The Effects of X- and γ -Radiation on Nucleic Acid Metabolism in the Rat *in vivo* and *in vitro*

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(Received 8 September 1955)

The effects of X-radiation on nucleic acid metabolism and on nucleoproteins have been investigated extensively (Euler & Hevesy, 1942; Holmes, 1947; Hevesy, 1955). Recently, the emphasis has shifted to an analysis of the differential effects on the base, sugar and phosphate components (Harrington & Lavik, 1955) and to the correlation of deoxyribonucleic acid (DNA) sensitivity with position in the mitotic cycle (Howard & Pelc, 1951; Holmes & Mee, 1954; Lajtha, Oliver & Ellis, 1954).

Previous experiments had demonstrated that there was no direct effect of γ -rays on oxidative phosphorylation by rat-spleen mitochondria *in vitro* (Ord & Stocken, 1955). We therefore decided to extend our search for a system which would be sensitive to radiation *in vitro* to a study of the effects of irradiation on nucleic acid metabolism, using incorporation of ^{32}P as an indicator. Totter (1954) and Passoneau & Totter (1954) reported an inhibition by γ -rays (^{60}Co) *in vitro* of the incorporation of [^{14}C]formate into rat bone-marrow and chick-embryo DNA. We have used spleen, thymus and lymph nodes for the experiments *in vitro* but, in extending the work to the effects of irradiation of the whole body *in vivo*, liver and regenerating liver have also been examined. Some ideas have been developed about the varying radiosensitivity of different tissues, which is such a striking factor after irradiation of the whole body.

METHODS

Animals. The laboratory strain of Wistar rats was used. For the experiments on thymus and lymph node male animals of 70–100 g. were used, and for the other experiments males of about 200 g., unless otherwise stated.

Partial hepatectomies were performed as described by Higgins & Anderson (1931). Control and hepatectomized rats were starved for 24 hr. before operation and given 5% glucose to drink for the following 24 hr.

The animals were maintained on 0.9% saline and were used on the fourth day when adrenalectomies had been performed.

Irradiation. X-radiation was carried out *in vivo* in the M.R.C. Radiobiological Research Unit, Harwell. Irradiation *in vitro* was given by ^{192}Ir sources circularly distributed 1 cm. below the Warburg incubation flasks (total volume 5 ml.) as described previously (Ord & Stocken, 1955). Calibration was by the FeSO_4 method (Miller, 1950). After

the tissues had been exposed for the required times (with new sources 1000 r. was delivered in about 10 min.) the flasks were removed from the bath and transferred to a second bath at the same temperature. The control vessels were kept in the shielded bath the whole time.

For the X-radiation of the lymph node cultures the closed chambers were kept in an insulated box at 37° for the duration of the exposure.

Incorporation of ^{32}P . Carrier-free ^{32}P was obtained from the Radiochemical Centre, Amersham, and was brought to pH 7 before use. Animals were injected intramuscularly with 25–50 μC /100 g. and killed 2 hr. after the injection. Unless otherwise stated, they were irradiated immediately after administration of ^{32}P .

In most experiments, blood was collected from the animals at death, and the specific activity of the blood inorganic phosphate was used as reference for the activities of the nucleic acids.

Liver nuclei were prepared initially by the method of Mirsky & Pollister (1946–47). In later experiments a method developed by Mr P. Esnouf (unpublished work) was used.

The lymph node cultures were set up by Dr Trowell in a medium modified from that previously described (Trowell, 1952*b*). To each chamber 60 μC of ^{32}P was added 6 ml. of incubation fluid after the cultures had been set up for 24 hr. The incubations were stopped after a further 2 hr.

The specific activity of inorganic phosphate in the medium was determined at the end of the incubation. Neither with the lymph nodes nor with blood in the experiments *in vivo* was any difference found between control and irradiated values. Since preliminary experiments suggested that very little incorporation into DNA took place in homogenates, tissue slices [about 100 mg. cut by a McIlwain & Buddle chopper (1953)] were used. The incubations were carried out at 37° in oxygenated Krebs–Ringer phosphate containing 25 μC of ^{32}P , and fortified with 0.3M glucose. Control experiments in which the flasks were kept at 0° gave DNA with only 3% of the activity found in the flasks incubated at 37°. The incubations were from 1 to 3 hr. and were stopped by the addition of 1 ml. of 30% trichloroacetic acid (TCA). The slices were removed from the vessels and dispersed in about 10 ml. of 6% TCA by means of a homogenizer (Potter & Elvehjem, 1936).

Extraction of nucleic acid. This was performed exactly as described by Deluca, Rossiter & Strickland (1953). The Hammarsten (1947) method of extracting nucleic acids by concentrated salt solution was used, followed by the Schmidt & Thannhauser (1945) separation of DNA and RNA (ribonucleic acid).

Analysis of nucleotides of DNA. In order to establish that ^{32}P incorporation into DNA had occurred it was

necessary to demonstrate the presence of ^{32}P in the individual nucleotides. DNA was therefore hydrolysed by DNA-ase (Kunitz, 1949) and the nucleotides were liberated by diesterase prepared from viper snake-venom (Hurst & Butler, 1951). The nucleotides were separated on Dowex 1 (Hurst, Marko & Butler, 1953), the fractions identified by their ultraviolet absorption (Hurst *et al.* 1953; Beaven, Holiday & Johnson, 1954) and the ^{32}P activity was measured.

Preparation of DNA. DNA was prepared by methods III 2 (*d*) and III 2 (*a*) of Chargaff (1954), sodium dodecyl sulphate and chloroform/amyl alcohol being used respectively for deproteinizing.

Analysis of DNA. Dry wt. determinations were performed in the way recommended by Chargaff (3 hr., 60°). Base analyses were carried out as described by Wyatt (1954) by hydrolysis with formic acid followed by chromatography in isopropanol-HCl-water.

Phosphorus. This was estimated by the method of Berenblum & Chain (1938).

Nitrogen. This was estimated by the Kjeldahl method by the distillation apparatus designed by Markham (1942).

Measurements of radioactivity. ^{32}P was counted in 10 ml. samples in a Geiger-Müller liquid counter (Veall, 1948). For measurements of specific activity, inorganic phosphate was extracted as phosphomolybdic acid into 10 ml. of isobutanol, counted and a suitable volume reduced to give the phosphorus content. Sufficient counts were recorded to give an error less than 3%.

EXPERIMENTAL AND RESULTS

Irradiation of spleen and thymus

Incorporation of ^{32}P into the nucleic acids of spleen and thymus occurred readily *in vitro*, but from a comparison of the ratios of the specific activities of RNA and DNA *in vivo* and *in vitro* it is evident that the pattern of uptake was altered. It is also clear (Table 1) that an exposure of 1000–2000 r. of

γ -radiation did not affect the ^{32}P uptake by spleen slices *in vitro*.

As thymus is rather more sensitive to radiation than spleen and has a less diverse cell population, it was decided to examine this tissue in the same way. A more active uptake of ^{32}P into DNA occurred than in spleen, and the uptake increased with time, similar to the results of Smellie, Humphrey, Kay & Davidson (1955) obtained *in vivo*. In two experiments with incubation for 2 and 3 hr. with ^{32}P , 1800 and 2000 r. produced respectively a 21 and 15% inhibition of uptake of ^{32}P into DNA. Uptake of ^{32}P into RNA was probably unaffected. The results, however, were not very reproducible, and histological examination of the incubated thymus demonstrated that 10 min. shaking caused considerable pyknosis of the nuclei, although respiration and uptake continued steadily over 3 hr. The damage was not avoided in other media which were tried.

Since thymus was histologically altered it was decided to study the comparative pattern of uptake of ^{32}P into DNA nucleotides *in vivo* and *in vitro*, and to demonstrate unequivocally that true incorporation of ^{32}P had occurred. The relative specific activities of the nucleotides are shown in Table 2, and since the recoveries from the columns accounted for 80–100% of the counts it is apparent that the method of isolation was suitable for demonstrating incorporation of ^{32}P into DNA (cf. Deluca *et al.* 1953).

The pattern of incorporation into the different nucleotides indicated that the incorporation of ^{32}P into purine compounds was reduced *in vitro* compared with that found *in vivo*, when the specific

Table 1. Incorporation of ^{32}P into rat nucleic acids after irradiation

Dose (r.)	Incubation time (hr.)	Relative specific activity			
		Control RNA/DNA	Irradiated		
			RNA/DNA	RNA	DNA
				(As % control)	
<i>In vitro</i> γ -radiation					
Spleen					
1095	2	11.6	10.5	92	100
2000	2	7.1	7.8	108	98
Thymus					
2000	1	6.9	7.5	109	100
1800	2	3.7	4.9	104	79
2000	2	4.0	3.3	78	93
2000	3	3.2	3.5	94	85
<i>In vivo</i> X-radiation					
Spleen					
1000	2	2.7	4.65	67	40
Thymus					
1000 (4 expts.)	2	1.7	2.6	55	36

Table 2. Incorporation of ^{32}P into rat-thymus deoxyribonucleotides

	Adenine	Guanine	Cytosine	Thymine	Whole DNA (counts/min./ $\mu\text{g. of P}$)
<i>In vitro</i>					
Control (adenine = 1.0)	1.0	1.2	2.9	2.9	4.1
	1.0	—	2.2	3.1	4.3
	1.0	0.85	2.3	2.4	3.8
<i>In vivo</i>					
Control (adenine = 1.0)	1.0	1.2	1.0	1.3	5.35
	1.0	1.4	0.6	1.5	4.4
Adrenalectomized	1.0	1.0	1.5	2.0	5.75
2 hr. after 1000 r. (as % control)	23	27	39	37	1.5
	20	37	58	65	1.4
Adrenalectomized	53	38	56	55	2.9

Table 3. Incorporation of ^{32}P into rat lymph nodes after 600 r. X-radiation

Time after radiation (hr.)	Relative specific activities			
	Control RNA/DNA	Irradiated		
		RNA/DNA	RNA (As % control)	DNA
<i>In vitro</i>				
0-2	6.7	10.5	124	79
	6.5	8.6	106	78
	7.9	14.4	110	60
	9.5	15.5	126	77
	13.2	17.9	100	73
	8.2	16.2	89	46
Mean*	8.7	13.9	109	69
3-5	7.0	14.8	82	39
	7.9	12.4	52	33
	5.3	9.2	72.5	41
Mean*	6.7	12.1	69	38
<i>In vivo</i>				
0-2	2.7	4.65	67	40

* Mean specific activity: control RNA 32.1 counts/min./ $\mu\text{g. of P}$; DNA 4.4 counts/min./ $\mu\text{g. of P}$.

activities of the purine and pyrimidine deoxynucleotides were nearly the same. This suggested that the system incorporating ^{32}P into purines might be more labile than that incorporating it into pyrimidines, and that our incubation conditions used for the radiation experiments *in vitro*, which produced histological damage, also damaged the system incorporating ^{32}P into purine deoxynucleotides.

Fractionation of thymus DNA following irradiation *in vivo* showed that ^{32}P incorporation into adenine and guanine deoxyribonucleotides was more inhibited at 2 hr. after 1000 r. than that into cytosine and thymine nucleotides. It is possible, therefore, that the injury sustained by the purine-incorporation system during the incubation *in vitro* also damages the purine components that are

sensitive to radiation, thus reducing any possible effect of irradiation.

In an attempt to establish at which point damage occurred to phosphate incorporation, it was decided to compare some properties of thymus deoxyribonucleic acid isolated from control rats and rats exposed to 1000 r. 2 hr. before sacrifice. No detectable difference was found between the ultraviolet absorption spectra which had maxima at 259 m μ . [$\epsilon(\text{P})$ 6700] and minima at 230-231 m μ . [$\epsilon(\text{P})$ 2800] (Chargaff & Zamenhof, 1948); although there was a small reduction in N/P ratio (cf. Limperos & Mosher, 1950), this was thought to be within our experimental error. Base analyses of control and irradiated specimens showed no difference in relative composition (94 % recovery) and the base ratios agreed with those previously reported in rat bone-marrow by Wyatt (1951). As has been pointed out by Chargaff (1954), however, very slight changes in composition, which would be within our experimental error, could affect considerably the biological behaviour of the nucleoprotein.

Lymph node experiments

As the conditions of the experiments *in vitro* with thymus and spleen appeared to reduce their sensitivity to radiation, it was decided to study the effects on lymph node cultures which can be maintained up to 96 hr. in a normal histological state and have been shown by Trowell (1952a, 1953) to be sensitive to radiation. From the RNA/DNA ratios *in vivo* and *in vitro* (Table 3), it is clear that DNA turnover is relatively slower *in vitro*, and this may be correlated with the very low mitotic rate of the lymphocytes (1:20 000; Trowell, 1952b), whereas *in vivo* mitoses are rather more frequent, particularly in rats of about 100 g. weight.

When the cells were incubated with ^{32}P and immediately irradiated (600 r.) it was found that after 2 hr. incubation there was a 30 % inhibition of the uptake of ^{32}P into DNA. Incubation with ^{32}P 3-5 hr. after radiation produced 69 % inhibition

in the uptake of P into DNA and about 30% inhibition of that into RNA. Cell counts at 2 and 5 hr. after 600 r. under similar conditions indicated 6.6 and 66% pyknosis respectively. *In vivo*, pyknosis is not present 2 hr. after 600 r. Although it was not demonstrated that the ^{32}P was incorporated into lymphocyte DNA and not into that of more radiation-resistant reticulum cells, the inhibition of uptake of ^{32}P into DNA by radiation may be correlated with previous findings that lymphocytes are exceptions to the law of Bergonie & Tribondeau (1906) linking sensitivity to radiation with dividing cells.

Liver

The results of experiments on liver *in vivo* similar to those already described for other rat tissues are shown in Table 4. Very little ^{32}P is incorporated into the DNA of normal liver, but the depression produced by 1000 r. has been observed by others (Hevesy, 1949; Kelly & Jones, 1950). It was also found that incorporation of ^{32}P into the RNA of normal liver was not reduced, and by isolating nuclei it was possible to show that

nuclear RNA was similarly unaffected. This was confirmed with regenerating liver where metabolism of DNA was inhibited while the nuclear RNA was not.

Dose-response curves

Hevesy (1955) and others had previously reported that in certain tissues such as regenerating liver a limit to the inhibition of metabolism of DNA was reached. This did not occur with the pyknotic response of lymph node cultures to irradiation (Trowell, 1952*b*) nor for the inhibition of spleen and thymus (Patt, 1955). We therefore decided to measure the inhibition of incorporation of ^{32}P into rat-thymus nucleic acids *in vivo* by exposures from 25 to 3200 r. (Table 5). RNA was generally less affected than DNA, except at low exposures (25–150 r.), when little difference in sensitivity was detectable. When the probit inhibition-log dose curve was plotted for DNA (Fig. 1) a linear response was obtained with a very similar sensitivity to that found by Patt for involution of the thymus 3 days after exposure.

Table 4. Incorporation of ^{32}P into rat-liver nucleic acids *in vivo* 2 hr. after 1000 r. X-radiation

Specific activity as counts/min./ $\mu\text{g.}$ of P $\times 10^3$ /specific activity of blood inorganic phosphate.

Cytoplasmic RNA		Effect as % control	Nuclear RNA			DNA			Control RNA/DNA
Control	Irradiated		Control	Irradiated	%	Control	Irradiated	%	
Normal liver									
1.6*	1.5*	94	5.3*	5.0*	94	0.18*	0.055*	31	8.9
18.8	16	85	—	—	—	3.6	3.5	97	5.2
21.8	28.2	129	—	—	—	3.5	1.5	43	6.2
8.3	8.5	102	—	—	—	—	—	—	—
Regenerating liver									
2.5*†	2.3*	92	8.7*	8.4*	97	1.08*	0.68*	63	2.1
44.5‡	36	81	—	—	—	42.5	11.0	26	1.05
—§	—	—	30	37.8	126	13.7	8.4	61	—

* Not relative to specific activity of blood inorganic phosphate.

† Hepatectomized 28 hr. before irradiation.

† Hepatectomized 47 hr. before irradiation.

§ Hepatectomized 42 hr. before irradiation.

Table 5. Effect of increasing exposure on incorporation of ^{32}P into rat-thymus nucleic acids

2 hr. after radiation. Specific activity relative to the specific activity of blood inorganic phosphate $\times 100$.

Dose (r.)	DNA		RNA	
	Specific activity	% inhibited	Specific activity	% inhibited
Control	5.9, 5.2, 5.8, 5.9	—	10.8, 11.9, 11.7, 11.0	—
25	5.1, 6.4	5	9.6, 11.4	9
50	5.0, 4.8	14	9.1, 9.3	22
100	4.4	23	7.9, 9.8	25
150	3.1, 3.8	40	6.8, 9.0	35
Control	2.8, 3.2, 5.2, 3.2	—	5.1, 6.4, 7.2, 6.0	—
100	1.8, 3.3	29.5	—	—
200	1.8	50	3.9, 5.8	22.5
400	1.6, 1.1	62	2.9, 4.8	38.5
800	1.0, 1.2	68	3.0, 2.9	52
1600	0.85, 0.84	76.5	2.2, 2.6	61
3200	0.57, 0.58	84	1.9, 2.2	67

Fig. 1. Probit inhibition-log dose curve for the effect of X-radiation on incorporation of ^{32}P into rat-thymus DNA.

DISCUSSION

As it was possible to isolate DNA nucleotides containing ^{32}P with the same specific activity as whole DNA it seemed probable that the isolation technique developed by Deluca *et al.* (1953) was reliable, although these authors were uncertain whether they had achieved an incorporation into the DNA of nervous tissue *in vitro*. The damage sustained by the cells of the thymus during incubation rendered it unlikely that mitosis could take place so that energy-dependent incorporation of ^{32}P into DNA in non-dividing tissue occurred. This is in agreement with the demonstrations of Howard & Pelc (1951) and Lajtha *et al.* (1954) that the incorporation of ^{32}P into DNA of bean-root tips or marrow takes place some time before division, during interphase. The pattern of incorporation *in vitro* has been shown to differ from that *in vivo* and the modification in uptake may be due either to trauma or to the removal of restraints.

The effects of irradiation on lymph node cultures suggested that very early damage to the DNA-incorporating system ensued. At 2 hr. after 600 r. the pyknotic count was not increased above the control level, although there was a 30% inhibition of uptake of ^{32}P into DNA. It has not yet been possible to correlate precisely the number of dividing cells in the cultures with the amount of ^{32}P incorporated, but if only the dividing cells are taking up phosphorus the inhibition produced by

radiation would affect an insignificant proportion of the cell population. However, by 5 hr. after 600 r. 69% of all the cells had pyknotic nuclei. This confirms that 'mammalian cells may be injured...by X-irradiation through mechanisms apparently unrelated to an aberration in cell division or in growth generally' (Patt, 1955).

Incorporation of ^{32}P occurred very readily *in vivo* into both RNA and DNA in spleen, thymus, regenerating liver and the lymph nodes of young rats. In all these tissues the ratio of the specific activities of RNA to DNA was low, which is probably connected with mitosis in these organs. In liver the ratio was higher, although not as high as has been reported previously (Hammarsten & Hevesy, 1946). When the incorporation into thymus DNA was analysed the relative values were similar to those found by Smellie *et al.* (1955) in rabbit tissues, though in our case the rate into guanine deoxynucleotide was slightly lower. Irradiation considerably depressed the incorporation into the purine deoxynucleotides, that into the pyrimidine nucleotides being less affected. Harrington & Lavik's (1955) data are difficult to compare with ours, as the DNA was examined at 24 hr. after 100 r. They did, however, obtain an inhibition of uptake of ^{32}P .

We have already suggested (Stocken & Ord, 1955) that at least two factors are likely to be concerned when results from different experiments are considered: the age of the animal will probably affect the mitotic count in any organ; secondly, in irradiation experiments the effects of total body radiation are not necessarily comparable with those obtained with direct exposure. A combination of these two effects may explain the quantitative differences which have been reported with regenerating liver. The dose-response curve found for ^{32}P uptake into thymus DNA shows that in the intact animal the inhibition in this tissue is a simple function of the exposure, as has also been found in lymphocytes (Trowell, 1952a; Patt, 1955).

Uptake of ^{32}P into nucleic acids *in vivo* will of course be affected by changes in the availability of the phosphate pool. This is extremely difficult to investigate. Sherman & Forssberg (1954) and Forssberg & Klein (1954) have described periodic fluctuations in acid-soluble phosphates of liver and ascites tumour cells immediately after irradiation. Unpublished experiments with rat liver at 2 hr. after 1000 r. and rat thymus at 1 and 2 hr. after 1000 r. showed that at these times both the specific activities and concentrations of inorganic phosphate and of acid-labile phosphates with insoluble barium salts were normal.

Inhibition of incorporation into RNA after radiation appears to depend on the tissue of origin. In liver the insensitivity of RNA confirms previous

studies (Payne, Kelly & Jones, 1952; Holmes & Mee, 1954; Smellie *et al.* 1955); we also observed that metabolism of nuclear RNA was unaffected when that of DNA had been reduced. In lymph nodes, spleen and thymus uptake of ^{32}P into RNA was reduced, but at 1000 r. the effect was less than that for DNA. These findings suggested that the radiation effects on RNA may be very closely connected with those on the nucleus, and although interference in DNA metabolism occurs in dividing cells the results with lymph node cultures indicate that nuclear damage embraces non-dividing cells also. The great sensitivity of dividing cells to radiation has been known for many years (Bergonie & Tribondeau, 1906), and it is obvious that in these cells interference in DNA synthesis would have serious effects which might well be communicated to the cytoplasm. The variation in sensitivity to radiation between normal liver and lymphocytes is well established. We suggest that the difference might reside in the larger amount of cytoplasm associated with the liver nucleus and its greater metabolic activity. Interaction with cytoplasmic metabolites might produce more destruction of free radicals, so that less damage is suffered by the nucleus; in addition, certain cytoplasmic functions of the liver might be less dependent on the nucleus and so able to continue apparently unperturbed.

SUMMARY

1. Uptake of ^{32}P by nucleic acids of spleen and thymus tissue *in vitro* has been demonstrated, and the pattern of this incorporation determined.

2. Uptake of ^{32}P by nucleic acids of lymph node cultures has been measured and has been found to be reduced by 600 r. X-radiation.

3. X-radiation *in vivo* affects incorporation of ^{32}P into DNA in the tissues examined; RNA susceptibility varies in different tissues.

4. The difference in sensitivity of animal tissues to X-radiation is discussed.

We would like to thank Dr J. F. Loutit, of the M.R.C. Radiobiological Research Unit, Harwell, for allowing us many facilities in his Department, and for arranging the supply of ^{192}Ir sources. We valued greatly the collaboration of Dr Trowell and his colleagues Mr W. R. Lush, Miss P. E. Law and Miss E. Peakman in the lymph node experiments. We are grateful to Mr M. J. Corp for irradiating the animals, Mr P. L. Strong for several adrenalectomies, and to Mrs J. Kinglerlee for her conscientious assistance. We also wish to express our thanks to Dr A. C. White, of The Wellcome Research Laboratories, for a gift of Stipven.

REFERENCES

Beaven, G. H., Holiday, E. R. & Johnson, E. A. (1954). *Nucleic Acids*, vol. 1, p. 493. New York: Academic Press.

Berenblum, L. & Chain, E. (1938). *Biochem. J.* **32**, 295.
 Bergonie, J. & Tribondeau, L. (1906). *C.R. Acad. Sci., Paris*, **143**, 983.
 Chargaff, E. (1954). *Nucleic Acids*, vol. 1, p. 307. New York: Academic Press.
 Chargaff, E. & Zamenhof, S. (1948). *J. biol. Chem.* **173**, 327.
 Deluca, H. A., Rossiter, R. J. & Strickland, K. P. (1953). *Biochem. J.* **55**, 193.
 Euler, H. von & Hevesy, G. (1942). *Biol. Medd., Kbh.*, **17**, 8.
 Forssberg, A. & Klein, G. (1954). *Exp. Cell Res.* **7**, 480.
 Hammarsten, E. (1947). *Acta med. scand.* **128** (Suppl. 196), 634.
 Hammarsten, E. & Hevesy, G. (1946). *Acta physiol. scand.* **11**, 335.
 Harrington, H. & Lavik, P. S. (1955). *Arch. Biochem. Biophys.* **54**, 6.
 Hevesy, G. (1949). *Nature, Lond.*, **163**, 869.
 Hevesy, G. (1955). *3rd Int. Congr. Biochem., Brussels* (in the Press).
 Higgins, G. M. & Anderson, R. M. (1931). *Arch. Path. (Lab. Med.)*, **12**, 186.
 Holmes, B. E. (1947). *Brit. J. Radiol., N.S.*, **20**, 450.
 Holmes, B. E. & Mee, L. K. (1954). *Radiobiology Symposium*, p. 220. London: Butterworth.
 Howard, A. & Pelc, S. R. (1951). *Exp. Cell Res.* **2**, 178.
 Hurst, R. O. & Butler, G. C. (1951). *J. biol. Chem.* **193**, 91.
 Hurst, R. O., Marko, A. M. & Butler, G. C. (1953). *J. biol. Chem.* **204**, 847.
 Kelly, L. S. & Jones, H. B. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 493.
 Kunitz, M. (1949). *J. gen. Physiol.* **33**, 349.
 Lajtha, L. G., Oliver, R. & Ellis, F. (1954). *Radiobiology Symposium*, p. 216. London: Butterworth.
 Limperos, G. & Mosher, W. A. (1950). *Amer. J. Roentgenol.* **63**, 691.
 McIlwain, H. & Buddle, H. L. (1953). *Biochem. J.* **53**, 412.
 Markham, R. (1942). *Biochem. J.* **46**, 790.
 Miller, N. (1950). *J. chem. Phys.* **18**, 79.
 Mirsky, A. E. & Pollister, A. W. (1946-47). *J. gen. Physiol.* **30**, 117.
 Ord, M. G. & Stocken, L. A. (1955). *Brit. J. Radiol., N.S.*, **28**, 279.
 Passaneau, J. V. & Totter, J. R. (1954). *Radiation Res.* **1**, 557.
 Patt, H. M. (1955). *Ann. N.Y. Acad. Sci.* **59**, 467.
 Payne, A. H., Kelly, L. S. & Jones, H. B. (1952). *Cancer Res.* **12**, 666.
 Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
 Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.
 Sherman, F. G. & Forssberg, A. (1954). *Arch. Biochem. Biophys.* **48**, 293.
 Smellie, R. M. S., Humphrey, G. F., Kay, E. R. M. & Davidson, J. N. (1955). *Biochem. J.* **60**, 177.
 Stocken, L. A. & Ord, M. G. (1955). *3rd Int. Congr. Biochem., Brussels* (in the Press).
 Totter, J. R. (1954). *Radiation Res.* **1**, 232.
 Trowell, O. A. (1952a). *J. Path. Bact.* **64**, 687.
 Trowell, O. A. (1952b). *Exp. Cell Res.* **3**, 79.
 Trowell, O. A. (1953). *Brit. J. Radiol.* **26**, 302.
 Veall, N. (1948). *Brit. J. Radiol.* **21**, 347.
 Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.
 Wyatt, G. R. (1954). *Nucleic Acids*, vol. 1, p. 243. New York: Academic Press.