ 5
11 (10)	31	56.1 $\pm$ 3.1	43.9 $\pm$ 5.3	92 $\pm$ 4	89 $\pm$ 8	87 $\pm$ 7
11 (4)	40	48.5 $\pm$ 3.8	45.4 $\pm$ 4.4	90 $\pm$ 5	90 $\pm$ 5	85 $\pm$ 4
16 (6)	26	88.2 $\pm$ 5.9	88.9 $\pm$ 3.7	97 $\pm$ 5	96 $\pm$ 4	92 $\pm$ 2
16 (6)	40	53.3 $\pm$ 3.8	47.4 $\pm$ 3.3	95 $\pm$ 3	99 $\pm$ 8	95 $\pm$ 6
20 (8)	20	100.0 $\pm$ 2.9	100.0 $\pm$ 5.7	100 $\pm$ 3	101 $\pm$ 2	100 $\pm$ 3
20 (6)	23	93.9 $\pm$ 5.4	103.3 $\pm$ 4.4	99 $\pm$ 2	98 $\pm$ 2	90 $\pm$ 4
20 (6)	26	101.8 $\pm$ 5.9	98.1 $\pm$ 4.6	101 $\pm$ 4	99 $\pm$ 4	91 $\pm$ 3
20 (12)	29	86.0 $\pm$ 1.6	91.5 $\pm$ 3.2	98 $\pm$ 2	97 $\pm$ 5	85 $\pm$ 6
20 (6)	32	88.7 $\pm$ 2.4	84.0 $\pm$ 3.4	99 $\pm$ 5	97 $\pm$ 4	92 $\pm$ 6
20 (8)	34	78.1 $\pm$ 2.4	80.8 $\pm$ 4.6	97 $\pm$ 4	94 $\pm$ 6	93 $\pm$ 5
20 (18)	40	69.2 $\pm$ 2.6	68.4 $\pm$ 3.9	96 $\pm$ 5	98 $\pm$ 6	90 $\pm$ 5
20 (6)	50	67.7 $\pm$ 2.5	66.7 $\pm$ 6.0	98 $\pm$ 7	97 $\pm$ 5	88 $\pm$ 6
20 (8)	53	65.7 $\pm$ 3.2	61.9 $\pm$ 5.9	99 $\pm$ 5	96 $\pm$ 4	90 $\pm$ 5
30 (4)	40	99.8 $\pm$ 3.1	95.0 $\pm$ 2.6	96 $\pm$ 6	101 $\pm$ 5	97 $\pm$ 4
30 (6)	50	90.2 $\pm$ 3.0	87.9 $\pm$ 2.7	98 $\pm$ 3	99 $\pm$ 5	95 $\pm$ 6
40 (6)	50	95 $\pm$ 6	99 $\pm$ 4.5	97 $\pm$ 5	99 $\pm$ 4	94 $\pm$ 6
40 (6)	60	99 $\pm$ 4	97 $\pm$ 3.8	99 $\pm$ 4	100 $\pm$ 3	93 $\pm$ 5
80 (4)	100	102 $\pm$ 4	106 $\pm$ 6	104 $\pm$ 3	105 $\pm$ 2	101 $\pm$ 5
80 (4)	120	101 $\pm$ 6	98 $\pm$ 5	102 $\pm$ 4	96 $\pm$ 4	98 $\pm$ 6
150 (3)	156	101 $\pm$ 3	100 $\pm$ 5	101 $\pm$ 3	104 $\pm$ 2	102 $\pm$ 2
150 (3)	162	103 $\pm$ 4	98 $\pm$ 3	101 $\pm$ 3	96 $\pm$ 4	99 $\pm$ 3
150 (3)	168	101 $\pm$ 6	100 $\pm$ 5	103 $\pm$ 4	97 $\pm$ 5	102 $\pm$ 4

approximately 10% lower than those of control animals. This indicates no major impairment of growth hormone secretion by the pituitary gland. A rat hypophysectomized at 20 days of age would only have about 25% of the weight of its control litter mates by 40 days of age.

Four other dehydrogenase enzymes were assayed on the same brain samples as glycerol phosphate dehydrogenase; the post-irradiation activities of lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37) and 'malic enzyme' (EC 1.1.1.40) were unaffected by irradiation at ages greater than 8 days, and with isocitrate dehydrogenase (EC 1.1.1.42) radioresistance was established after 16 days of age. At earlier ages, however, these enzymes were radiosensitive (Table 2) but not as much as after irradiation at 2 days of age as previously reported (de Vellis *et al.* 1967). The time-dependence of the radiation effect observed for

glycerol phosphate dehydrogenase seemed also to apply to the above-mentioned enzymes, except that the period between the onset of radioresistance and maximum development of enzyme activity varied. Lactate dehydrogenase, malate dehydrogenase and 'malic enzyme' developments were radioresistant by 8 days of age, whereas their total activities continued to increase until about 35–40 days, as could be calculated from their specific activities published by de Vellis *et al.* (1967). On the other hand, the development of isocitrate dehydrogenase, whose total activity ceased to increase by 20 days of age, was radiosensitive up to 16 days of age. X-irradiation inhibited almost totally any increase of this enzyme.

Effect of radiation dose. The effect of radiation dose on glycerol phosphate dehydrogenase development was determined by irradiating rats at 20 days and assaying enzyme activity at 40 days of age.

Table 2. *Isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), 'malic enzyme' (ME) and malate dehydrogenase (MDH) activities in the developing rat brain after irradiation at various ages*

Enzyme activities per pair of irradiated cerebral hemispheres (XC) and per irradiated brain stem (XBS) are expressed as a percentage of values obtained with control litter mates. These dehydrogenases were assayed in the same brain samples as in corresponding treatments in Table 1, where results for glycerol phosphate dehydrogenase are shown. Results are given as means $\pm$ s.d. For other details see Table 1.

		Relative enzyme activity (% of control values)							
Age (days)		IDH		LDH		ME		MDH	
Irradiation	Death	XC	XBS	XC	XBS	XC	XBS	XC	XBS
8	26	74	69	85	81	101	86	88	95
8	30	85	69	97	90	98	85	95	91
8	42	86	84	94	98	95	93	94	97
11	31	82 $\pm$ 5	73 $\pm$ 3	90 $\pm$ 3	91 $\pm$ 4	92 $\pm$ 6	97 $\pm$ 3	97 $\pm$ 5	100 $\pm$ 4
16	40	92 $\pm$ 4	93 $\pm$ 3	102 $\pm$ 4	101 $\pm$ 5	107 $\pm$ 7	110 $\pm$ 7	101 $\pm$ 6	105 $\pm$ 5
20	20	99 $\pm$ 3	101 $\pm$ 2	99 $\pm$ 2	100 $\pm$ 3	101 $\pm$ 4	98 $\pm$ 5	96 $\pm$ 5	107 $\pm$ 6
20	29	94 $\pm$ 4	95 $\pm$ 7	98 $\pm$ 5	100 $\pm$ 6	97 $\pm$ 5	98 $\pm$ 4	97 $\pm$ 5	105 $\pm$ 5
20	40	95 $\pm$ 7	94 $\pm$ 5	100 $\pm$ 5	97 $\pm$ 4	94 $\pm$ 6	103 $\pm$ 4	98 $\pm$ 5	100 $\pm$ 4
80	100	107 $\pm$ 5	98 $\pm$ 3	96 $\pm$ 4	100 $\pm$ 5	104 $\pm$ 3	99 $\pm$ 5	100 $\pm$ 3	101 $\pm$ 4
80	120	105 $\pm$ 5	104 $\pm$ 3	101 $\pm$ 4	106 $\pm$ 5	100 $\pm$ 2	97 $\pm$ 3	98 $\pm$ 4	99 $\pm$ 2

Fig. 2. Relationship between radiation dose and inhibition of glycerol phosphate dehydrogenase formation between 20 and 40 days of age in cerebral hemispheres (○) and the brain stem (●). X-irradiation was administered at 20 days as described in the Materials and Methods section. Vertical bars represent s.d., depicted as either + or -. Results from male and female rats were pooled, since no sex difference in response to radiation was observed. Each point represents the mean of 6–20 animals.

Significant decreases in enzymic activity were noted after administration of doses greater than 100 r (Fig. 2). The cerebral hemispheres displayed a slightly greater radiosensitivity than the brain

stem. A dose of 750 r inhibited 50% of the normal increase in glycerol phosphate dehydrogenase activity in the cerebral hemispheres and 40% in the brain stem. However, similar biphasic dose-response curves were obtained in both regions. The other dehydrogenases mentioned above were assayed along with glycerol phosphate dehydrogenase. As could be expected from the results shown in Table 2 for 750 r, no radiation effects were noted in the dose range used.

Effect of partial body irradiation. Since irradiation to the head has been reported to inhibit the synthesis of liver enzymes (Hietbrink & DuBois, 1966; Nair & Zeitlin, 1967) it was decided to test whether the brain was similarly a target of such abscopal effects. Irradiation (500 r) to the body, with the head shielded, at 20 days of age had no effect on the post-irradiation formation of glycerol phosphate dehydrogenase. Lactate dehydrogenase, which at this age was not affected by head irradiation, was similarly not decreased by body irradiation. The experiment was conducted on five control and five irradiated litter mate male rats.

Effect of actinomycin D. Actinomycin D, an inhibitor of DNA-directed RNA synthesis (Hurwitz, Furth, Malamy & Alexander, 1962), was used to ascertain whether or not the increase in activity of glycerol phosphate dehydrogenase during development was due to enzyme synthesis requiring genetic transcription. Actinomycin D was injected intracerebrally at the usual dose of 1 μ g./g. At 20 days of age the cerebral hemispheres weighed approximately 1 g. This age was chosen so that comparison could be made of the effects of this drug with those

Table 3. *Effect of actinomycin D on the development of glycerol phosphate dehydrogenase (GPDH), isocitrate dehydrogenase (IDH) and lactate dehydrogenase (LDH) activities in the rat brain*

A dose (0.5 μ g.) of actinomycin D was injected into each cerebrum at 20 days of age (1 μ g. total/animal). All three enzymes were assayed on the same brain samples as described in the Materials and Methods section. The numbers of rats injected with actinomycin D are given in parentheses. There was an equal number of control litter mate animals. Results are given as means $\pm$ s.d.

Age of assay (days)	Brain region	Enzyme activity (% of control values)		
		GPDH	IDH	LDH
26 (4)	Cerebrums	99 $\pm$ 3	98 $\pm$ 4	102 $\pm$ 6
	Brain stem	100 $\pm$ 5	99 $\pm$ 3	98 $\pm$ 4
34 (4)	Cerebrums	80 $\pm$ 7	94 $\pm$ 1	94 $\pm$ 6
	Brain stem	97 $\pm$ 8	98 $\pm$ 1	92 $\pm$ 1
41 (4)	Cerebrums	65 $\pm$ 12	92 $\pm$ 2	88 $\pm$ 7
	Brain stem	83 $\pm$ 7	89 $\pm$ 5	88 $\pm$ 4

of X-irradiation. Glycerol phosphate dehydrogenase, isocitrate dehydrogenase and lactate dehydrogenase were assayed at 26, 34 and 41 days of age. Glycerol phosphate dehydrogenase activity was normal 6 days after the administration of actinomycin, but at later ages the activity was diminished in the cerebral hemispheres. The brain stem showed a significant inhibition only at 41 days of age. This could have been due to failure of actinomycin D to diffuse efficiently from the cerebrums to the brain stem. Activities of the other two enzymes were not significantly affected in either region (Table 3).

DISCUSSION

The data presented show that during development there is a good correlation between the rise in activity of glycerol phosphate dehydrogenase and its radiosensitivity. Once it has reached its maximum value, its activity is not affected by irradiation. This observation and the fact that inhibition of enzyme activity during the radio-sensitive period is observed only after a delay indicate that radiation does not affect (directly or indirectly) the enzyme by causing its denaturation, but rather by inhibiting its synthesis. Furthermore, electrophoretic studies show that the predominant isoenzyme synthesized between 20 and 40 days of age for glycerol phosphate dehydrogenase is normally of form III. In irradiated brains there is merely less of this isoenzyme present with no change in electrophoretic mobility (de Vellis, 1967).

To support the above interpretation of our data, other possible actions of ionizing radiations, such as cell death, need to be ruled out. Since the enzymes studied are known to be present in neuronal and glial cells, the fact that four dehydrogenases were unaffected by irradiation at 20 days of age indicates

that the brain-cell population remains unchanged. Further, brain glutamine synthetase (EC 6.3.1.2) is also unaffected after irradiation at 20 days of age (J. de Vellis, unpublished work). It is also noteworthy that the protein content remains the same in irradiated as in control animals under these experimental conditions. Zeman & Curtis (1962) reported that even 100 000 r produced no effect on certain oxidative enzymes of central-nervous-system cells unless they became necrotic. Consequently, the radiation effect on glycerol phosphate dehydrogenase cannot be attributed to selective cell death. This conclusion is further substantiated by the work of Brownson, Suter & Diller (1963), who showed that head X-irradiation induced very few pyknotic brain cells at the doses used in this study. Further, this radiation-induced pyknosis was directly proportional with dose up to 10 000 r, but the inhibition of glycerol phosphate dehydrogenase shows a biphasic dose-response curve increasing very slowly after 375 r.

The fact that actinomycin affects glycerol phosphate dehydrogenase formation in a manner similar to ionizing radiation suggests that radiation interferes at the level of transcription, possibly by inhibiting the synthesis of specific messenger RNA or other DNA-dependent RNA syntheses required for the formation of glycerol phosphate dehydrogenase. This conclusion seems also to be pertinent to isocitrate dehydrogenase and to the other dehydrogenases studied if one invokes for the latter the existence of particularly stable templates (Spirin, 1966). Finally, the finding that irradiation of the heads of rats at 20 days of age causes immediate brain DNA degradation (12% loss from brain cells at 750 r) (de Vellis, 1967) leads to the tentative conclusion that the radiation-induced inhibition of the formation of glycerol phosphate dehydrogenase and perhaps other enzymes in the

developing rat brain is related to radiation damage to DNA, as proposed by Pollard & Barone (1966) for β -galactosidase induction in *Escherichia coli*. The fact that the dose-response curve for the loss of DNA from brain cells (J. de Vellis & K. De Wees, unpublished work) shows a strong similarity to that presented here for glycerol phosphate dehydrogenase inhibition by X-rays gives some support to this suggestion.

The decreased radiosensitivity of 'malic enzyme', malate dehydrogenase, lactate dehydrogenase and isocitrate dehydrogenase with age corresponds well with similar age-dose relationships demonstrated for neurological and histological parameters by Yamazaki, Bennett & Clemente (1962). On the other hand, the metabolic consequences of the insufficiency of glycerol phosphate dehydrogenase induced by irradiation at 20 days of age are not associated with an inhibition of myelination, as occurs in the case of irradiation at 2 days of age (de Vellis *et al.* 1967; Schjeide, Yamazaki, Haack, Ciminelli & Clemente, 1966). There is only a slight decrease in lipids and in proteolipid protein after irradiation at 20 days of age (J. de Vellis, unpublished work). However, a study of radioisotope incorporation into phospholipids might be more meaningful in this respect.

The authors thank Dr C. D. Clemente for his continued support and helpful discussions and Mrs L. Koste for assistance on part of this study. These studies were supported by Contract AT(04-1) Gen-12 between the Atomic Energy Commission and the University of California, and Public Health Service Grant no. NB-01065 from the National Institute for Neurological Diseases and Blindness.

REFERENCES

- Brownson, R. H., Suter, D. B. & Diller, D. A. (1963). *Neurology*, **13**, 181.
- Clayton, R. K. & Adler, H. I. (1962). *Biochim. biophys. Acta*, **56**, 257.
- de Vellis, J. (1967). *Biochem. J.* **104**, 39P.
- de Vellis, J., Schjeide, O. A. & Clemente, C. D. (1967). *J. Neurochem.* **14**, 499.
- Greengard, O., Baker, G. T., Horowitz, M. L. & Knox, W. E. (1966). *Proc. nat. Acad. Sci., Wash.*, **56**, 1303.
- Hietbrink, B. E. & DuBois, K. P. (1966). *Radiat. Res.* **27**, 669.
- Hurwitz, J., Furth, J. J., Malamy, M. & Alexander, M. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1222.
- Mishkin, E. P., Shore, M. L. & Davis, A. K. (1967). *Fed. Proc.* **26**, 668.
- Myers, D. K. (1962). *Canad. J. Biochem. Physiol.* **40**, 619.
- Nair, V. & Zeitlin, E. (1967). *Radiat. Res.* **31**, 609.
- Pitot, H. C., Peraino, C. & Lamar, C., jun. (1965). *Science*, **150**, 901.
- Pollard, E. C. (1964). *Science*, **146**, 927.
- Pollard, E. C. & Barone, T. F. (1966). *Radiat. Res. Suppl.* **6**, p. 194.
- Schjeide, O. A., Yamazaki, J., Haack, K., Ciminelli, E. & Clemente, C. D. (1966). *Acta radiol.* **5**, 185.
- Spirin, A. S. (1966). In *Current Topics in Developmental Biology*, vol. 1, p. 2. Ed. by Moscona, A. A. & Monroy, A. New York: Academic Press Inc.
- Van Lancker, J. L. (1962). *Fed. Proc.* **21**, 1118.
- Yamazaki, J. N., Bennett, L. R. & Clemente, C. D. (1962). In *Response of the Nervous System to Ionizing Radiation*, p. 59. Ed. by Maley, T. J. & Snider, R. S. New York: Academic Press Inc.
- Zeman, W. & Curtis, H. J. (1962). In *Proc. 4th int. Congr. Neuropathology*, p. 141. Ed. by Jacob, H. Stuttgart: Georg Thieme Verlag.