THE EXCEPTIONALLY SMALL SIZE OF THE SCRAPIE AGENT

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Scrapie, a progressive encephalopathy of sheep, is transmissible with cell-free filtrates, and on these grounds the agent has been classed as a virus (Andrewes, 1964). However, it has some unusual properties, for example the high temperature required for inactivation (Stamp, 1962), and extraordinary resistance to treatment by formalin (Pattison, 1965).

Quantitative studies of the scrapie agent have been facilitated by the development of methods for titrating infective material in mice (Chandler, 1963). However, the agent has as yet not been purified, nor has the particle size been determined, because it evidently adheres strongly to cell fragments (Hunter and Millson, 1964; Mould, Dawson and Smith, 1965).

The sizes of molecules and macromolecules possessing biological activity may be estimated from the manner in which they are inactivated by ionizing radiation. The validity of the approach has been demonstrated with a wide range of biological materials for which independent determinations of size are available (Hutchinson and Pollard, 1961). We have therefore made use of this technique with the scrapie agent, using as our source of

radiation the electron beam from the Medical Research Council 8 MeV Linear Accelerator (Batchelor, et al, 1959).

Calculation of "target size" from radiation inactivation data are most reliable when inactivations are due only to energy deposited within the molecules under investigation, i.e. when there is no contribution by "indirect action" (Dale, 1940), due to radiolysis products formed in a solvent or suspending medium. The scrapie infective material was therefore irradiated dry.

Materials and Methods

Brains were collected from mice showing advanced signs of scrapie following intracranial injections of the Chandler (1963) strain. They were suspended in distilled water (10% W/V) and the mixtures centrifuged twice at 1800Xg. for 10 minutes. The supernatant fluid was then freeze-dried in glass ampoules. Oxygen was admitted before the ampoules were sealed, since the absence of oxygen may protect against ionizing radiation, and estimates of target size are based fundamentally on the assumption that there is a high probability of inactivation by each average energy-loss event which occurs within the "target volume".

The electron doses, delivered at about 0.3 megarad/minute, were measured by simultaneously exposing pieces of specially prepared perspex, enclosed in ampoules similar to those containing the scrapie material, (Boag, Dolphin and Rotblat, 1958).

After irradiation, the scrapie material was reconstituted in distilled water. In the first two experiments tenfold serial dilutions in buffered saline were made, and 0.03 ml of each dilution injected into each of 7 mice. In the third experiment somewhat greater accuracy was attained by using serial semi-logarithmic dilutions, with 12 mice to test each dilution.

An experiment was also carried out to determine the susceptibility of the scrapie agent to ultraviolet radiation at 2537A. The material was exposed as a saline suspension, diluted 10 times as compared with the freeze-dried material. The source of UV was a "Hanovia" 15 watt germicidal lamp, with about 90% of the energy at 2537A. The dose rate was about 540 ergs/mm ^{2}/sec.

Results and interpretation

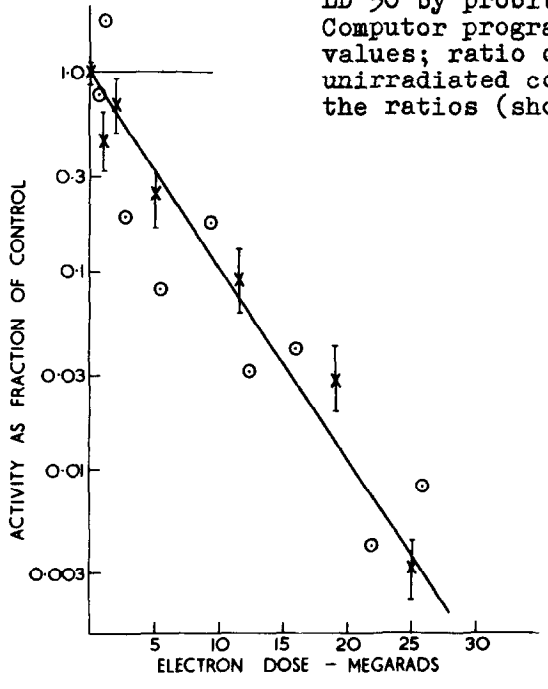
1. Ionizing radiation

In a pilot experiment, the maximum dose of 2 megarads was insufficient to give a measurable loss of activity. In two further experiments doses up to 25 megarads were given.

Figure 1: Two experiments to determine the value of Do for the agent of scrapie

⊙, First, less accurate, experiment. Values of LD 50 by method of Kärber (1931). Errors not calculated.

X, Second, more accurate, experiment. Values of LD 50 by probit analysis (Finney, 1952). Computor program, by M. C. Pike, gave LD 50 values; ratio of each LD50 value to that for unirradiated control; and standard errors of the ratios (shown by vertical lines).



The curves relating surviving activity to dose were exponential, and had equal slopes within experimental error (Fig. 1). The mean dose (Do) to give 36.8% (e^{-1}) of the initial activity was 4.3 ± 0.3 megarads.

The value of the Do for the scrapie agent is compared in Table 1 with values for various materials in which radiation damage could be measured in terms of loss of biological activity. All of these were irradiated dry, in the presence of oxygen. Also given are the "target sizes" derived from the Do values

Table 1

Material	Do Megarads	Ref:	Target Size (Lea, 1946) Mol.wt.x10^{-4}	Independent Estimate Mol.wt.x10^{-4}	Ref:
Ribonuclease	22	a	3	1.27	f
Lysozyme	33	b	1.8	1.7	f
Trypsin	25	b	2.5	2.38	f
Desoxyribonuclease	17	b	3.9		
	7.8	c	8	6.3	f
Scrapie agent	4.3		15		
Marker, Transforming Principle	1.6		45		
	2.7	d	29		
Bacteriophage R17 (RNA phage)	0.78	e	92	100	g
Bacteriophage ØX 174 (free DNA irradiated)	0.34	e	220	170	h
Bacteriophage T3	0.08		diameter 28mμ	diameter head 47mμ DNA 34mμ	i

References

a. Hunt, Till & Williams, 1962
b. Brustad, 1961
c. Okada & Fletcher, 1960
d. Tanooka & Hutchinson, 1965
e. Ginoza, 1963
f. Dixon & Webb, 1964
g. Zinder, 1965
h. Sinsheimer, 1959
i. Adams, 1959.

in accordance with the calculations of Lea (1946). Independent estimates of size are available for many of the materials, and the agreement is good enough to suggest that the "target molecular weight" is unlikely to differ from the independent estimate by more than a factor of 3.

According to Lea's calculations, a Do value of 5 megarads will be observed with a particle whose target diameter is about 7mμ, i.e. whose molecular weight is about $1.5X10^5$. The value of Do quoted for dry bacteriophage T3 was obtained in the same conditions of irradiation as for the scrapie agent: a suitable small volume of bacteriophage suspension was mixed with mouse-brain suspension, which was then dried in ampoules as described. This was done to check the possibility that the suspended brain material might, when dried, afford protection against ionizing radiation. The value of Do for bacteriophage T3 dried in a mouse-brain suspension was about the same as that obtained in another series of experiments in which bacteriophage DNA was irradiated soon after it had been injected into wet host cells.

2. Ultraviolet light at 2537A

There was no significant loss in the activity of the scrapie agent exposed to doses up to $2.4X10^4$ ergs/mm^2. When bacteriophage T3 was mixed with an equivalent mouse-brain suspension, a dose of 10^3 ergs/mm^2 reduced the bacteriophage activity to 1%. With single-stranded phage S13 this level of survival would be obtained after exposure in similar conditions to 600 ergs/mm^2.

Discussion

The smallest known virus particles are probably the single-stranded DNA phages ØX174 and S13 (Sinsheimer, 1959)

and the RNA phages (Loeb and Zinder, 1961). The nucleic acid content of these bacteriophages would, if regarded as single macromolecules, have molecular weights of one to two million (Sinsheimer, 1959; Zinder, 1965). Their Do values, for irradiation in the dry state, are about one-tenth that found for the agent of scrapie. If, therefore, the target for radiation damage to this agent is a nucleic acid moiety, its molecular weight is unlikely to be greater than about 2.10^5, which would mean about 800 bases.

Since the scrapie agent multiplies in the host animal, it has been assumed that nucleic acid must be a part of its structure. However, the evidence that no inactivation results from exposure to a huge dose of ultraviolet light, of wavelength specifically absorbed by nucleic acids, suggests that the agent may be able to increase in quantity without itself containing nucleic acid. This possibility is supported by the data from electron irradiations, since these yield a target size which is implausibly small as a nucleic acid code. In any event, our data strongly support the conclusion of Pattison (1965) that this agent is likely to be of an unusual nature.

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