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# The Scrapie Agent: Evidence Against its Dependence for Replication on Intrinsic Nucleic Acid

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SUMMARY

Exposure of the scrapie agent to u.v. light at various wavelengths has shown that light of 237 nm is 4 to 5 times as effective in inactivating it as 'germicidal' wavelengths (250 to 270 nm); whereas with systems that depend on RNA or DNA for function, inactivation is most effective by wavelengths in the germicidal range and there is a minimum of response in the wavelength region round 240 nm. The action spectrum for the scrapie agent is reminiscent of the absorption spectrum for purified bacterial endotoxin, identified as a lipopolysaccharide complex.

Dilute aqueous suspensions of scrapie agent were exposed to ionizing radiations in the presence or absence of oxygen. In dilute suspensions of test systems depending on the integrity of nucleic acid or protein, oxygen is almost invariably protective, but it was extremely sensitizing for inactivation of the scrapie agent, to an extent approached only in the case of membranous systems like lysosomes.

Results of these two methods argue against dependence of the scrapie agent on an intrinsic nucleic acid moiety for ability to replicate. They suggest that a lipid fraction is an important component and to that extent provide additional support for the 'membrane hypothesis'.

## INTRODUCTION

Scrapie is one of a group of four transmissible subacute spongiform encephalopathies (two of them afflicting humans). Because of their transmissibility, and proliferation in the animal host, they have been classified as slow virus diseases. However, the agents of all four diseases respond to numerous physical and chemical agents in ways that distinguish them sharply from viruses which, by current definition, depend for replication on the integrity of a nucleic acid moiety (see Hunter, 1974, for review).

A common characteristic of the group is the very long incubation period. Quantitative work on the scrapie agent became possible only after the disease had been successfully transmitted to mice (Chandler, 1961). The mouse-adapted strain of scrapie with which our experiments were done causes clinical signs in mice about 14 to 30 weeks after infection, depending on the titre of the inoculum. This makes progress possible, but it cannot be fast, since the only test of effect of any treatment is loss in infective titre. The agent has not been identified by electron microscopy, nor purified. The source of infective material is crude

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preparations of tissue (usually brains) from affected animals. Some 'purification' can be achieved, i.e. debris removed, but always at the sacrifice of activity (Haig *et al.* 1977).

We hoped to gain insight into the nature of the scrapie agent by drawing inferences from its response to ionizing and u.v. irradiations. The 'target molecular weight', estimated from the dose for inactivation by ionizing radiation, was $10^5$ to $1{\cdot}5 \times 10^5$ (Alper *et al.* 1966). This seemed too small for a nucleic acid virus core carrying enough information to code for its own replication. In experiments carried out concurrently, we found that the agent was almost unaffected by large doses of u.v. light at 254 nm. For those reasons we tentatively inferred that replication of the agent did not depend on an intrinsic nucleic acid moiety (Alper *et al.* 1967) and two models were subsequently proposed for modes of replication of an infective particle of this exceptional nature (Gibbons & Hunter, 1967; Griffith, 1967).

Nucleic acid was at that time so firmly established as the only biological compound with 'self-replicating' properties that it was clearly desirable to find some means of testing our inference. The opportunity to construct an outline action spectrum was afforded by the development, at the Institut du Radium in Paris, of a system for filtering the light from a high pressure mercury vapour lamp through a system of liquid filters which passed only a narrow band of wavelengths (Muel & Malpiéce, 1969). Results of a first set of experiments were reported (Latarjet *et al.* 1970) and we now summarize the results from four separate sets of experiments.

The methods used in radiation chemistry provide another possible way of drawing inferences about the nature of an agent of unknown composition. When macromolecules or viruses are irradiated in dilute aqueous solution or suspension, loss of biological function is attributable mainly to 'indirect' action, i.e. to interaction of the target molecules with the radiolysis products of water, so-called 'active radicals.' Both oxidizing and reducing species are engendered. If dissolved oxygen is present it reacts with the reducing radicals at a high rate, so that the target molecules are protected against reductive reactions; and the product of the reaction [radical + $O_2$] may itself increase the overall oxidizing effect of the irradiation. Thus the presence of oxygen in an irradiated aqueous solution or suspension will, in general, enhance such damage to target molecules as is caused by oxidation; whereas if they are more readily inactivated by a reducing process, the presence of oxygen during irradiation will be protective.

When nucleic acid preparations, or enzymes, have been tested for biological or biochemical activity after irradiation in dilute suspension or solution, oxygen has almost always been protective. Therefore we examined the effect of oxygen on the inactivation of scrapie agent in suspensions that were dilute enough for 'indirect action' to contribute most of the inactivating effect of radiation.

## METHODS

*Preparations of the scrapie suspensions, and assay after treatment.* These were as previously described (Alper *et al.* 1966). For the reason given in the introduction, no purpose would have been served by attempts to 'purify' the preparations, so brain homogenates were used in appropriate dilution after centrifugation twice for 10 min at 1800 *g*, to remove debris.

*Exposure to near-monochromatic u.v. irradiation.* The supernatant fluids obtained from 10 % brain suspensions after centrifugation were diluted a further 40-fold for exposure to u.v. light. The methods for obtaining near monochromatic light have been described by Muel & Malpiéce (1969). Methods for irradiation and dosimetry were described by Latarjet *et al.* (1970). In the course of three series of irradiations by these methods, exposures were

made at wavelengths 237, 252, 254, 267 and 280 nm, the half-band widths at those wavelengths ranging from 9 to 15 nm (Muel & Malpiéce, 1969). Reasonable dose rates at shorter wavelength were not achieved. It was hoped that this could be done by using the powerful monochromator at the Department de Radio-agronomie, Centre d'Études Nucléaires, Cadarache. Here we gave two doses at each of the wavelengths 210, 225, 237 and 254 nm. The experiments were only partially successful; the experimental conditions called for irradiation of volumes that were rather too small to provide enough material for the injection of undiluted as well as diluted irradiated material; and some of the samples proved to have been inactivated to too great an extent to demonstrate surviving activity in the diluted samples. The results were adequate, therefore, only for a rough estimate of the efficacy of the two lowest wavelengths.

*Exposure of suspension to ionizing radiation.* The object was to compare the effectiveness of radiation in the presence and absence of oxygen, under conditions such that inactivation was attributable mainly to radiolysis products of water. In general, in such experiments the relative contribution of the so-called 'indirect' action is the greater, the more dilute the suspension, until further dilution no longer results in an increased effect. We needed to use a scrapie suspension that was dilute enough so that a substantial contribution of indirect action could be anticipated, but still concentrated enough for the activity of the agent to be measurable after considerable inactivation. Homogenized brain tissue diluted 500-fold seemed suitable by those criteria. We used that dilution for three separate series of experiments and in a final series we irradiated the material in 50- and 5000-fold dilution, so that an estimate could be made of the extent to which indirect action had contributed to inactivation in the main series.

The suspensions were irradiated in vessels designed for irradiation and sample removal was under continuous gas bubbling (Alper, 1955). We used oxygen-free nitrogen or pure oxygen. Irradiation was by the electron beam from the Medical Research Council, 7 MeV linear accelerator, from which sufficiently high dose rates were obtainable.

To check on effects of irradiation in similar conditions on an entity that depends on the integrity of nucleic acid for reproductive capacity, bacteriophage $\phi$X174 was irradiated in mouse brain suspension diluted 500-fold. Suspensions were irradiated in parallel under continuous flushing with oxygen or oxygen-free nitrogen. After irradiation samples were suitably diluted and plated together with an appropriate host strain, by the agar layer technique (Adams, 1959).

## RESULTS

### *Ultraviolet light 'action spectrum'*

The results of the first experiment, reported by Latarjet *et al.* (1970) were confirmed and extended. An example of raw data from one of the experiments is given in Table 1. This shows that doses at 237 nm were equivalent in their effect to doses four to five times as high at 267 and 280 nm.

Activities of irradiated suspensions relative to controls were reduced exponentially with dose. In no case was it possible to resolve the inactivation curves in the range 252 to 280 nm (Fig. 1), so the effectiveness of u.v. light at 237 nm was in each series compared with that of all wavelengths in the 'germicidal' range used in that series. The effectiveness at 237 nm relative to wavelengths in the range 250 to 280 nm was defined by the inverse of the ratio of doses to give the same inactivation. The ratios were 5·7, 4·5 and 3·9 in the three experiments, giving a mean value of $4{\cdot}7 \pm 0{\cdot}4$ (0·4 is the standard error of the mean).

In the experimental run at Cadarache, previous inactivation curves at 237 nm were

Table 1. *Exposure of scrapie brain suspensions, diluted 1/400, to u.v. light at different wavelengths*

| | | Wavelength peak, nm | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 237 | | 254 | | 267 | | | | 280 | | Unirradiated controls, pooled |
| | | Incident dose, $J/m^2 \times 10^{-4}$ | | | | | | | | | | |
| | | 1·11 | 2·3 | 9·4 | 14·5 | 4·67 | 7·80 | 11·7 | 17·4 | 4·1 | 11·0 | |
| Dilution, $\log_{10}$ | −2·6 | | 12/12 | | 11/11 | | | | 14/14 | | | |
| | −3·1 | | 8/8 | | 9/9 | | | 12/12 | 9/9 | | 13/13 | |
| | −3·6 | | | 13/13 | 8/8 | | 12/12 | 9/9 | 6/7 | | 9/9 | |
| | −4·1 | 14/14* | 7/7 | 8/8 | | | 9/9 | 8/8 | | | 7/7 | |
| | −4·6 | 8/8 | | 7/7 | 2/8 | | 8/8 | | 1/7 | | | |
| | −5·1 | 8/8 | 5/8 | | 2/7 | 11/11 | | 2/8 | 1/9 | 12/12 | 6/8 | |
| 0·05 ml/mouse | −5·6 | | | 2/9 | 2/10 | 9/9 | 6/8 | 2/7 | 1/13 | 8/9 | 3/9 | |
| | −6·1 | 7/8 | 1/9 | 2/9 | | 6/8 | 4/8 | 1/14 | | 4/8 | 0/11 | 41/41 |
| | −6·6 | 2/9 | 1/14 | 0/8 | | 0/8 | 0/14 | | | 2/6 | | 24/26 |
| | −7·1 | 2/10 | | | | 0/14 | | | | 0/7 | | 9/19 |
| | −7·6 | | | | | | | | | 0/12 | | 5/19 |
| | −8·1 | | | | | | | | | | | 1/34 |
| Dilution for $LD_{50}$, $\log_{10}$ by probit analysis | | −6·5 | −5·5 | −5·5 | −4·7 | −6·2 | −5·9 | −5·1 | −4·4 | −6·2 | −5·3 | −7·2 |
| 95 % confidence interval | | −6·8 | −5·8 | −5·8 | −5·0 | −6·5 | −6·2 | −5·4 | −4·7 | −6·5 | −5·6 | −7·4 |
| | | −6·2 | −5·1 | −5·1 | −4·4 | −5·9 | −5·6 | −4·8 | −4·0 | −5·9 | −5·1 | −7·1 |
| Residual activity as fraction of control $\log_{10}$ | | −0·7 | −1·7 | −1·8 | −2·5 | −1·0 | −1·3 | −2·1 | −2·9 | −1·0 | −1·9 | |
| 95 % confidence interval | | −0·4 | −1·4 | −1·4 | −2·2 | −0·7 | −1·0 | −1·8 | −2·5 | −0·7 | −1·6 | |
| | | −1·0 | −2·2 | −2·1 | −2·9 | −1·3 | −1·6 | −2·5 | −3·2 | −1·3 | −2·2 | |

* Cases of scrapie/number of mice per group.

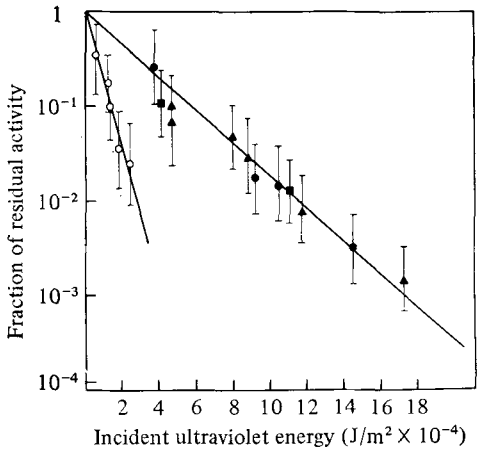


Fig. 1. Inactivation of scrapie agent by u.v. light. ○, 237 nm; ●, 254 nm; ▲, 267 nm; ■. 280 nm. Vertical bars show 95 % confidence intervals.

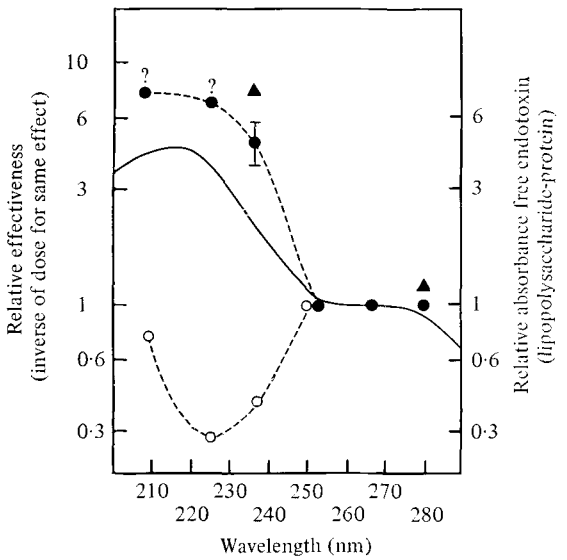


Fig. 2. ●---●, Relative effectiveness of u.v. light at various wavelengths for inactivation of scrapie. Points at 210 and 225 nm are estimates. Vertical bar, 237 nm, represents standard error of mean for 3 experiments; ○---○, relative effectiveness of u.v. light at various wavelengths for inactivation of bacteriophage $T_2$ experiment run concurrently with one of the scrapie experiments; ▲, relative effectiveness of u.v. light at 237 and 280 nm for inactivation of 'partially purified' scrapie suspension (points ○ and ▲ personally communicated by Dr R. Latarjet; ——, absorbance of lipopolysaccharide-protein complex purified from free endotoxin (Marsh & Crutchley, 1967).

confirmed. Irradiations at 210 and 225 nm yielded results that could be interpreted only qualitatively. Inactivation was about twice as effective at the lower wavelengths as at 237 nm.

Points showing relative effectiveness of the various wavelengths are plotted in Fig. 2 and also the results (R. Latarjet, personal communication) on a 'partially purified' scrapie suspension, prepared and assayed by Dr D. C. Gajdusek and Dr C. J. Gibbs and irradiated

Table 2. *Exposure of scrapie brain suspensions, diluted 1/500 and continuously flushed with oxygen or oxygen-free nitrogen, to an electron beam*

| | | Electron dose (kilograys) | | | | | | | | | | Unirradiated control, including suspensions flushed with oxygen or oxygen-free nitrogen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Oxygen | | | | Oxygen-free nitrogen | | | | | | |
| | | 1·5 | 2·5 | 5·0 | 10 | 15 | 25 | 35 | 60 | 150 | 200 | |
| Dilution, $\log_{10}$ | −2·7 | | | | 3/7 | | | | | | | |
| | −3·2 | | | | 1/8 | | | | | 8/8 | 6/6 | |
| | −3·7 | 8/8* | 6/6 | 4/8 | 1/7 | | | | | 8/8 | 5/6 | |
| | −4·2 | 8/8 | 7/7 | 0/8 | 1/7 | | | 8/8 | 8/8 | 8/8 | 8/8 | |
| | −4·7 | 8/8 | 5/8 | 0/8 | 0/8 | 7/7 | 8/8 | 8/8 | 8/8 | 8/8 | 4/8 | |
| | −5·2 | 8/8 | 1/8 | 0/8 | 0/8 | 6/6 | 7/7 | 8/8 | 7/7 | 2/8 | 3/8 | |
| 0·05 ml/mouse | −5·7 | 6/8 | 0/8 | 0/6 | 0/8 | 6/7 | 8/8 | 8/8 | 6/6 | 2/7 | 0/8 | 36/36 |
| | −6·2 | 1/6 | 0/7 | 0/8 | | 5/8 | 4/7 | 5/8 | 4/6 | | 0/8 | 37/39 |
| | −6·7 | 1/8 | | | | 0/7 | 2/7 | 0/8 | 2/7 | | | 26/38 |
| | −7·2 | | | | | 0/8 | 0/8 | | | | | 11/39 |
| | −7·7 | | | | | | | | | | | 0/38 |
| Dilution for $LD_{50}$, $\log_{10}$, by probit analysis | | −6·0 | −4·8 | −3·6 | −2·9 | −6·2 | −6·4 | −6·3 | −6·5 | −5·2 | −4·8 | −6·9 |
| 95 % confidence interval | | −6·3 | −5·1 | −3·9 | −3·2 | −6·5 | −6·6 | −6·5 | −6·8 | −5·5 | −5·0 | −7·0 |
| | | −5·8 | −4·6 | −3·3 | −2·6 | −5·9 | −6·1 | −6·0 | −6·2 | −5·0 | −4·6 | −6·8 |
| Residual activity as fraction of control, $\log_{10}$ | | −0·9 | −2·1 | −3·3 | −4·0 | −0·7 | −0·5 | −0·6 | −0·5 | −1·7 | −2·1 | |
| 95 % confidence interval | | −0·6 | −1·8 | −3·0 | −3·7 | −0·4 | −0·2 | −0·4 | −0·1 | −1·4 | −1·8 | |
| | | −1·1 | −2·4 | −3·7 | −4·3 | −1·0 | −0·8 | −0·9 | −0·8 | −1·9 | −2·4 | |

* Cases of scrapie/number of mice per group.

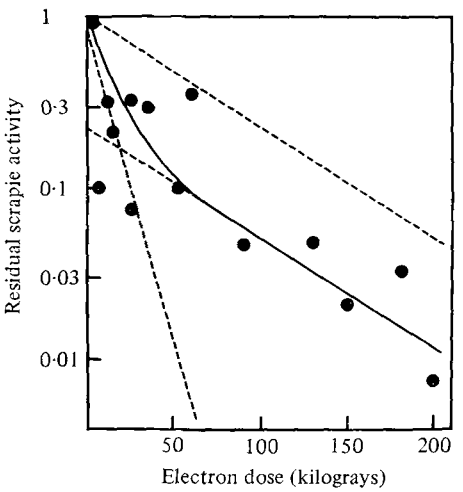


Fig. 3. Inactivation of scrapie agent exposed to ionizing radiation in dilute suspension, anoxic. The solid curve describes the equation

$$f = 0{\cdot}8e^{-D/12} + 0{\cdot}2e^{-D/68}$$

where $f$ is the residual fractional scrapie activity after dose $D$ kilograys. Upper dashed line reproduces inactivation curve for dry, anoxic scrapie agent (Alper & Haig, 1968).

at the Institut du Radium. A reference in qualitative terms to that result was made by Gajdusek & Gibbs (1975). Also shown are the results of R. Latarjet and B. Muel (personal communication) on the irradiation of coliphage $T_2$, carried out concurrently with, and as a control for, the irradiations of the scrapie agent at Cadarache.

### *Irradiation by electrons*

Inactivation curves for the scrapie agent were exponential when the material was irradiated dry (Alper *et al.* 1966; Alper & Haig, 1968; Field *et al.* 1969), but when suspensions were irradiated, the results could not be fitted by exponential curves. The results from four sets of experiments on anoxic preparations, exemplified by part of Table 2, are shown in Fig. 3. Despite considerable scatter, there is evidence of at least two modes of inactivation. Back-extrapolation of the 'resistant tail' gives the size of the more resistant fraction as about 20 %. The lower dotted curve of Fig. 3, referring to the remaining 80 %, was obtained by subtraction of the back-extrapolated curve from the smooth curve fitted to all the points. Inactivation doses calculated for the two exponentials (i.e. doses required to give residual activities of $e^{-1}$ of each fraction) were respectively 12 and 68 kilograys the latter corresponding with the inactivation dose by direct effect on dry anoxic preparations (Alper & Haig, 1968). When the brain was diluted 5000-fold the size of the resistant fraction was reduced to about 10 %, but there was no detectable increase in the sensitivity of the fraction inactivated by indirect effect.

Suspensions irradiated in the presence of oxygen were more sensitive and a much smaller fraction was protected against indirect action. An example of raw data from one experiment is given in Table 2. Fig. 4 shows results of irradiation on oxygenated suspensions from four separate runs (including inactivation of the agent in suspensions diluted 1/5000). An exponential curve may be fitted to the points to a level of residual activity of less than $10^{-3}$. The dose required to reduce activity to $e^{-1}$ of the control was about 950 gray. Thus the

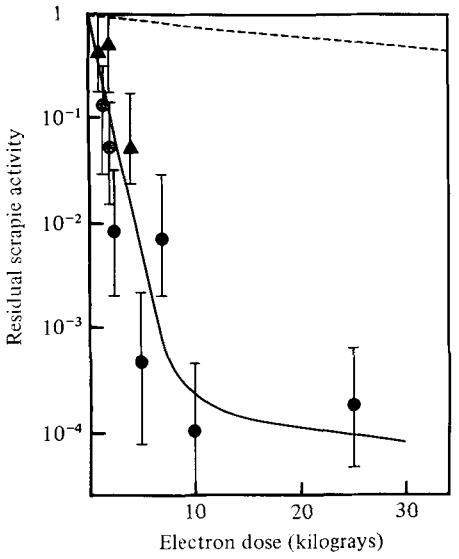


Fig. 4. Inactivation of scrapie agent exposed to ionizing radiation in dilute suspension, oxygenated. Initial straight line describes the equation

$$f = e^{-D/950}$$

where $f$ is the residual fractional scrapie activity after dose $D$ grays. ●, irradiated suspension 1/500 of original brain suspension; ▲, irradiated suspension 1/5000 of original brain suspension. Upper dashed curve, inactivation of dry oxygenated scrapie agent (Alper & Haig, 1968).

initial ratios of doses in anoxic and oxygenated conditions to give the same inactivation by 'indirect effect' was 12000/950, i.e. about 12·5. That 'oxygen enhancement ratio' (o.e.r.) is considerably higher than has been recorded for any radiation-induced loss of biological or biochemical function other than by lysosomes, with which release of bound enzymes were measured (Watkins, 1970).

Most of the inactivation in oxygenated suspensions diluted 500-fold could be attributed to indirect action, since dilution by a further factor of 10 did not result in greater sensitivity.

*Control for possibility of inactivation by long-lived radiation products*

When pure water containing only dissolved oxygen is irradiated, hydrogen peroxide is formed; and with biological material in suspension (as in our scrapie preparations) or in solution, organic peroxides are also formed. These long-lived products may be toxic to a biological system being tested for the effects of short-lived active radicals, as has been observed in the inactivation of bacteriophage (Alper, 1948) enzymes (Anderson, 1954) and animal viruses (Johnson, 1965). It was particularly important to check for possible post-irradiation effects in the inactivation of scrapie agent since there was usually an interval of many hours between the end of irradiation and the injection of irradiated samples into mice

Normal mouse brain suspensions were irradiated under oxygen with doses of 5 and 30 kilogray, which had left residual scrapie activity respectively of about $10^{-3}$ and $10^{-4}$. Unirradiated scrapie suspensions were immediately added to give a final dilution of 1/500, and assays were made 4 h and 22 h later. No significant loss of activity was found in the suspension that had received 5 kilogray; in the one that had received 30 kilogray, activity was somewhat reduced by 22 h (Table 3). Since the calculation of initial o.e.r. is based on

Table 3. *Post-irradiation inactivation of scrapie agent*

| | Titrated, h after mixing | |
|---|---|---|
| | 4 | 22 |
| Scrapie agent + unirradiated normal mouse brain | −6·5* | −6·6 |
| | −6·6 | −6·7 |
| Scrapie agent + brain irradiated 5 kilogray | −6·3 | −6·6 |
| | −6·6 | −6·4 |
| Scrapie agent + brain irradiated 30 kilogray | −6·1 | −5·7 |
| | −6·4 | −5·8 |

* Dilution to give $\log_{10}$ $LD_{50}$/0·05 ml/mouse, titrations in duplicate.

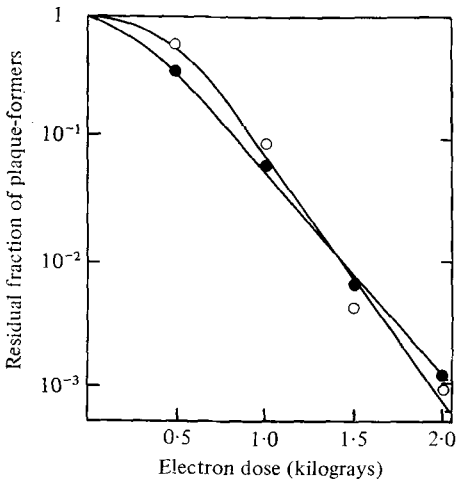


Fig. 5. Inactivation of bacteriophage $\phi$X174 irradiated in mouse brain suspension diluted 1/500: ○—○, in presence of oxygen; ●—●, anoxic.

doses of less than 5 kilogray to the oxygenated suspension, no correction for post-irradiation inactivation was required.

In contrast, a brain suspension irradiated with oxygen present was found to be very toxic to bacteriophage $\phi$X174. When a sample was introduced into a mouse brain suspension that had been exposed to 2 kilogray under oxygen, the phage activity was reduced to 34 % of the controls, when measured as soon as possible after mixing, and to less than 1 % after a further 45 min. Suspensions that had been irradiated in the absence of oxygen had no toxic effect.

### *Irradiation of bacteriophage $\phi$X174 in mouse brain suspension*

Effects of the short-lived active radicals formed in the irradiation of anoxic and oxygenated mouse-brain suspension are exemplified in Fig. 5. In the dose range within which up to 98 % of the phage particles were inactivated, oxygen was protective. With increasing dose, that protective effect was counteracted by the lethal effects of long-lived toxic products, which, as described above, were formed only when oxygen was present during irradiation. This caused the slope of the inactivation curve to increase with increasing dose. Comparable results were observed when phage S13 was irradiated in phosphate buffer (Alper, 1954; 1955): except that, in that case, inactivation curves were exponential when

the dose-rate was high enough so that toxic action during the course of irradiation was negligible.

Johnson (1965), working with the viruses of foot-and-mouth disease and vesicular stomatitis, observed inactivation in buffer solution, and the inactivating effect of the hydrogen peroxide formed in modes entirely consonant with those reported for bacteriophage S13 (Alper, 1954, 1955). Thus our results with the scrapie agent differed in two respects from those obtained both with animal viruses and bacteriophages: slopes of inactivation curves for scrapie decreased with increasing dose; and the scrapie was inactivated to only a negligible extent by the long-lived products formed in the oxygenated suspension. The effect of removing oxygen from animal virus suspensions was not tested by Johnson (1965) but the most striking difference between the responses of scrapie agent and bacteriophage lay in the high sensitization of the former by oxygen, which increased with increasing dose.

## DISCUSSION

Biological damage to whole cells by ionizing radiation is usually enhanced by the presence of oxygen. The o.e.r. is given by the ratio of doses required to deliver the same effect to anoxically and aerobically irradiated cells. When proliferative death is the test of the damage, the o.e.r. has been found to have values ranging from about 2 to a little over 3 with higher cells, and up to about 4 or 5 with some strains of bacteria, but oxygen fails to act as a radiosensitizer of nucleic acid preparations irradiated extracellularly in suspension when damage is assessed by loss of function. No sensitizing action of oxygen was found for the inactivation of bacteriophage, or transforming DNA, when the irradiated preparations contained scavenger material (e.g. proteins) that protected against 'indirect action' (e.g. Hewitt & Read, 1950; Ephrussi-Taylor & Latarjet, 1955). In more dilute suspension, such that there was significant contribution to inactivation from active radicals, oxygen was found to protect bacteriophages S13 and T1 (Alper, 1954; Bachofer & Pottinger, 1954); naked bacteriophage DNA (Van der Schans, quoted by Blok & Loman, 1973), and also RNA, damage to which was tested by loss of transfer or messenger activity (Ekert & Grunberg-Manago, 1966; Ekert & Latarjet, 1971).

In contrast, the presence of oxygen during irradiation greatly enhances the damaging effect of ionizing radiation on biological membranes. This is illustrated by the work of Watkins (1970) on lysosomes irradiated in aqueous suspension. Assessing damage to the membrane by the release of lysosomal enzymes he found that, depending on the enzyme, the o.e.r. ranged from 5 to about 20. Effects of oxygen on radiation-induced damage to some sub-cellular systems irradiated in fairly dilute solution or suspension are summarized in Table 4. Since the oxidative and reductive effects of active radicals are dependent on pH, results are included only for suspensions irradiated at pH values near 7, i.e. corresponding with our preparations of scrapie agent.

Ultraviolet light action spectra for mutation induction as well as killing in the case of micro-organisms were recognized as being similar to the nucleic acid absorption spectrum some years before DNA was established as the component responsible for carrying the genetic code (Gates, 1930). Such results demonstrate that disruption of biological functions of cellular DNA by radiant energy depends on its absorption by specific chromophores, despite the firm attachment of the DNA to cell membranes (Jacob *et al.* 1966; Tremblay *et al.* 1969). Similarly, action spectra for virus inactivation demonstrate a peak in the region of greatest absorption of u.v. light by nucleic acid (250 to 270 nm), a trough in the region of 240 nm, and considerably reduced effectiveness at 280 nm and above,

Table 4. *Ratios of radiation doses to yield the same biological or biochemical effects in anoxic and well oxygenated dilute aqueous solutions or suspensions or sub-cellular test systems, from experiments in which pH values were near 7*

| Test system | Test of damage | Dose ratio, anoxic: oxygenated | Reference |
|---|---|---|---|
| Bacteriophage S13 | Loss of infective activity | 0·50 | Alper (1955) |
| Bacteriophage T1 | Loss of infective activity | 0·35 | Bachofer & Pottinger (1954) |
| DNA of $\phi$X174 | Loss of infective activity | 0·56 | Van der Schans, quoted by Blok & Loman (1973) |
| Poly-uracil | Loss of phenylalanine synthesising ability | 0·74 | Ekert & Grunberg-Manago (1966) |
| Transfer-RNA, *Escherichia coli* | Loss of transfer activity, lysyl-t-RNA | 0·47 | Ekert & Latarjet (1971) |
| Transforming DNA* (Pneumococcus) | Loss of transforming activity | 0·8–1·1 | DeFilippes & Guild (1959) |
| Lysozyme | Loss of enzyme activity | 0·80 | Brustad (1967) |
| Ribonuclease† | Loss of enzyme activity | 0·74 | Adams *et al.* (1971) |
| $\alpha$-Chymotrypsin† | Loss of esterase activity | 0·43 | Adams *et al.* (1973) |
| Lima bean protease inhibitor | Loss of inhibitory activity towards $\beta$-trypsin | 0·73 | Lynn & Raoult (1976) |
| Lysosomes | Release of acid phosphotase | about 6 | Watkins (1970) |
| | Release of $\beta$-glucuronidase | 10–20 | |
| Scrapie | Loss of infective activity to 10% | about 12 | |
| | Greater loss of activity | Maximum measured about 80 | |

* The concentration of DNA used for these measurements was high enough for the contribution from indirect action to be reduced.

† Anoxia achieved by flushing with argon; in other experiments with oxygen-free nitrogen.

although the nucleic acid cores are coated with protein. When action spectra on whole viruses and extracted nucleic acid have been compared, the only significant difference has been the even lesser effectiveness of u.v. light at about 240 nm for the latter (e.g. Kassanis & Kleczkowski, 1965), so that the contrast with the action spectrum for the scrapie agent is even more marked. One strain of tobacco mosaic virus, U1, is an exception, in that the action spectrum for the whole virus failed to show the peak corresponding to maximum absorption by nucleic acid (Hollaender & Duggar, 1936), the effectiveness of u.v. light at about 240 nm being 2·3 times as great as at 250 nm. As with other viruses, however, u.v. light at 280 nm was less effective than at 250 nm, in that case by the factor 2·4. It has been suggested that the action spectrum for U1 virus irradiated intact 'bears some similarities to that of the scrapie agent', but with the latter there was no detectable difference in effectiveness throughout the range 250 to 280 nm (Fig. 1, 2). In view of the obvious and numerous disparities between a plant virus and the scrapie agent, and of the importance of the protein coat of TMV(U1) as a determinant of its anomalous action spectrum (Kleczkowski & McLaren, 1967), it would seem unrealistic to disregard the clues provided by the action spectrum for scrapie agent on account of the one anomaly. It is interesting in this connection

to note that evidence of the nucleic acid nature of the genome in micro-organisms was provided by early action spectra for killing and mutation induction, but the correct interpretation was not made because it was generally accepted at that time that only proteins had the chemical properties requisite for gene specificity (Professor Charlotte Auerbach, personal communication; see also Huxley, 1949).

As shown in Fig. 2, the absorption spectrum for the toxic fraction of bacterial endotoxin (Marsh & Crutchley, 1967) is reasonably similar to our outline action spectrum for the scrapie agent. From a series of chemical observations, combined with bio-assay, those authors identified the toxic fraction as a lipopolysaccharide-protein complex.

The marked susceptibility of scrapie agent to radiation-induced oxidative reactions is evidence that the component essential for replication contains a lipid fraction. This provides support for the original 'membrane hypothesis' of Gibbons & Hunter (1967); and the support is augmented by the apparent similarity between the absorption spectrum for endotoxin (a constituent of bacterial membrane) and the action spectrum for scrapie agent. Studies on the distribution of the agent in subcellular fractions isolated from brain and spleen of mice affected with experimental scrapie have shown that a large proportion of the infectivity is associated with the plasma membrane and endoplasmic reticulum of the cell (Millson *et al.* 1971). Observations with the cell line SMB (Clarke & Haig, 1970) have shown that distribution of the agent coincides with the enzyme 5′-nucleotidase, indicating association of the agent with plasma membrane (Clarke & Millson, 1976). From studies based on centrifugation of hamster brain homogenates prepared during the course of infection, Malone *et al.* (1978) confirmed that the greatest activity was associated with developing membranes, but concluded that infectivity was due in great measure to the products of membrane dissolution, rather than to structural components.

Diener's suggestion (1972; 1973) that the scrapie agent is a viroid, i.e. a small fragment of RNA, probably influenced the formulation of the subsidiary hypothesis that replication of the scrapie agent requires the presence of a moiety of nucleic acid attached to membrane (e.g. Hunter, 1974; Gajdusek & Gibbs, 1975) and searches have been made, without success, for infectious nucleic acid associated with the transmission of scrapie (Marsh *et al.* 1974; Ward *et al.* 1974; Hunter *et al.* 1976). Less attention seems to have been paid to Diener's enumeration (1973) of 'fundamental differences' between viroids and the scrapie agent than of similarities, the most important of the latter being the very low mol. wt. of viroids. An example of one significant difference is that the clue which led to the identification of the agent of potato spindle tuber virus (PSTV) was its inactivation by ribonuclease (Diener & Raymer, 1969) whereas the scrapie agent is well-known to be unaffected by nucleases. A similarity quoted by Diener (1972, 1973) between PSTV and scrapie agent was their firm attachment to cell debris. Although this attachment clearly failed to protect PSTV against attack by ribonuclease, it was nevertheless invoked to account for the lack of inactivating effect of nucleases on scrapie agent.

In one important respect there is, indeed, something in common between viroids and the scrapie agent. Without some modification, the 'central dogma', as enunciated by Crick (1970), cannot accommodate the initiation of 'self-replication' by pathogens of either kind: on the one hand, an agent for which no evidence of a nucleic acid moiety can be adduced, despite considerable effort devoted to the attempts to do so; and, on the other, a fragment of RNA that is too small to code for even one protein.

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