Wis. 51–20 C and BRL 700. (With the exception of strain BRL 700, these strains are all available from the American Type Culture Collection; results using strain BRL 700, a high yielding industrial strain, were obtained through collaboration with Beecham Research Laboratories, Brockham Park, Betchworth, Surrey, who also supplied this strain.) These penicillin producing strains are interrelated and are all derived from strain NRRL 1951 by mutation and strain selection procedures. The relationships between the various strains are illustrated diagrammatically in Fig. 1.

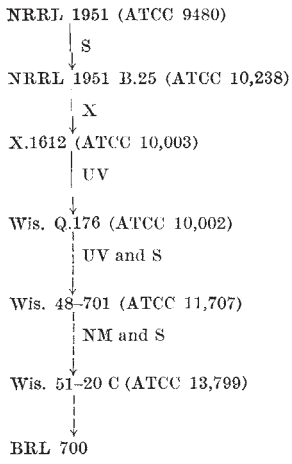

Fig. 1. Simplified diagrammatic representation of derivation of various strains of *P. chrysogenum*. S indicates derivation by strain selection without mutation; X, UV and NM indicate mutation by means of X-irradiation, ultraviolet irradiation and nitrogen mustards, respectively. Single-step derivation is represented by a continuous line, multiple-step derivation by a broken line.

Strains of *P. chrysogenum* were grown in submerged culture in shaken flasks at 24° C for approximately 48–60 h, when maximum growth of the fungi was recorded; the medium used was a simple one containing 4·0 per cent maltose, 1·0 per cent bacteriological peptone and 2·4 per cent malt extract, adjusted to *p*H 7·0. Particle preparations were obtained by disrupting the mycelia (approximately 10 g wet weight) in a Pascall Triple Roll Mill No. 1 and extracting with ten times this weight of 0·03 M phosphate buffer *p*H 7·0 containing 0·1 per cent thioglycollic acid. Cell debris was removed by centrifugation at 15,000*g* for 30 min and the particles sedimented from the clarified extract by ultracentrifugation at 78,480*g* for 90 min. The pellets were re-suspended in 1·0 ml. each of 0·03 M phosphate buffer *p*H 7·0 (without thioglycollic acid) and debris again removed by low speed centrifugation at 7,000*g* for 20 min. The resultant crude preparations were examined by electron microscopy (J.E.M. Model 7) and shown to contain polyhedral virus-like particles approximately 35 mmicrons in diameter. The particles obtained from the seven different strains of *P. chrysogenum* were morphologically indistinguishable by the techniques used. An electron micrograph of a particle preparation from strain BRL 700 is shown in Fig. 2. The crude particle preparation could be further purified by density gradient ultracentrifugation, a well defined, blue-grey, light-scattering zone being formed in a sucrose gradient column (10–50 per cent) after centrifugation at 69,000*g* for 120 min, at a level corresponding to 20–22 per cent sucrose; the formation of such a zone is a characteristic of virus particles.

Investigations into the composition, structure, serological and possible interferon stimulating properties of these virus-like particles are continuing.

An interesting feature of this work is that the seven strains of *P. chrysogenum* found by us to contain virus-like particles are penicillin producing strains from which all modern high yielding industrial strains are derived. Because the presumed virus has survived treatment with various mutagenic agents (see Fig. 1), it is possible that many industrial strains are similarly infected. The presence of virus might be expected to influence profoundly host metabolism and hence the biosynthesis of penicillin. The effect of any confirmed virus infection upon penicillin biosynthesis will be examined.

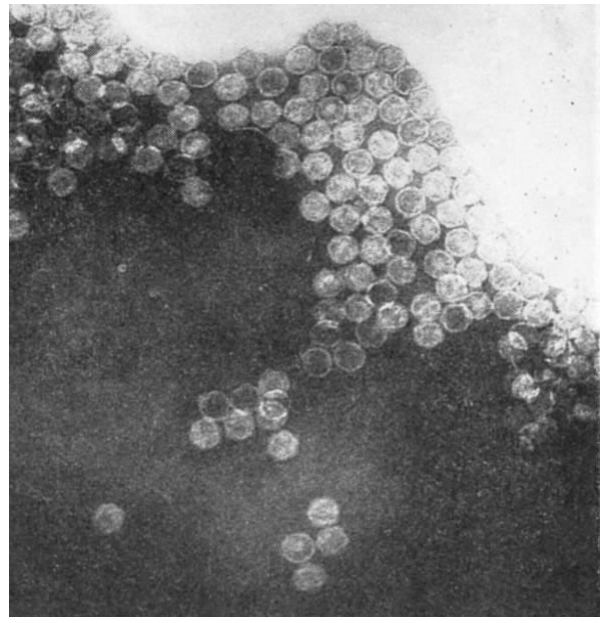

Fig. 2. Electron micrograph of virus-like particle preparation from *P. chrysogenum*, strain BRL 700. ×110,000.

G. T. Banks
K. W. Buck
E. B. Chain
Joan E. Darbyshire
F. Himmelweit

Department of Biochemistry,
Imperial College of Science and Technology,
London SW7.

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[1] Hollings, M., *Nature*, **196**, 962 (1962).
[2] Ellis, L. F., and Kleinschmidt, W. J., *Nature*, **215**, 649 (1967).
[3] Kleinschmidt, W. J., and Ellis, L. F., *CIBA Found. Symp.*, 39 (1968).
[4] Banks, G. T., Buck, K. W., Chain, E. B., Himmelweit, F., Marks, J. E., Tyler, J. M., Hollings, M., Last, F. T., and Stone, O. M., *Nature*, **218**, 542 (1968).
[5] Kleinschmidt, W. J., Ellis, L. F., Van Frank, R. M., and Murphy, E. B., *Nature*, **220**, 167 (1968).
[6] Belgian Patent No. 691,881, Glaxo Laboratories Ltd (1968).

Susceptibility of Scrapie Agent to Ionizing Radiation

The infective agent of scrapie, a progressive encephalopathy endemic in sheep, has recently been the subject of much speculation. Its remarkable stability in a variety of physical and chemical conditions and in particular its resistance to ionizing radiation have given rise to a number of heterodox ideas as to the nature and mode of action of the agent[1–3]. The resistance of the scrapie agent to both ionizing and ultraviolet irradiation has led Alper and her colleagues[4] to state that "it does not depend on a nucleic acid for its ability to replicate". Here we present independent confirmation of Alper's results but also discuss the difficulties in accepting her far-reaching conclusions.

Brains were removed from animals 6 months after intracerebral inoculation of mouse adapted scrapie (sixth passage); these animals showed typical advanced clinical

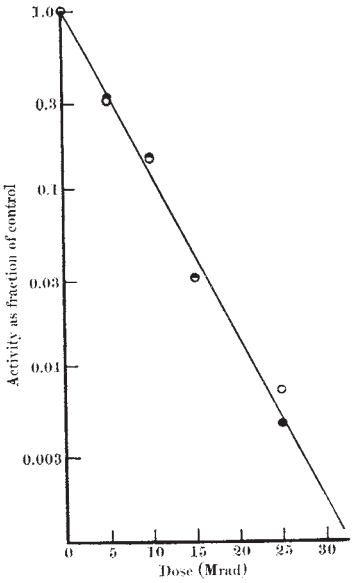

Fig. 1. Inactivation of the scrapie agent. Relation between radiation dose and loss of biological activity. ●, Experiment 1; ○, experiment 2.

scrapie with the characteristic histopathology of the disease. The brains were homogenized in distilled water to give a 10 per cent (w/v) suspension, which was centrifuged twice at 1,800*g* for 10 min and the supernate freeze dried. For irradiation 10 mg amounts of dried scrapie brain were weighed into open thin wall ampoules of 10 mm diameter, tamped down with a flattened glass rod and placed over a 4,000 Ci ^{60}Co source. Oxygen saturation was maintained by passing a stream of dry oxygen over the material through a capillary. Samples were irradiated at a dose rate of 43,000 rad/min for 116–582 min to give total doses ranging from 5–25 Mrad. The powdered samples were then homogenized with saline to give a 10^{-1} dilution and further ten-fold dilutions prepared down to 10^{-7} using fresh disposable pipettes for each dilution. For each series a similar powdered but unirradiated sample was prepared and diluted in the same manner to determine the initial scrapie activity. Biological activity was assayed by injecting 0·05 ml. of each dilution intracerebrally into groups of six mice and observing these animals for 8 months in the first experiment and 12 months in the second, after which the animals were examined histologically

The relation between the residual scrapie activity after irradiation and the total dose is shown in Fig 1. The LD_{50} values were computed by the Spearman–Kärber method (Table 1)[5] and standard errors by the method of Irwin and Cheezeman[6]. There is excellent agreement between the duplicate experiments (Fig. 1), and the mean dose D_o required to reduce the initial activity by a factor of e^{-1} (36·8 per cent) was 4·6 Mrad.

The result of duplicate experiments fully confirms the observations of Alper[7] and gives the same value of the mean inactivating dose (D_o). Irradiation was carried out in an atmosphere of oxygen, as anoxia has been shown to exert a protective effect on materials similarly irradiated in the dry state[8]. A recent study by Alper and Haig[9] to determine the oxygen enhancement ratio for scrapie agent compared with more conventional "viral" agents gave values similar to those for herpes simplex and μ2

Table 1. ELECTRON INACTIVATION OF SCRAPIE AGENT

Electron dose 10^{-7} (Mrad)	Dilution of injected suspension: 10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	LD_{50} ± standard error
	Cases of scrapie/No. of mice in group						
25	—	0/6	0/6	2/6	4/6	6/6	6/6	4·49 ± 0·30
15	0/6	0/6	0/6	4/6	6/6	6/6	6/6	5·16 ± 0·26
10	0/6	0/6	3/6	5/6	6/6	6/6	6/6	5·83 ± 0·33
5	0/6	0/6	4/6	6/6	6/6	6/6	6/6	6·16 ± 0·26
0	1/6	1/6	5/6	6/6	6/6	6/6	6/6	6·67 ± 0·27

Raw data from experiment 1.

bacteriophage and therefore failed to contribute any new information on the chemical nature of scrapie.

Interpretation of these results on the basis of conventional target theory leads to the conclusion that the agent is small, with a molecular weight of about $1·5 \times 10^5$ assuming no anomalies. Even if a deviation of the same order as that described by Alper and Haig[9] for T1 and T3 bacteriophage had occurred the molecular weight would still be exceptionally small for a conventional virus (about $4–5 \times 10^5$). In a nucleic acid virus Alper *et al.* suggest this would entail an improbably small nucleotide code, although it would be adequate for large peptides or small proteins. The rejection of nucleic acids solely on the basis of the length of possible message must, however, be treated with caution. Small circular DNA ($0·8 \times 10^6$ molecular weight) has been demonstrated in *M. lysodeikticus* although its biological function is uncertain.

Moreover, a number of factors may invalidate classical target theory in this case. The purity of materials has a marked effect on irradiation inactivation[11] and scrapie agent may indeed be a special case. So far it has not been detached from subcellular components (membranes)[12] and this firm combination may exert a greater protective effect than the mere presence of contaminating subcellular particles[7]. Finally, irradiation in the dry state may hinder radicals combining on steric grounds[13] and lead to high inactivation dose. Even failure to obtain inactivation of scrapie agent with ultraviolet irradiation does not provide absolute proof that nucleic acids are not involved.

There have been a number of speculations on the nature of the agent; it might be polysaccharide[14], a basic protein[15], a small conventional virus stabilized with a polysaccharide coat[16], and there are also the membrane hypotheses of Gibbons and Hunter[17] and Adams and E. J. F.[18].

If the irradiation technique provides a true measure of stability and size of a biological agent, the choice of structure and nature of the scrapie agent remains between a rather primitive small virus, retaining full informational sequence in a short polynucleotide chain[17], and a non-nucleic replicating material probably inducing its own replication by conformational changes in the cell membrane.

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E. J. FIELD
F. FARMER
E. A. CASPARY
GRETA JOYCE

MRC Demyelinating Diseases Unit,
13 Framlington Place,
Newcastle upon Tyne 2.

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1 *Lancet*, ii, 705 (1967).
2 *Nature*, **214**, 755 (1967).
3 *Nature*, **220**, 15 (1968).
4 Alper, T., Cramp, W. A., Haig, D. A., and Clarke, M. C., *Nature*, **214**, 764 (1967).
5 Finney, D. J., *Statistical Methods in Biological Assay* (Griffin, London, 1952).
6 Irwin, J. O., and Cheezeman, E. A., *J. Hyg. (Cambridge)*, **39**, 574 (1939).
7 Alper, T., Haig, D. A., and Clarke, M. C., *Biochem. Biophys. Res. Commun.*, **22**, 278 (1966).
8 Alexander, P., *Radiat. Res.*, **6**, 653 (1957).
9 Alper, T., and Haig, D. A., *J. Gen. Virol.*, **3**, 157 (1968).
10 Lee, C. S., and Davidson, N., *Biochem. Biophys. Res. Commun.*, **32**, 757 (1968).
11 Braams, R., Hutchinson, F., and Ray, D., *Nature*, **182**, 1506 (1958).
12 Hunter, G. D., and Millson, G. C., *J. Gen. Microbiol.*, **37**, 251 (1964).
13 Alexander, P., Lett, J. T., and Ormerod, M. G., *Biochim. Biophys. Acta*, **51**, 207 (1961).
14 Field, E. J., *Brit. Med. J.*, ii, 564 (1966).
15 Pattison, I. H., and Jones, K. M., *Vet. Rec.*, **80**, 2 (1967).
16 Adams, D. H., and Caspary, E. A., *Brit. Med. J.*, ii, 173 (1967).
17 Gibbons, R. A., and Hunter, G. D., *Nature*, **215**, 1041 (1967).
18 Adams, D. H., and Field, E. J., *Lancet*, ii, 714 (1968).