702380

AMB

# FACTORS IN THE RADIOSENSITIVITY OF MAMMALIAN CELLS

*By*
Harvey M. Patt

Reprinted from
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 59, Article 4, Pages 649-664
February 3, 1955

REPOSITORY Argonne - CHR
COLLECTION A. Brues
BOX No. 15
FOLDER 1

0018317

PARTIAL DOCUMENT

# FACTORS IN THE RADIOSENSITIVITY OF MAMMALIAN CELLS

*By* Harvey M. Patt
*Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.*

The selective action of ionizing radiations was recognized very early in the history of their application to biological investigation. It was learned that radiosensitivity is frequently associated with proliferative activity and that the most dramatic effects of the radiations are related to their influence on growing or developing systems. Since the initial energy transfer is chemically nonspecific, sensitivity must obviously depend upon differences in the subsequent mechanisms of energy dissipation, or on the constitutional and physiological requirements of the responding cell or tissue. It is the purpose of this paper to discuss the influence of environmental factors on the response of mammalian cells to ionizing radiations. Some attention will also be given to the general problem of radiosensitivity by way of introduction. This area has been reviewed recently.[1-3] Although the expression radiosensitivity has attained wide usage, its meaning is ambiguous unless it is defined in terms of a standard dose response for a specific biological effect. Yet, even with this qualification, it is often a matter of conjecture as to whether sensitivity implies differences in the extent of the initial injury or in the capacity for recovery. In general, radiosensitivity, as discussed here, will refer to the destruction or degeneration of cells as living entities.

It is generally agreed that the proliferative capacity of a tissue is an important factor in its radiosensitivity. A relationship of this sort is understandable, since the cell in mitosis is usually more susceptible to injury than the cell at rest, and degenerative changes are prone to occur at the time of cell division regardless of the period of irradiation. Tissue sensitivity to X rays may be expressed as an inverse function of the intermitotic time in certain instances.[4] It may be recalled that the duration of mitotic inhibition in various tissues bears a direct relationship to the duration of interphase.[5, 6] Indeed, this relationship may give rise to the apparent paradox that tissues believed to be radiosensitive by histologic criteria may show less interference of mitotic activity than somewhat more resistant tissues.[6] The nature of the relationship between sensitivity and cell division is obscure. Peaks of respiratory and synthetic activity occur during the mitotic cycle, and one may inquire to what extent these and other changes, for example in chromosomal surface and cross section and in local oxygen tension, contribute to the sensitivity of premitotic and mitotic cells.[1]

It must be emphasized that there are many departures from the simple condition relating radiosensitivity to growth and differentiation. Perhaps the most glaring example, about which more will be said later, is the fact that small lymphocytes are quite sensitive and yet largely nonmitotic. We may note also that rapidly growing anaplastic tumors may be either more or less resistant than slowly growing neoplasms. There are other impressive exceptions to the general thesis that proliferation and primitiveness are common denominators



0018318

for differences in radiosensitivity. These exceptions have been pointed out by Bloom,[7] Mole,[8] and others. It is sufficient to note for our purpose that mammalian cells may be injured and killed by moderate gamma or X irradiation through mechanisms apparently unrelated to an aberration in cell division or in growth generally. It may also be stated that sensitivity dependencies of this sort are, in general, less obvious with the high ion density radiations.

Brues[9] has suggested that radiosensitivity may be related to the ratio of the nuclear and cytoplasmic nucleic acids, since the highest ratio is found in lymphoid tissue and the lowest in resistant cells. Proliferating tissues probably show, in general, an increase in DNA per cell.[10] It is well known, however, that PNA also tends to be increased in proliferating cells. There is no obvious relationship between susceptibility of different tissues and their metabolic rate, nor is there any reason to believe that differences in oxygen tension of the various tissues are of a sufficient magnitude to influence sensitivity to X rays appreciably, although the liver may be a possible exception. On the other hand, tissue oxygen concentration is probably an important consideration in the radiosensitivity of poorly vascularized tumors, as may be inferred from the work of Gray[11] and Hall.[12] Although polyploidy may protect simple organisms against radiation damage,[13, 14] this characteristic would not seem to be an important factor in radiosensitivity of the several animal tissues. It may be applicable, however, to certain instances of tumor sensitivity.

There is growing awareness that radiosensitivity can be influenced, to a considerable extent, by the physiological interplay in the system as a whole, whether this represents a cell, tissue, or animal. It may be noted, for example, that X irradiation of isolated nuclei results in negligible change in nuclear structure[15] and in the properties of nucleoproteins.[16] There accordingly appears to be a metabolic requirement even for certain primarily local radiation effects.

### *Radiosensitivity of Lymphoid Tissues* in Vivo *and* in Vitro

We may turn now to a more detailed consideration of the role of environmental factors in the radiosensitivity of mammalian cells. For this purpose, we shall draw mainly on experiences with lymphoid tissues. Although the lethal dose of X radiation varies among the different animal species, it is of interest that lymphopenia and lymphoid involution are more nearly independent of species, and that they reflect the amount of radiation instead of the lethal effect.[7, 17, 18] The great sensitivity of the lymph-node lymphocyte and of the thymus lymphocyte may be recalled. The fact that these cells rarely divide, and yet are as sensitive as mitotic cells has prompted Schrek and Ott[19] to suggest that a common physiological process may be affected in the nucleus of the lymphocyte and in the mitotic nucleus in general. This process is considered by many investigators to involve an aberration in nucleoprotein metabolism. Klein and Forssberg[20] have shown recently that there is a decrease in DNA per cell with time during the mitotic block of irradiated ascites tumor cells. The average cell volume, PNA and nitrogen contents increase, however, in proportion to the estimated growth rate before irradiation. A decrease in

0018319



TABLE 1

COMPARATIVE EFFECTS OF X IRRADIATION ON LYMPHOID CELLS

| Cell or tissue | Effect* | Time after X irradiation | $ED_{50}$† roentgens | Regression (log arc sine) | Reference |
|---|---|---|---|---|---|
| *In vitro* | | | | | |
| Lymph-node lymphocytes | Pyknosis | 5 hours | 305 ± 6 | 0.99 ± 0.02 | 21 |
| Blood lymphocytes | Pyknosis | 5 hours | 2218 ± 780 | 0.59 ± 0.07 | 21 |
| Thymus lymphocytes | Eosin stainability | 6 hours | 518 ± 10 | 0.94 ± 0.08 | 27 |
| Thymus lymphocytes | Eosin stainability | 22 hours | 114 ± 13 | 1.56 ± 0.12 | 24 |
| *In vivo* | | | | | |
| Lymph-node lymphocytes | Pyknosis | 5 hours | 160 ± 4 | 1.67 ± 0.05 | 21 |
| Duodenal lymphocytes | Pyknosis | 5 hours | 2805 ± 499 | 1.02 ± 0.09 | 21 |
| Blood lymphocytes | Lymphopenia | 3 days | 137 ± 8 | 1.84 ± 0.12 | 26 |
| Thymus | Involution | 3 days | 180 ± 16 | 1.49 ± 0.19 | 26 |
| Thymus | Involution | 5 days | 194 ± 6 | 1.34 ± 0.04 | 25 |
| Spleen | Involution | 3 days | 270 ± 19 | 1.14 ± 0.09 | 26 |
| Spleen | Involution | 5 days | 242 ± 15 | 1.05 ± 0.07 | 25 |

* Expressed as percentage of nonirradiated.
† Dose required for 50% effect.

the DNA/PNA ratio per cell has also been observed in the X-irradiated thymus.[20A]

Trowell[21] has contributed a significant paper on the radiosensitivity of lymphocytes in which he emphasizes the nonspecific nature of the cytological damage produced by X rays. The dose required to produce nuclear pyknosis in 50 per cent of rat-lymph-node lymphocytes five hours after irradiation ($ED_{50}$) is about 300 r. in tissue culture and 160 r. *in vivo*.* This difference may be obscured somewhat by differences in oxygen concentration and in metabolism in the two situations. The results, however, compare favorably with the observations by Schrek[22, 23] and Patt *et al.*[24] on the *in vitro* sensitivity of rabbit thymus lymphocytes as estimated by eosin stainability and with the 50 per cent dose for lymphopenia and thymus and spleen involution, after whole body X irradiation of the mouse, as shown by Carter[25] and Patt.[26, 27] The data are summarized in TABLE 1.

These effects are reasonably linear with the log of the dose as indicated in FIGURES 1 and 2.† The linearity of these relationships is suggestive of a symmetrical distribution of sensitivities. Variations in slope reflect differences in the range of sensitivities. It may be noted, in particular, that the slope for the lymph node lymphocyte response is steeper *in vivo* than *in vitro*. This effect is probably not a result of differences in the mean oxygen concentration, since the slopes do not vary with oxygen tension.[28] There is no difference,

* The data reported by Trowell[21] were corrected for pyknosis in the nonirradiated samples.
† An arc-sine transformation was used; essentially similar relationships appear with a probit transformation. The regression coefficients were kindly determined by Sylvanus Tyler.

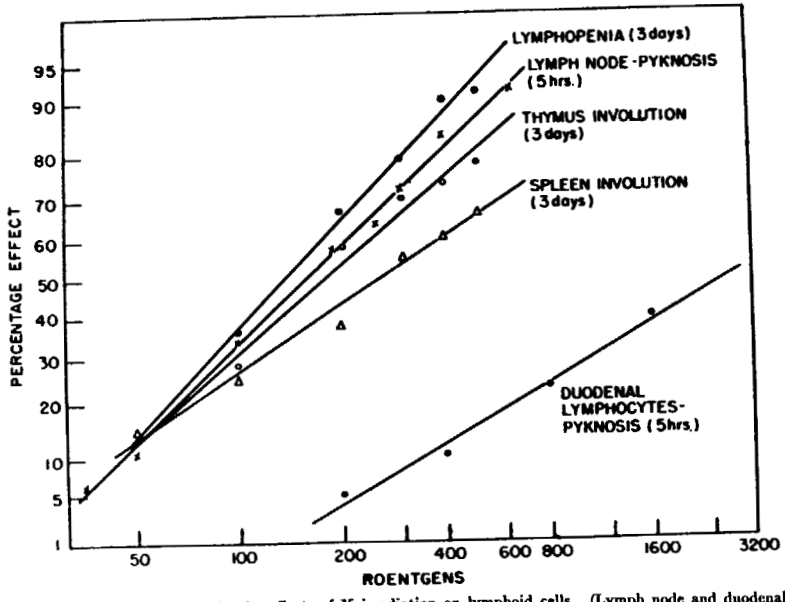


FIGURE 1. Comparative *in vivo* effects of X irradiation on lymphoid cells. (Lymph node and duodenal lymphocyte data from Trowell[21]; blood lymphocyte and thymus and spleen data from Patt *et al.*[26, 27])

moreover, between the slopes for thymus lymphocyte suspensions, in which the oxygen gradient is comparatively small, and for lymph node lymphocyte cultures at comparable times (FIGURE 2). The apparent difference in range of sensitivities for the lymph node may reflect differences in the reaction time *in vivo* and *in vitro*. It is noteworthy that the slope for thymocytes is 0.94 at six hours after X irradiation and 1.57 at 22 hours. The latter may be compared with a slope of 1.67 for lymph node pyknosis at five hours *in vivo*.

The wider range of sensitivity for blood lymphocytes than for lymph node or thymus lymphocytes at comparable times *in vitro* may be attributed to the fact that these cells represent a more heterogeneous population in terms of maturity and differentiation. Trowell[21] has suggested that this may also account for their lower average sensitivity. Lymphocytes of the duodenal villi appear to be much more resistant to X rays than lymph node lymphocytes *in vivo*. The relative resistance of scattered intestinal lymphocytes may be related perhaps to their age or to environmental factors, *e.g.*, oxygen. It is possible also that pyknotic lymphocytes are removed more rapidly from the intestinal mucosa than from a lymph node, and this difference may account, in part, for the apparent resistance. A situation of this sort has been described for pyknotic blood lymphocytes *in vivo*.[21]

It has been estimated that lymph node lymphocytes are about 11 times more sensitive *in vivo* than *in vitro* from the point of view of equivalent oxygen concentration.[28] The validity of such comparison is questionable, however,

0018320



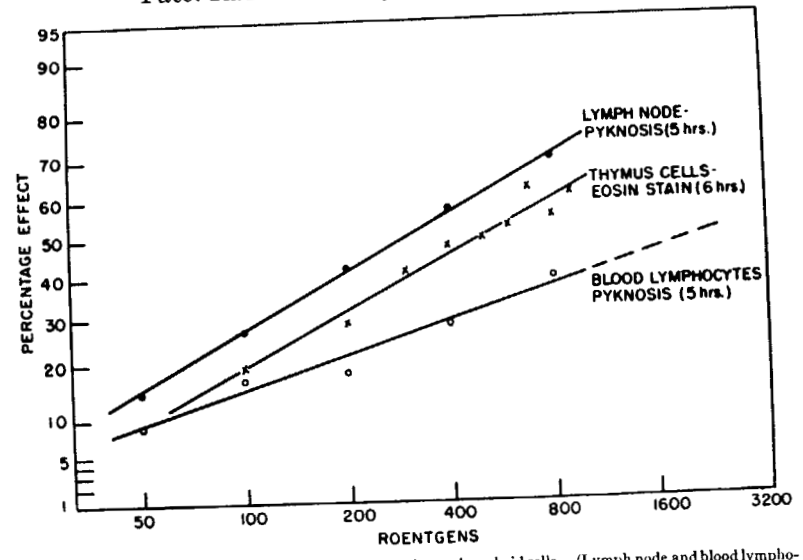


FIGURE 2. Comparative *in vitro* effects of X irradiation on lymphoid cells. (Lymph node and blood lymphocyte data from Trowell[21]; thymocyte data from Patt *et al.*[34])

when we recall that successful culturing of lymph nodes requires pure oxygen.[28, 29] Under these conditions, the average oxygen concentration exceeds that of nodes *in vivo* by a factor of 10. The greater oxygen requirement for lymphocyte survival *in vitro* points to differences in metabolism and in the accumulation of metabolic products. The comparatively small difference in sensitivity *in vivo* and *in vitro* when optimal culturing conditions are employed may be attributed, in part, to lactate accumulation in the cultures and, more generally, to differences in reaction time. The 22 hour $ED_{50}$ for thymocytes *in vitro* is 114 r. which is consistent with the dosages required *in vivo*.

While the killing of lymphocytes appears to be mainly a direct consequence of their radiosensitivity, remote effects on lymphoid tissue have been observed from time to time. Price[30] has shown that a distant effect can be demonstrated, particularly when the volume of irradiated tissue is small. The relative magnitude of direct and remote effects on lymphoid tissue depends, for the most part, on the integral dose.

### *Temperature Effects*

The effect of temperature on radiosensitivity has received considerable attention. There is no reason to believe, from a physical point of view, that the primary energy transfer resulting in ionization and excitation is temperature dependent. Extreme temperature changes may influence the response in certain instances, however, perhaps because of altered formation and diffusion of free radicals or of some alteration in configuration of the various biological

targets. Other temperature dependencies during irradiation can generally be attributed to concomitant changes in cell division, in oxygen tension, or in blood flow, or to effects on recovery processes. When these considerations are not pertinent, it is generally observed that the immediate reaction of cells to ionizing radiation has a low temperature coefficient. This is the case with thymus lymphocytes, as shown by Schrek.[23]

The biological expression of the energy transfer, on the other hand, appears to be rate sensitive, the ultimate outcome depending on the respective temperature coefficients of the ensuing reactions of injury and of recovery for a given effect. This is illustrated in FIGURE 3, where it will be noted that the 50 per cent survival time of X-irradiated thymic cells is increased when the incubation temperature is decreased below 37° C.[23] The temperature coefficient for survival of nonirradiated cells is about three over the range of 17 to 42° C. Incubation temperatures in excess of 42° C. kill thymocytes before X-ray lethality can be manifest. Maximal protective effects appear when the

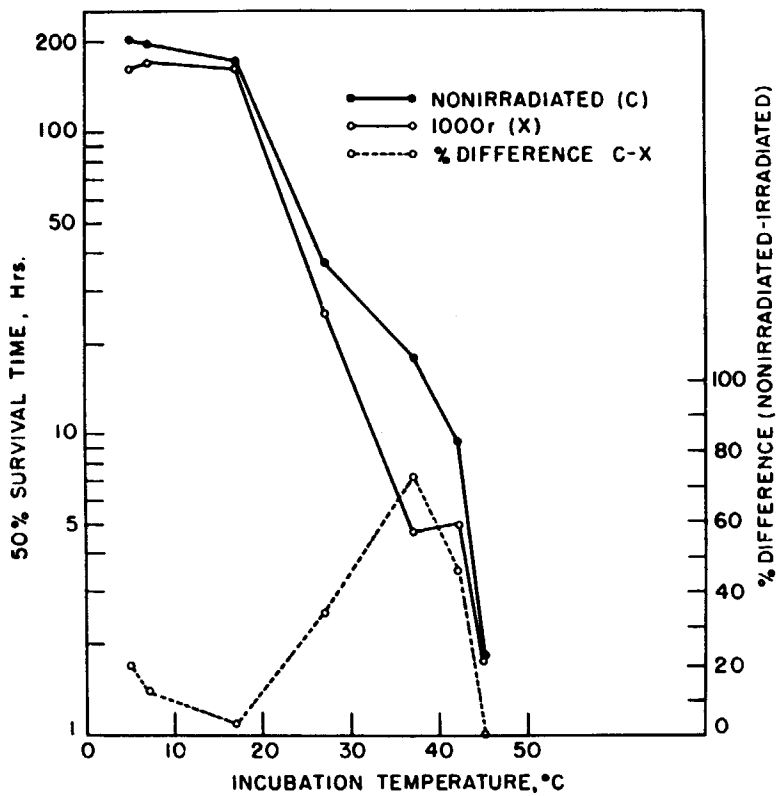


FIGURE 3. Survival time of thymus lymphocytes as a function of incubation temperature.[23]

0018321



temperature is about 17° C., and it may be recalled that an optimum of 18° C. has been observed by Stapleton *et al.*[31] for *Escherichia coli* in the presence of certain media. This similarity may be fortuitous, but it indicates, at any rate, that recovery can be influenced preferentially by appropriate temperature changes.

These results may be compared with our experiences with Amphibia, which showed that survival was greatly enhanced when frogs were kept at 6° C. continuously after X irradiation.[32] Survival was not influenced by altering the body temperature during irradiation. The protective effect of postirradiation cold was due apparently to a decrease in the rate of development of radiation damage rather than to any appreciable recovery, since removal of the animals from the cold after periods of 60 to 130 days resulted in a typical radiation picture. One wonders whether certain types of recovery, even in the frog, might not be influenced preferentially at intermediate temperatures. This question we are presently investigating.

### *Effects of pH*

Changes in hydrogen ion concentration probably occur in the vicinity of an ionization track and may contribute to the development of radiation injury. The pH may also play a role in radiosensitivity by modifying the physicochemical characteristics of the various biological components or the rates of reaction subsequent to irradiation.

Marshak[33] has observed that the frequency of chromosome aberrations in plant cells is reduced when ammonium hydroxide is applied prior to X-ray exposure. The sensitivity of fern spores, *Drosophila* eggs, and *Paramecia* has also been shown by Zirkle[34-36] to depend on the pH during irradiation. The relationships are complex, with distinct sensitivity maxima and minima as the hydrogen ion concentration is increased or decreased. The effects do not correspond particularly to changes in the yield of radiochemical products with pH.[37] They may perhaps be related more to alterations in the behavior of the various protoplasmic colloids.

Significant pH effects have also been described by Schrek[23] for thymocytes, and by Trowell[28] for lymphocytes. These effects differ, however, from the effects described above since the changes are attributable to the pH after exposure to X rays. The pH dependency is remarkably similar in the case of thymus and lymph-node cells, even though the hydrogen ion concentration was changed after irradiation of the former and before irradiation of the latter. The results presented in FIGURE 4 reveal that sensitivity decreases sharply as the pH is diminished from 7.0 to 6.0. There is little change in sensitivity from pH 7.0 to about 8.5. Alteration in the pH of thymic cell suspensions from 6.6 to 7.8 during irradiation does not modify their survival. Nonirradiated cells will not survive incubation below pH 6.0. Survival is not altered by pH's of 6.0 to 8.5. The postirradiation effects of pH are apparently unrelated to autolytic phenomena, although other interpretations are not available. It may be remarked that the pH and temperature effects for thymus lymphocytes appear to differ with nitrogen mustard and X rays.[38] It is well known that there are many similarities in the action of these toxic agents.

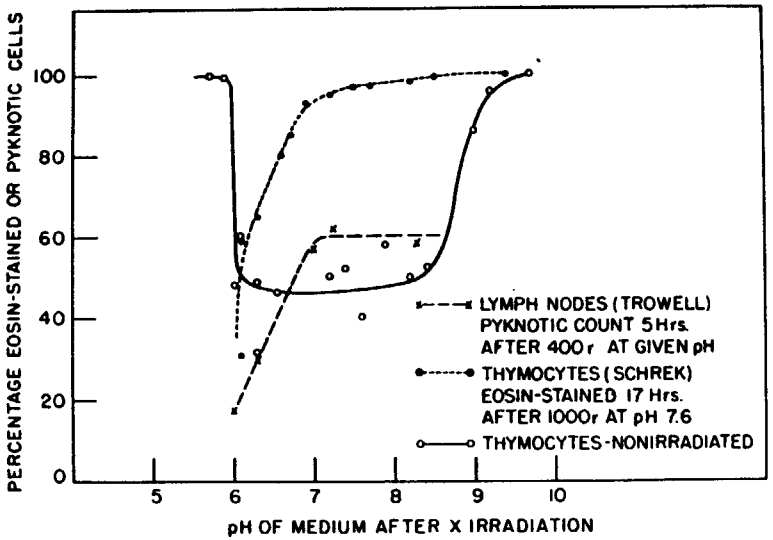


FIGURE 4. Effect of hydrogen ion concentration on radiosensitivity.[23, 28]

### *Effect of Oxygen*

Certain forms of radiation sensitivity are known to be intimately related to oxygen tension, and it is appropriate to examine this relationship as it pertains to mammalian cells. Hypoxia during irradiation has been shown to reduce many of the effects of whole body or local exposure to gamma and X rays.[3] The pattern of the X-ray-oxygen dependence for rabbit thymus and rat-lymph-node lymphocytes and for mouse ascites tumor cells is presented in FIGURE 5. The effects observed with thymus cell and ascites tumor cell suspensions resemble closely those previously described for microorganisms, insects, and plants,[11] the sensitivity in air being about 2.5 to 3 times that in nitrogen. The oxygen effect, in general, increases sharply from 0 to 10 per cent oxygen and then plateaus at levels approaching the physiological or normal ambient tension. While this general picture also obtains for cultures of lymph-node lymphocytes,[28] the differences in sensitivity are considerably greater, and the range of the effect with oxygen tension is more extensive. Trowell[28] has suggested that the greater range may be an artifact. Since a rather steep oxygen gradient exists from the surface to the center of the lymph-node culture, the average oxygen tension in a system of this sort is considerably lower than the ambient tension.

These considerations are germane to many of the peculiarities in radiosensitivity of tumors and tumor fragments. Hall[12] has observed, for example, that the X-ray sensitivity of tumor fragments in air is an inverse function of their size, the implication being that the more centrally located cells are relatively anoxic, and that the anoxic area varies directly with the size of the



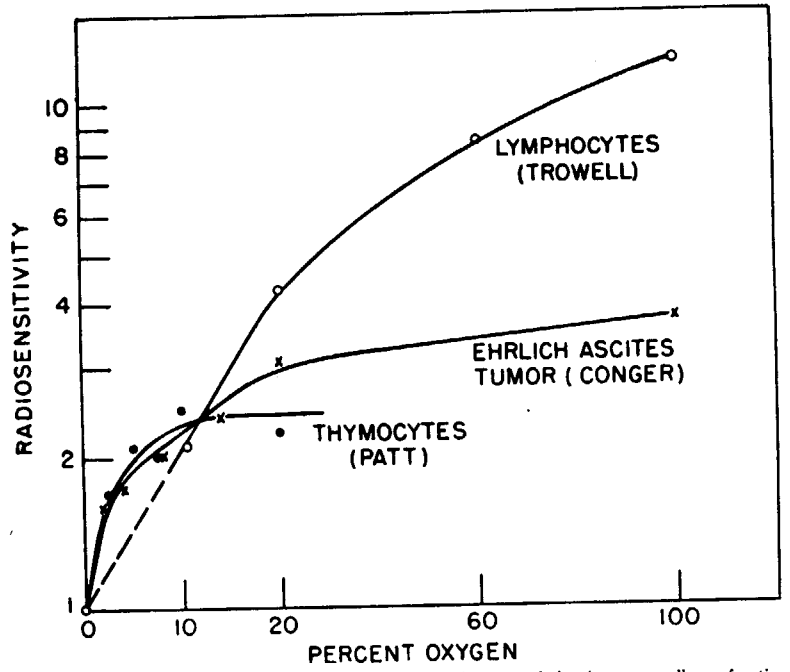


FIGURE 5. Radiosensitivity of lymph node and thymus lymphocytes and of ascites tumor cells as a function of oxygen tension.[11, 27, 28]

fragments. In further support of this observation, it was noted that the *in vivo* sensitivity of well-vascularized, rapidly growing tumors to local X irradiation is comparable to the *in vitro* sensitivity of tumor fragments irradiated in air when their oxygen consumption is depressed by cold or cyanide. The importance of oxygen tension in the response of certain solid tumors *in vivo* is obvious from the recent experiments by Gray *et al.*[11] Since it may be assumed that only a small oxygen gradient exists from the medium to individual cells in suspension, factors of oxygen consumption and diffusion would be of little consequence in cell suspensions. The fact that cyanide can potentiate X-ray sensitivity of various tissues, but not of cells in suspension, may be interpreted on this basis.[3]

The magnitude of the oxygen effect in rat lymphocytes, greater than in the other systems thus far investigated, is not readily explained, particularly in terms of the usual interpretation of effects of this sort.[3] Trowell[28] has speculated that chemical changes incidental to brief anoxia may play a greater role in the sensitivity of mammalian tissues than of plant or insect tissues, owing perhaps to the faster rate of metabolism of the former. Yet we may recall that suspensions of mouse ascites tumor cells exhibit a more or less typical oxygen-X ray sensitivity relationship.[11] For what it is worth, the sensitivity

0018322

of rat-lymphocyte cultures is decreased somewhat by the addition of sodium lactate but not of glucose prior to X irradiation.[28] Intracellular accumulation of lactate in the lymph-node culture may perhaps complement other effects of anoxia, *e.g.*, on the yield of toxic breakdown products from cellular water. This would not appear to be sufficient, however, to account for the increased protective effect. It is of interest that sensitivity is not influenced appreciably when the cultures are irradiated in Tyrodes solution or in 0.65 per cent serum. Similar findings have been obtained by us with thymus lymphocyte suspensions, which suggests that the medium makes a negligible contribution to the toxic effect of X rays in these situations.

That the oxygen effect can not always be interpreted entirely in terms of immediate oxidative reactions in irradiated water is apparent also from other considerations. The data in FIGURE 6 reveal that protection does not occur if thymic-cell suspensions are chilled to 2° C. during equilibration with commercial nitrogen (*ca.* 0.1 per cent $O_2$) over a 15- or 30-minute period immediately prior to X irradiation. While this effect may perhaps represent an artifact, due to the difficulty in removing intracellular oxygen under these conditions, we have found that chilling, even for a brief period after irradiation, decreases significantly ($p < .01$) the protective effect of hypoxia. Other data emphasize

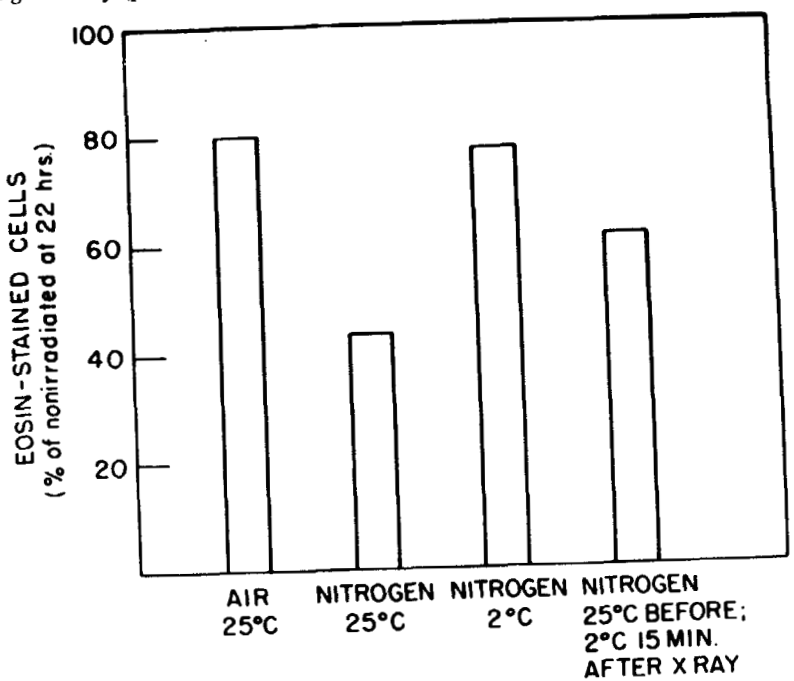


FIGURE 6. Effect of temperature on protection afforded X irradiated thymus lymphocytes by hypoxia.[46] (Nitrogen equilibration for 15 to 30 minutes at 2° or 25° C. before 200 r.)

0018323



the difficulties of interpretation. It has been observed, for example, that anoxic conditions after exposure to X rays exert almost as great a protective effect on the hematopoietic cells of tadpoles as similar treatment before and during the exposure.[39] Certain protective effects of prolonged anaerobiosis on suspensions of leukemic cells appear to be due mainly to the situation after irradiation.[22] There is the suggestion also that oxygen presence during X irradiation may affect the recovery of mitosis in grasshopper neuroblasts rather than its immediate depression.[22A] There is some uncertainty, however, concerning the dose-response relationships for mitotic block in this instance.

It is significant that oxygen plays a comparatively small part during irradiation of Ehrlich ascites tumor cells with fast neutrons in contrast to the marked effect with X rays.[11] An inverse relationship between the oxygen effect and ionization density has also been observed with *Vicia faba* roots[40] and *Tradescantia* microspores.[41] These facts parallel the greater oxygen dependence of radiochemical reactions resulting from the less densely ionizing radiations and provide a powerful argument that chemical intermediates derived from water, play a substantial role in the induction of radiation injuries. While we tacitly assume that oxygen modifies the nature of the chemical intermediates that are formed during exposure to gamma or X rays, alternative and complementary actions of oxygen must be involved in certain instances.

### *Protective Effect of Cysteine*

There are numerous examples of chemical protection, and one is inclined generally to interpret them in radiochemical rather than in biological terms.[3] This interpretation implies, as in the case of anoxia, that the protective phenomena are due mainly to a decrease in the effectiveness of the radiation rather than to a basic change in the sensitivity of the biological system. We shall be concerned here with the effects of cysteine which, in certain respects at least, may be thought of as a characteristic example of protection by hydrogen donors.

The sensitivity of thymus lymphocytes is decreased by a factor of 2 to 3 when cysteine is added to the suspension prior to X irradiation (FIGURE 7). Similar protective effects occur with ascites tumor cells and with the whole animal. Protection of thymocytes depends upon cysteine concentration and time of administration but, significantly, is not a simple function of the sulfhydryl level during exposure.[24] Cysteine addition 15 to 30 minutes before irradiation is optimum, which suggests that some interaction is necessary. Unlike the picture with bacteria and animals, there is a definite, although lesser, protective effect when cysteine is added to the thymocyte suspension immediately after irradiation. This effect can be accounted for only to a slight extent by the persistence of toxic substances in the medium. The apparent discrepancy in the time course of protection of the various systems may be related perhaps to differences in the kinetics of reactions with cysteine and in the time constants for development of irreversible injury. It may be recalled that a postirradiation effect of cysteine has also been described for onion epidermis cells.[42]

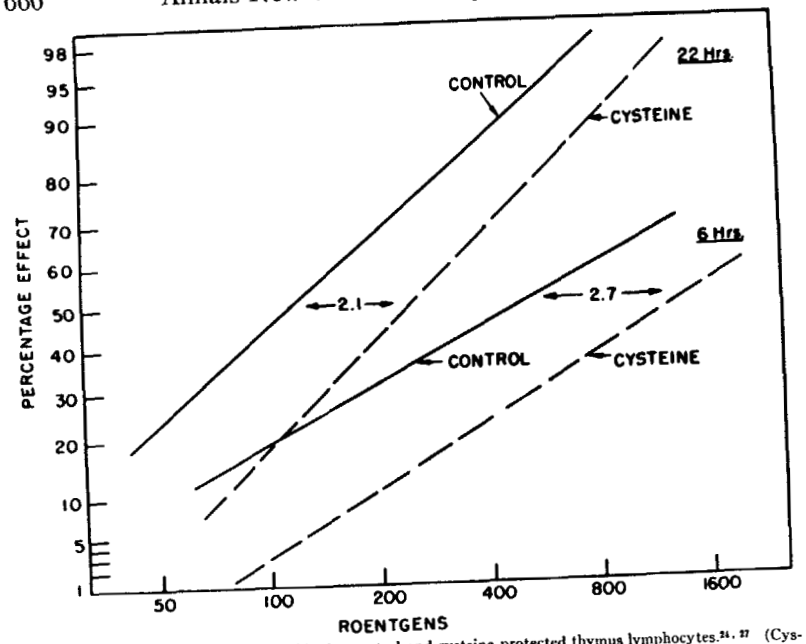


FIGURE 7. Dose-response relationship for control and cysteine protected thymus lymphocytes.[24, 27] (Cysteine, final concentration 0.02 M, added 15 minutes before X irradiation.)

In contrast to the finding that —SH compounds give some protection to bacteria in oxygen-free phosphate buffer,[43] it may be noted that cysteine does not protect thymic cells in the absence of oxygen. The resistance of packed thymocytes and the failure of cysteine to protect them may be attributed to their hypoxic state. Cells equilibrated with oxygen before packing by centrifugation appear to be as sensitive as cells in suspension, and they are readily protected by cysteine.[24] This is interpreted as evidence that cysteine acts at the level of the cell. A cellular site of action is implicated also from studies with tumor fragments.[44] It may be noted that there is a rapid uptake of oxygen when cysteine is added to the thymic cell suspension. There is also an increase in lactic acid.[45] These results suggest perhaps that cysteine action is related to the availability of intracellular oxygen.

Mechanisms other than the immediate oxidative reactions induced by activated water must be involved, however. It has been observed[46] that protection of thymocytes in suspension is dependent upon temperature during the first 30 to 60 minutes after irradiation (FIGURE 8). A brief period of chilling at 2° C. immediately after X-ray exposure completely reverses the protection. Temperature dependence of cysteine action before irradiation has also been shown. The latter may be thought of in terms of a decreased rate of reaction with oxygen in the cold. An explanation of the postirradiation temperature dependence for cysteine protection of thymus lymphocytes is not readily

0018324



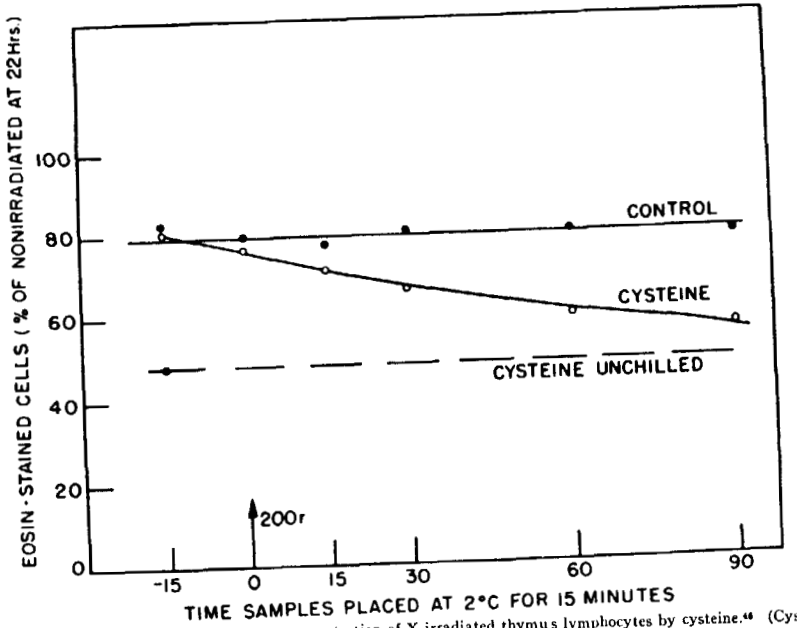


FIGURE 8. Effect of temperature on protection of X-irradiated thymus lymphocytes by cysteine.[46] (Cysteine, final concentration 0.02 M, added 15 minutes before X irradiation.)

apparent. It is clear, however, that we must inquire about the role of delayed and of chain reactions in the interpretation of these phenomena. The importance of considerations of this sort is indicated from recent work on the chemical aftereffects of ionizing radiations.[47] The pathways for reaction of organic radicals formed during irradiation may depend upon oxygen availability in addition to other factors. It is possible, for example, that decreased oxidation of cysteine at low temperature frees oxygen for reaction with persisting radicals to form organic peroxides. It is of interest that increased oxygen uptake was maintained for several hours with the cysteine concentration used, but that the critical postirradiation period for modification of the protection was about an hour. Cysteine protection is apparently unrelated to neutralization of hydrogen peroxide. In fact, cysteine potentiates or activates the effect of hydrogen peroxide on DNA solutions.[48] We may recall that a qualitatively similar, though lesser, temperature effect has been observed with hypoxia. Since the suspensions are not completely deoxygenated when commercial nitrogen is used, immediate postirradiation chilling may also increase intracellular oxygen above a critical level for reaction with chemical intermediates. The results with thymocytes may be contrasted with the chemical protective effects in bacteria.[49] Several modes of action apparently prevail in the protection of the various systems, owing perhaps to differences in the nature of the chemical intermediates and their reactions, the time required for develop-

TABLE 2

COMPARATIVE PROTECTION WITH CYSTEINE FOR SEVERAL EFFECTS ON MAMMALIAN SYSTEMS

| Object | Effect | Per cent dose reduction | Reference |
|---|---|---|---|
| Thymocytes* | Eosin stainability | 53 | 24 |
| Ascites tumor* | Growth† | 55 | 27 |
| Solid tumor* | Transplantability† | 18 | 44 |
| Solid tumor* | Growth delay† | 34 | 44 |
| Lymphocytes† | Lymphopenia | 31 | 26 |
| Granulocytes† | Granulocytopenia | 56 | 26 |
| Spleen† | Involution | 42 | 26 |
| Mouse | Lethality—30 day | 42 | 26 |

* *In vitro.*
† *In vivo.*

ment of irreversible injury and the contribution of the medium to the over-all effect.

In concluding, it is appropriate to refer to the pattern of cysteine protection for various effects in mammalian tissues. There is considerable evidence that the protection is a fairly general phenomenon, and that both destructive and regenerative processes can be modified.[3] This is apparent particularly when the effects can be quantitated in terms of radiation dose biological response parameters.[26] Uniformity of the protection is shown in TABLE 2. The comparatively low value obtained for transplantability of solid tumors may be related to the uncertainty concerning the dose-response curves. It is worth noting that *d* and *l* cysteine are equally effective in mice, but that all sulfhydryl substances are not protective to animals, perhaps because of differences in their biological fate. The action of cysteine and $\beta$ mercaptoethylamine appears to be similar.[50] The latter is more efficient but is also more toxic than the former. Maximally tolerated doses of each give equivalent protection against acute radiation lethality. Suboptimal amounts seem to be completely additive.

Of considerable interest is the fact that cysteine protection against the lethal effects of fast neutron irradiation in mice is about half of that found with gamma irradiation.[52] This finding parallels the oxygen effect in irradiated systems and is supported by other data (TABLE 3). It may be remarked that the biological additivity of two briefly spaced X-ray doses in mice is a direct function of the cysteine dose preceding each exposure.[26] A similar additivity has been observed for the oxygen effect on broad bean roots.[51]

Uniformity of protection by cysteine suggests that the decisive action occurs at an early stage in the chain of events but does not necessarily imply protection against all radiation changes since different primary mechanisms may be involved in their development. For the present, these effects may be interpreted best in terms of the physicochemical ramifications of energy transfer in irradiated water. It would appear that protective chemicals of this sort may alter the effective radical concentration (*e.g.*, of OH and $HO_2$) either directly or indirectly by relative depletion of oxygen. The mechanisms may be more complex in certain instances and involve also effects on chain reactions initiated

0018325



TABLE 3

DEPENDENCE OF PROTECTIVE EFFECT OF ANOXIA OR CYSTEINE ON RADIATION QUALITY

| Object | Radiation | | | Reference |
|---|---|---|---|---|
| | Alpha | Fast neutron | Gamma or X | |
| Vicia faba | + | | ++++ | 40 |
| Tradescantia | | ++ | ++++ | 41 |
| Allium* | + | | ++++ | 53 |
| Ehrlich ascites tumor | | + | ++++ | 11 |
| Mouse* | | ++ | ++++ | 52 |

* Refers to effect of cysteine.

by the primary interactions. The experimental findings, in general, are consistent with such assumptions, and there is little basis for postulating a more selective protection of one or another physiological mechanism.

## *Conclusions*

There is no obvious common denominator to account for differences in radiosensitivity of the various mammalian cells and tissues. Although these differences can be related in certain instances to mitosis and growth, there are a number of exceptions to this condition. The over-all relationships are complex and are influenced by the interplay in the system as a whole.

The killing of lymphocytes appears to be mainly a direct consequence of their radiosensitivity. Sensitivity *in vivo* and *in vitro* is remarkably similar when extenuating factors are considered. The influence of environmental factors on sensitivity of lymphoid and other tissues is discussed.

The idea that radiation effects may be mediated by chemical vectors seems firmly established. Many of the protective effects of hypoxia and substances such as cysteine can be interpreted in terms of chemical intermediates derived from water and their reactions. It is clear, however, that we can not confine our thinking in this area.

## *References*


1. SPARROW, A. H. 1951. Ann. N. Y. Acad. Sci. **51:** 1508–1540.
2. GRAY, L. H. 1951. Progress in Biophysics and Biophysical Chemistry. **2:** 240–305. J. A. V. Butler & J. T. Randall, Eds. Academic Press. New York, N. Y.
3. PATT, H. M. 1953. Physiol. Revs. **33:** 35–76.
4. KNOWLTON, N. P., JR. & W. R. WIDNER. 1950. Cancer Research. **10:** 59–63.
5. FRIEDENWALD, J. C. & S. SIGELMAN. 1953. Exptl. Cellular Research. **4:** 1–31.
6. KNOWLTON, N. P., JR. & L. H. HEMPELMANN. 1949. J. Cellular Comp. Physiol. **33:** 73–91.
7. BLOOM, W. 1947. Radiology. **49:** 344–348.
8. MOLE, R. H. 1953. Brit. J. Radiology. **26:** 234–241.
9. BRUES, A. M. & L. RIETZ. 1951. Ann. N. Y. Acad. Sci. **51:** 1497–1507.
10. PRICE, J. M. & A. K. LAIRD. 1950. Cancer Research. **10:** 650–658.
11. GRAY, L. H., A. D. CONGER, M. EBERT, S. HORNSEY & O. C. A. SCOTT. 1953. Brit. J. Radiology. **26:** 638–648.
12. HALL, B. V. 1952. Cancer Research. **12:** 268.
13. CLARK, A. M. & E. M. KELLY. 1950. Cancer Research. **10:** 348–352.
14. LATARJET, R. & B. EPHRUSSI. 1949. Compt. rend. **229:** 306–308.
15. DURYEE, W. R. 1949. J. Natl. Cancer Inst. **10:** 735–795.

16. VON EULER, H. & L. HAHN. 1946. Acta Radiol. **24:** 269–280.
17. DEBRUYN, P. P. H. 1948. Anat. Record. **101:** 373–405.
18. BRUES, A. M. & L. RIETZ. 1948. Argonne Natl. Lab. Report 4227. : 183–187.
19. SCHREK, R. & J. N. OTT, JR. 1952. Arch. Path. **53:** 363–378.
20. KLEIN, G. & A. FORSSBERG. 1954. Exptl. Cellular Research. **6:** 211–220.
20A. WEYMOUTH, P. P., R. J. DOELL, D. G. LETE & H. S. KAPLAN. 1953. Proc. Am. Assoc. Cancer Research. **1:** 59.
21. TROWELL, O. A. 1952. J. Path. Bact. **64:** 687–704.
22. SCHREK, R. 1946. Radiology. **46:** 395–410.
22A. GAULDEN, M. E., M. NIX & J. MOSHMAN. 1953. J. Cellular Comp. Physiol. **41:** 451–470.
23. SCHREK, R. 1946. J. Cellular Comp. Physiol. **28:** 277–304.
24. PATT, H. M., M. E. BLACKFORD & R. L. STRAUBE. 1952. Proc. Soc. Exptl. Biol. Med. **80:** 92–97.
25. CARTER, R. E., P. S. HARRIS & J. T. BRENNAN. 1950. Los Alamos Natl. Lab. Report 1075.
26. PATT, H. M., S. H. MAYER, R. L. STRAUBE & E. M. JACKSON. 1953. J. Cellular Comp. Physiol. **42:** 327–342.
27. PATT, H. M. & M. E. BLACKFORD. Unpublished observations.
28. TROWELL, O. A. 1953. Brit. J. Radiology. **26:** 302–309.
29. TROWELL, O. A. 1952. Exptl. Cellular Research. **3:** 79–107.
30. PRICE, C. H. G. 1951. Brit. J. Radiology. **24:** 556–567.
31. STAPLETON, G. E., D. BILLEN & A. HOLLAENDER. 1953. J. Cellular Comp. Physiol. **41:** 345–357.
32. PATT, H. M. & M. N. SWIFT. 1948. Am. J. Physiol. **155:** 388–393.
33. MARSHAK, A. 1938. Proc. Soc. Exptl. Biol. Med. **38:** 705–713.
34. ZIRKLE, R. E. 1936. Am. J. Roentgenol. **35:** 230–237.
35. ZIRKLE, R. E. 1940. J. Cellular Comp. Physiol. **16:** 301–311.
36. ZIRKLE, R. E. 1941. J. Cellular Comp. Physiol. **17:** 65–70.
37. BONET-MAURY, P. & M. LEFORT. 1950. Nature. **166:** 981–982.
38 SCHREK, R. 1948. Acta Unio Intern. contra Cancrum. **6:** 848–856.
39. ALLEN, B. M., O. A. SCHJEIDE & R. PICCIRILLO. 1951. Univ. California at Los Angeles Report 149.
40. THODAY, J. M. & J. READ. 1949. Nature. **163:** 133–134.
41. GILES, N. H., JR., A. V. BEATTY & H. P. RILEY. 1952. Genetics. **37:** 641–649.
42. BELLOCK, S. & A. T. KREBS. 1951. Army Medical Research Lab. Report 6-64-12-06-(43).
43. HOLLAENDER, A., W. K. BAKER & E. H. ANDERSON. 1951. Cold Spring Harbor Symposia Quant. Biol. **16:** 315–326.
44. HALL, B. V. 1952. Cancer Research. **12:** 787–792.
45. PATT, H. M. & T. N. TAHMISIAN. Unpublished observations.
46. BLACKFORD, M. E. & H. M. PATT. 1952. Argonne Natl. Lab. Report. 4840. : 47–49.
47. Selected papers: "The Chemistry of Biological After-Effects of Ultraviolet and Ionizing Radiations." 1954. Brit. J. Radiology. **27:** 36–80, 117–144.
48. CONWAY, B. E. & J. A. V. BUTLER. 1952. J. Chem. Soc. : 834–838.
49. STAPLETON, G. E., D. BILLEN & A. HOLLAENDER. 1952. J. Bact. **63:** 805–811.
50. STRAUBE, R. L. & H. M. PATT. 1953. Proc. Soc. Exptl. Biol. Med. **84:** 702–704.
51. READ, J. 1952. Brit. J. Radiology. **25:** 336–338.

0018326