long incubation period, suggests that the biochemical changes associated with scrapie are likely to be relative subtle and hence difficult to study.

- Avery, R. J., Mills, J. A. & Darcel, C. le Q. (1960). *Canad. J. comp. Med.* **24**, 241.
- Caspary, E. A. & Sewell, F. M. (1968a). *Experientia*, **24**, 793.
- Caspary, E. A. & Sewell, F. M. (1968b). *Biochem. J.* **108**, 37 r.
- Chandler, R. L. (1963). *Res. vet. Sci.* **4**, 276.
- Chandler, R. L. & Smith, K. (1968). *Res. vet. Sci.* **9**, 228.
- Darcel, C. le Q. & Avery, R. J. (1960). *Canad. J. comp. Med.* **24**, 17.
- Darcel, C. le Q., Avery, R. J. & Carson, R. B. (1961). *Nature, Lond.*, **189**, 410.
- Darcel, C. le Q., Merriman, M., Beauregard, M., Avery, R. J. & Kasting, R. (1963). *Canad. J. comp. Med.* **27**, 81.
- Field, E. J., Caspary, E. A. & Windsor, G. D. (1966). *Res. vet. Sci.* **7**, 72.
- Gibbons, R. A. & Hunter, G. D. (1967). *Nature, Lond.*, **215**, 1041.
- Heitzman, R. J. (1968). *Lancet*, i, 427.
- Heitzman, R. J. & Skipworth, S. (1969). *J. Neurochem.* **16**, 121.
- Hunter, G. D. & Millson, G. C. (1966). *J. Neurochem.* **13**, 375.
- Hunter, G. D., Millson, G. C. & Vockins, M. D. (1967). *Biochem. J.* **102**, 43 p.
- Kasting, R. & Darcel, C. le Q. (1963). *Res. vet. Sci.* **4**, 518.
- Kimberlin, R. H. (1968). *J. comp. Path.* **78**, 237.
- Kimberlin, R. H. (1969). *Res. vet. Sci.* (in the Press).
- Kimberlin, R. H. & Anger, H. S. (1969). *J. Neurochem.* **16**, 543.
- Kimberlin, R. H. & Hunter, G. D. (1967). *J. gen. Virol.* **1**, 115.
- Kimberlin, R. H. & Millson, G. C. (1967). *J. comp. Path.* **77**, 359.
- Mackenzie, A., Millson, G. C. & Wilson, A. M. (1968a). *J. comp. Path.* **78**, 43.
- Mackenzie, A. & Wilson, A. M. (1966). *Res. vet. Sci.* **7**, 45.
- Mackenzie, A., Wilson, A. M. & Dennis, P. F. (1968b). *J. comp. Path.* **78**, 489.
- Millson, G. C. (1965). *J. Neurochem.* **12**, 461.
- Millson, G. C. & Hunter, G. D. (1968). *J. Neurochem.* **15**, 447.
- Millson, G. C., West, L. C. & Dew, S. M. (1960). *J. comp. Path.* **70**, 194.
- Mould, D. L., Dawson, A. McL., Slater, J. S. & Zlotnik, I. (1967). *J. comp. Path.* **77**, 393.
- Slater, J. S. (1965a). *Res. vet. Sci.* **6**, 92.
- Slater, J. S. (1965b). *Res. vet. Sci.* **6**, 155.

The Size and Intracellular Location of the Scrapie Agent

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Early work on goat and sheep scrapie (reviewed by Stamp, 1962) had clearly shown that the scrapie agent would pass through filters of average pore diameter 200nm., but no other unequivocal information relating to the size of the scrapie agent was available. Further progress has been made with the mouse-adapted agent: Eklund, Hadlow &

Kennedy (1963) and Haig & Clarke (1965) were able to filter preparations of the agent through filters of average pore diameter 100nm., and Gibbs, Gajdusek & Morris (1965) showed that even filtrates obtained with filters of 43nm. average pore diameter exhibited some biological activity. No activity was found in filtrates obtained with filters of average pore diameter 27nm. Thus the published filtration evidence suggests a size for the scrapie agent in the range 20–40nm., a conclusion consistent with the deductions made by D. L. Mould (unpublished work) from the results of sedimentation studies of extracts containing the scrapie agent. It has, however, been suggested by Pattison & Sansom (1964) that the scrapie agent may in certain circumstances be diffusible on dialysis, although Mould & Dawson (1968), using a somewhat different technique, were unable to confirm their experimental findings.

The situation was radically altered as a result of the observations by Alper, Haig & Clarke (1966), who exposed preparations containing the scrapie agent to the action of ionizing radiation. The large amount of radiation required for inactivation indicated a very small target size of the order of 10^5 daltons, presumably the size of the scrapie agent. The experimental findings have been confirmed in other laboratories, but recently we have re-examined the suggestion that the immediate radiation target is necessarily to be exactly equated with the scrapie agent because it seemed that it was not immediately reconcilable with the membrane hypothesis of scrapie (Gibbons & Hunter, 1967). In fact, five recent experiments carried out by R. H. Kimberlin, G. C. Millson & G. D. Hunter (unpublished work) have shown that ultrasonic treatment of certain preparations of the scrapie agent produces finely dispersed suspensions that can readily be passed through small filters with reasonable recoveries of protein nitrogen. In all these experiments, most of the scrapie activity was recovered in filtrates obtained with filters of average pore diameter 50nm., but none at all in extracts that had passed through filters of average pore diameter 20–35nm. or smaller. Electron-microscopic examination showed that finely divided particles can pass through filters of average pore diameter 20–35nm. and 50nm., and thus suggested that there was a lower limit to the size of membrane fragment with which scrapie activity was associated. This conclusion has been strengthened by employing exclusion chromatography on agarose columns, where in three separate experiments the scrapie agent has been essentially excluded by Bio-Gel A-50m., indicating a molecular weight for the scrapie agent greater than 5×10^7 daltons. The apparent major discrepancy between this conclusion and that to be drawn from the radiation data may not be a

real one; the latter can be interpreted otherwise (Hunter, Kimberlin & Gibbons, 1968). The radiation target may not be the whole scrapie molecular complex, but an individual molecule that plays a key role in maintaining the structural integrity of the membrane complex.

Turning now to the question of the intracellular location of the scrapie agent, the early studies by Hunter, Millson & Meek (1964) and Mould, Smith & Dawson (1965) showed that the bulk of the scrapie agent in brain was associated with particulate subcellular elements, and even the very small amount of activity associated with the cell sap could be almost completely removed by prolonged ultracentrifugation (D. L. Mould, unpublished work). The early work further showed that the highest titres were to be found in the crude mitochondrial fractions, although microsomal fractions also possessed high specific activities of scrapie agent. Most of the scrapie activity can be removed from nuclear preparations, however, by repeated cycles of washing and sedimentation. More refined cellular fractionation studies (Hunter & Millson, 1966) revealed that the scrapie activity could not readily be associated with any one component of the whole mitochondrial fraction, but it was at any rate clear that there was no specific association between the scrapie agent and lysosomal particles.

Nevertheless these early experiments did suggest that rather high scrapie activities were present in the lighter subfractions obtainable from crude brain mitochondrial preparations. Those indications have recently been pursued further (G. C. Millson, G. D. Hunter & R. H. Kimberlin, unpublished work), and it has been shown that relatively small amounts of scrapie agent are present in well-washed mitochondria or mitochondrial membranes. The highest titres are associated with lighter fractions containing myelin, plasma membrane, synaptosomes and endoplasmic reticulum. Experiments with well-washed and purified myelin preparations have shown that the scrapie activity can be greatly decreased here also, so that the major intracellular locus for the scrapie agent residues in one or all of the plasma membrane, endoplasmic reticulum and synaptosomes. Unfortunately, the degree of separation that has been achieved among this group derived from brain corresponds fairly closely to the degree of accuracy of the biological titration assay for the scrapie agent. However, we have attempted to distinguish between these possibilities and have made use of a number of presumed enzymic markers: 5'-nucleotidase and $\text{Na}^+ + \text{K}^+$ -activated adenosine triphosphatase for plasma membrane, uridine diphosphatase and glucose 6-phosphatase for endoplasmic reticulum, acetylcholinesterase for synaptosomes and 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride reductase for

mitochondria. A limited success has been achieved in that fractions rich in synaptosomes usually possess rather less scrapie activity than fractions apparently rich in endoplasmic reticulum and plasma membrane. The titres of well-washed fractions containing mainly endoplasmic-reticulum membranes are not highly reproducible, but also tend to be somewhat decreased; thus a very tentative conclusion has been arrived at: in brain, the bulk of the scrapie agent may be associated with cellular plasma membrane.

Although the amounts of scrapie activity are about 50-fold lower in spleen than in clinically affected brain (Hunter & Millson, 1964), cellular fractionation of spleen homogenates is somewhat simpler than similar manipulations carried out on brain. We have therefore attempted to obtain further information about the intracellular location of the scrapie agent from studies carried out on spleen homogenates. A simple differential separation showed that, as in brain, high scrapie activity was present in all crude particulate fractions. Initial attempts to apply the liver method of Neville (1960) to the preparation of plasma membrane from crude spleen nuclei were not successful. It was, however, possible to obtain a subfraction rich in the enzyme 5'-nucleotidase from crude spleen mitochondria. Titration studies have shown that this fraction does indeed possess a high specific activity of scrapie agent, but the activity in other subfractions rich in endoplasmic reticulum is almost as high. This experimental approach is being pursued, but it is not yet possible to distinguish between plasma membrane and endoplasmic reticulum as major locations of scrapie activity in spleen.

- Alper, T., Haig, D. A. & Clarke, M. C. (1966). *Biochem. biophys. Res. Commun.* **22**, 278.
 Eklund, C. M., Hadlow, W. J. & Kennedy, R. C. (1963). *Proc. Soc. exp. Biol., N.Y.*, **112**, 974.
 Gibbons, R. A. & Hunter, G. D. (1967). *Nature, Lond.*, **215**, 1041.
 Gibbs, C. J., Gajdusek, D. C. & Morris, J. A. (1965). *Monogr. nat. Inst. nerv. Dis. & Blindn.* no. 2, p. 195.
 Haig, D. A. & Clarke, M. C. (1965). *Monogr. nat. Inst. nerv. Dis. & Blindn.* no. 2, p. 215.
 Hunter, G. D., Kimberlin, R. H. & Gibbons, R. A. (1968). *J. theoret. Biol.* **20**, 355.
 Hunter, G. D. & Millson, G. C. (1964). *Res. vet. Sci.* **5**, 149.
 Hunter, G. D. & Millson, G. C. (1966). *J. Neurochem.* **13**, 375.
 Hunter, G. D., Millson, G. C. & Meek, G. (1964). *J. gen. Microbiol.* **34**, 319.
 Mould, D. L. & Dawson, A. M. (1968). *J. comp. Path.* **78**, 115.
 Mould, D. L., Smith, W. & Dawson, A. M. (1965). *J. gen. Microbiol.* **40**, 71.
 Neville, D. M. (1960). *J. biophys. biochem. Cytol.* **8**, 413.
 Pattison, I. H. & Sansom, B. F. (1964). *Res. vet. Sci.* **5**, 340.
 Stamp, J. T. (1962). *Vet. Rec.* **74**, 357.