mRNA has multiple components which differ with respect to half-life; the data appear to be adequately explained by a single value of $T_{1/2}$.

The 10 h half-life of poly A-containing mRNA in L-cells estimated by the kinetics of approach to steady state labelling does not agree with the 3–4 h half-life of mRNA estimated from the rate of decay of polyribosomes in a high dose of actinomycin D[6,7]. Three possible explanations for this discrepancy suggest themselves: first, poly A-containing mRNA is not representative of total mRNA; second, actinomycin D converts growing cells to non-growing cells which have an intrinsically higher rate of mRNA turnover; third, actinomycin D directly causes abnormal decay of polyribosomes. The first possibility can be eliminated, as it is known that nearly all (>90%) of mRNA in L-cells contains poly A[5]. It is not yet possible to distinguish between the other two possibilities.

The only mammalian mRNA known to lack poly A is histone mRNA[5,14] which seems to turn over more rapidly than other types of mRNA[7,15,16]. The absence of poly A from histone mRNA and its presence in other, longer-lived types of mRNA suggest that poly A may have a role in determining mRNA stability, and, therefore, in controlling the translation of mRNA.

Equation (1) was derived as follows: in an exponentially-growing population in which the amount per cell of a component C is constant, the amount of C at time t is given by $C = Co\ e^{(\ln 2/T_D)t}$ where Co is the amount of C at $t=0$, and T_D is the doubling time of the population. If C turns over stochastically, then in a labelling experiment the unlabelled molecules initially present will disappear at a rate determined by their half-life. Therefore, the amount of unlabelled material Cu present at time t is $Cu = Co\ e^{-(\ln 2/T_{1/2})t}$ where Co is the amount present at $t=0$ and $T_{1/2}$ is the half-life. It is assumed that all molecules synthesized at $t>0$ have the same average specific activity. The fraction of C unlabelled at time t is $Cu/C = e^{-\ln 2(1/T_D + 1/T_{1/2})t}$. The fraction of C labelled at t is $1-Cu/C$. If the radioactive precursor is drawn from a pool of constant specific activity, then $1 - Cu/C = A/A_\infty = 1 - e^{-\ln 2(1/T_D + 1/T_{1/2})t}$.

This work was supported by grants from the National Science Foundation and the US Public Health Service, and an appropriation from the Commonwealth of Pennsylvania to Dr R. P. Perry. I thank Mr Gerald Sterling for technical assistance.

JAY R. GREENBERG

Institute for Cancer Research, Philadelphia, Pennsylvania 19111

Received May 10, 1972.

1 Kates, J., *Cold Spring Harbor Symp. Quant. Biol.*, **35**, 743 (1970).
2 Lee, S. Y., Mendecki, J., and Brawerman, G., *Proc. US Nat. Acad. Sci.*, **68**, 1331 (1971).
3 Edmonds, M., Vaughan, jun., M. H., and Nakazoto, H., *Proc. US Nat. Acad. Sci.*, **68**, 1336 (1971).
4 Darnell, J. E., Wall, R., and Tushinski, R. J., *Proc. US Nat. Acad. Sci.*, **68**, 1321 (1971).
5 Perry, R. P., Greenberg, J. R., Kelley, D. E., LaTorre, J., and Schochetman, G., *Gene Expression and Its Regulation* (Plenum Press).
6 Penman, S., Scherrer, K., Becker, Y., and Darnell, J. E., *Proc. US Nat. Acad. Sci.*, **49**, 654 (1963).
7 Craig, N., Perry, R. P., and Kelley, D. E., *Biochim. Biophys. Acta*, **246**, 493 (1971).
8 Cheevers, W. P., and Sheinin, R., *Biochim. Biophys. Acta*, **204**, 449 (1970).
9 Singer, R. H., and Penman, S., *Nature New Biology* (in the press).
10 Rake, A. V., and Graham, A. F., *J. Cell. Comp. Physiol.*, **59**, 139 (1962).
11 Perry, R. P., and Kelley, D. E., *J. Mol. Biol.*, **35**, 37 (1968).
12 Weber, M. J., *Nature New Biology*, **235**, 58 (1972).
13 Perry, R. P., LaTorre, J., Kelley, D. E., and Greenberg, J. R., *Biochim. Biophys. Acta*, **262**, 220 (1972).
14 Adesnik, M., and Darnell, J. E., *J. Mol. Biol.* (in the press).
15 Borun, T. W., Scharff, M. D., and Robbins, E., *Proc. US Nat. Acad. Sci.*, **58**, 1977 (1967).
16 Gallwitz, D., and Mueller, G. C., *J. Biol. Chem.*, **244**, 5947 (1969).

Rapid Diagnosis of Scrapie in the Mouse

RESEARCH into scrapie, an enigma of veterinary medicine which may embody principles of considerable interest for human disease, commonly involves demonstration of agent activity or its titration. A great step forward was made by Chandler[1] when he showed the disease could be produced in mice, and biological titration in these animals is currently widely employed. This takes 6–8 months. From a recent study of multiple sclerosis, with which scrapie has been linked[2], we were led into an immunological study of scrapie itself. We found that brain or spleen from a scrapie mouse when injected into a guinea-pig (adult Hartley) led to the appearance of blood lymphocytes which were more highly sensitized to scrapie brain or spleen than to normal brain or spleen.

In early experiments, guinea-pigs were inoculated intracutaneously with 0.1 ml. of 10^{-1} suspension of scrapie brain or scrapie spleen in sterile saline. Control animals were similarly injected with brain or spleen from normal mice which had been injected some weeks previously with normal brain suspension. At intervals after 5 days, 2–3 ml. of blood was removed by cardiac puncture and lymphocyte sensitization to the scrapie and normal suspensions estimated by the sensitive and highly specific macrophage electrophoretic migration (MEM) method[3]. In principle the method depends on the release by sensitized lymphocyte of a protein which slows migration of normal guinea-pig macrophages in an electric field (macrophage slowing factor (MSF)—which may be identical with macrophage inhibitory factor (MIF)). Our measurements have been carried out in a Zeiss cytopherometer and full experimental details have been given[4].

Guinea-pig blood lymphocytes were prepared by the method of Coulson and Chalmers[5], as modified by Hughes and Caspary[6], and normal guinea-pig macrophages by washing out the peritoneal cavity with heparinized Hanks solution 6–8 days after injection of sterile liquid paraffin. In order to obviate a two-way mixed lymphocyte reaction (at least for the duration of the test) the normal exudate was subjected to 100 rad γ-irradiation[3].

10^{-1} scrapie brain, scrapie spleen, normal brain and normal spleen suspensions were used as test antigen and for later testing lymphocyte sensitization. 0.5×10^6 guinea-pig blood lymphocytes were incubated for 90 min at 20° C with 0.1 ml. of a 10^{-1} suspension of scrapie mouse brain or spleen (cleared by spinning at 1,800g for 10 min) in the presence of 10^7 irradiated normal macrophages. Control tubes comprised lymphocytes and macrophages without antigen. The migration time of macrophages in each specimen was measured by timing ten cells (readily identified by their size and paraffin droplet content) in each direction of the potential difference so that a mean (with SD) from twenty readings could be calculated. A full protocol from one specimen is given by Caspary and Field[4]. All measurements were made "blind" and results unscrambled later. If t_c = migration time of control macrophage in the absence of antigen and t_e = migration time when antigen is present; then in general $t_e > t_c$ and $t_e - t_c/t_c \times 100$ is a measure of lymphocyte sensitization to the antigen.

The scrapie brain and spleen with which the guinea-pigs were inoculated were titred out by inoculation into groups of 6 mice at dilutions of 10^{-1} through 10^{-7}. The animals were observed for eight months and all clinical scrapie diagnoses were checked histologically.

Guinea-pigs were immunized with either scrapie or normal mouse brain and spleen, 0.1 ml. of a 10^{-1} suspension being inoculated intracutaneously in the dorsum of the right foot (Table 1*a*, *b*). The guinea-pig lymphocyte sensitization to normal brain and normal spleen is always greater when the animal has been injected with scrapie material than with normal (as pointed out by Gardiner[7] with respect to circulating antibody in rabbit experiments). Moreover, the scrapie-normal difference (when scrapie brain or spleen is used as test antigen

for the lymphocytes) is greater in the guinea-pig immunized with scrapie brain than with normal brain. The difference appears to be greatest between five and thirteen days. Table 1*b* shows that the same is true when guinea-pigs immunized with scrapie or normal spleen are tested for cellular sensitization to scrapie and normal brain and spleen. The results with spleen are particularly interesting since this organ whilst rich in agent ($LD_{50}=10^{-4.7}$) shows no morphological change[8] so that the test antigen being used might well be the scrapie agent itself (or scrapie-altered but morphologically normal membrane).

Table 1 Demonstration of Scrapie Activity in Mouse Brain and Spleen

	a, Mouse scrapie brain injected guinea-pig 10^{-1}				*b*, Mouse scrapie spleen injected guinea-pig 10^{-1}			
	5 days	13 days	22 days	34 days	7 days	11 days	25 days	35 days
EF	12.0	15.3	11.1	4.7	6.6	9.2	11.3	2.2
Normal brain	11.3	12.5	10.3	3.4	3.4	8.9	7.6	0.8
Scrapie brain	16.5	17.9	14.3	5.4	10.1	14.2	12.3	2.6
Brain difference	5.2	5.4	4.0	2.0	6.7	5.3	4.7	1.8
Normal spleen	5.2	6.1	6.1	1.7	11.5	12.2	10.8	1.8
Scrapie spleen	9.6	11.1	10.4	3.5	17.4	17.9	15.3	3.9
Spleen difference	4.4	5.0	4.3	1.8	5.9	5.7	4.7	2.1
	Mouse normal brain injected guinea-pig				Mouse normal spleen injected guinea-pig			
	5 days	13 days	22 days	34 days	7 days	11 days	25 days	35 days
EF	12.0	13.5	9.6	3.0	6.2	8.7	8.2	2.4
Normal brain	9.9	13.7	9.4	2.5	4.0	6.2	6.9	1.5
Scrapie brain	12.1	15.5	10.3	3.2	5.7	8.2	8.4	2.4
Brain difference	2.2	1.8	0.9	1.3	1.7	2.0	1.5	0.9
Normal spleen	4.0	5.9	6.2	1.2	13.9	13.9	11.4	2.7
Scrapie spleen	6.2	7.7	7.6	2.2	14.6	15.7	13.3	3.5
Spleen difference	2.2	1.8	1.4	1.0	0.7	1.8	1.9	0.8

Adult Hartley guinea-pigs inoculated intracutaneously in the dorsum of the right foot with 0.1 ml. 10^{-1} crapie brain or spleen (titre 10^{-6}; $10^{-4.7}$ respectively) and lymphocyte sensitization to scrapie and normal brain measured at intervals by the macrophage electrophoresis method of Field and Caspary[3]. Results expressed as percentage slowing (*loc. cit.*).

In the case of inoculation with scrapie brain, however, the difference may perhaps be attributed to morphological changes (especially astrocyte increase) in the inoculated material. Having established the quantitative difference in response to scrapie as opposed to normal tissue we used this method to titre out scrapie activity.

Guinea-pigs were injected with scrapie brain at dilutions of 10^{-1} to 10^{-6} and the lymphocytes examined for sensitization to scrapie brain or spleen and normal brain or spleen at six or seven days and at seventeen or eighteen days. For comparison a similar study was made in guinea-pigs injected with normal brain (Tables 2 and 3). In the scrapie brain sensitized guinea-pig the difference in lymphocyte sensitization to scrapie as compared with normal brain is 2.5% ($P<0.01$) even at 10^{-6} original inoculum level when the guinea-pig is tested at six days but falls to 2.0% ($P=0.1-0.05$) by sixteen days. When sensitization to scrapie spleen and normal spleen in these animals is compared, the difference is significant only in guinea-pigs which have received 10^{-4} scrapie brain.

Animals injected with normal brain showed no significant difference when their lymphocytes were tested with scrapie as opposed to normal brain or spleen (though in general the values with the former were higher).

Guinea-pigs were sensitized by injecting scrapie spleen at 10^{-1} to 10^{-6} and their lymphocytes tested for sensitization to scrapie brain and spleen (Table 3). A significant difference between sensitization to scrapie and normal spleen still exists at 10^{-5} ($P<0.01$) with spleen as antigen but barely with brain (2.1% difference; $P=0.05$), showing apparent scrapie antigenicity in the 10^{-5} dilution.

The titre of the scrapie brain inoculated into the test guinea-pigs was calculated to be $LD_{50}=10^{-5.6}$. We noted that

Table 2 Titration of Scrapie Activity from Mouse Brain

Antigen	Lymphocytes tested at 6 days					Lymphocytes tested at 16 days		
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-6}	10^{-2}	10^{-4}	10^{-6}
	% macrophage slowing							
Scrapie brain								
Normal mouse brain	11.3	11.0	8.1	5.7	3.7	11.1	5.9	3.9
Scrapie brain	16.5	14.1	11.7	9.6	5.2	14.7	9.9	5.9
Brain difference	5.2	3.1	3.6	3.9	2.5	3.6	4.0	2.0
Normal mouse spleen	5.2	6.1	4.7	3.9	1.7	5.9	4.7	2.9
Scrapie mouse spleen	9.6	10.8	8.1	7.4	3.2	10.1	8.1	4.4
Spleen difference	4.4	4.7	3.4	3.5	1.5	4.2	3.4	1.5
Normal brain								
Normal mouse brain		8.9		7.1	5.9	9.6	5.5	4.4
Scrapie mouse brain		10.3		7.9	6.7	10.1	6.5	5.2
Brain difference		1.4		0.6	0.8	0.5	1.0	0.8
Normal mouse spleen		5.4		2.5	3.0	6.1	4.4	3.5
Scrapie mouse spleen		6.2		3.8	4.2	7.2	5.7	4.5
Spleen difference		0.8		1.3	1.2	1.1	1.3	1.0

Guinea-pigs inoculated with scrapie mouse brain with a titre of $10^{-5.6}$. 0.1 ml. inoculated at different dilutions.

Table 3 Titration of Scrapie Activity from Mouse Spleen

Antigen	Lymphocytes tested at 7 days						Lymphocytes tested at 17 days		
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-2}	10^{-4}	10^{-6}
	% macrophage slowing								
Scrapie spleen									
Normal mouse brain	3.4	4.5	2.7	1.0	1.7	0	4.7	1.5	0.7
Scrapie brain	10.1	10.3	7.2	4.4	3.8	1.2	10.1	4.9	1.5
Brain difference	6.7	5.8	4.5	3.4	2.1	1.2	5.4	3.4	0.8
Normal mouse spleen	11.5	12.6	10.4	10.6	8.1	4.5	11.1	10.0	4.4
Scrapie mouse spleen	17.4	17.0	15.3	14.4	10.8	6.3	15.0	13.8	6.2
Spleen difference	5.9	4.4	4.9	3.8	2.7	1.8	3.9	3.8	1.8
Normal spleen									
Normal mouse brain		4.5		1.5		1.0	6.4	3.0	1.8
Scrapie mouse brain		5.9		2.9		1.2	7.6	4.4	2.9
Brain difference		1.4		1.4		0.2	1.2	1.4	1.1
Normal mouse spleen		11.1		9.1		4.7	10.5	7.9	4.7
Scrapie mouse spleen		12.4		10.2		6.4	11.5	8.9	5.6
Spleen difference		1.3		1.1		1.7	0.9	1.0	0.9

Guinea-pigs inoculated with spleen suspension with a titre of 10^6. 0.1 ml. inoculated at different dilutions.

1/6 mice developed scrapie at 10^{-6} and 1/6 at 10^{-7} and the immunological test suggests that some activity is still present at 10^{-6}. The titre of spleen used for immunizing the guinea-pigs was $LD_{50}=10^{-5}$. The results of *in vivo* titration therefore agree with the *in vitro* immunological assay, and the significance of these results is two-fold. (1) Attempts to show the existence of circulating antibody and/or specific antigen in scrapie disease have not been successful[7,8]. Gardiner did show by inoculation of rabbits with scrapie and non-scrapie spleen material that the latter had increased antigenicity *in vivo*. The present work extends these very important findings in that it suggests that this is true also of lymphocyte sensitization and that the reactivity is greater against scrapie material (both brain and spleen) than against normal material. This suggests that some specific antigen—perhaps the agent itself, or specifically agent-altered material since the spleen is so potent as a testing tissue—may be active. The macrophage electrophoretic migration (MEM) method is exquisitely responsive to minor changes in antigenic determinant structure and appears to be able to distinguish between normal and scrapie tissue. It is, of course, not possible to decide whether the difference resides in the presence of a specific scrapie agent or whether we are dealing

with a structural (antigenic) change induced by the agent. However, if scrapie infected brain is split into a neuronal and glial compartment by the method of Giorgi (unpublished) then, contrary to expectations based on the precocious glial hypertrophy, higher scrapie titre is associated with the neuronal rather than the glial fraction and it is in the former that particles with the size and structural characters postulated for scrapie agent have been found[9]. (2) Lymphocyte sensitization test enables the presence of scrapie to be established and titration carried out within 8 days, an important saving in time during the study of this fascinating condition. Further experiments are in progress involving the use of Freund's adjuvant to boost the responses. A full account of these experiments will be published elsewhere.

E. J. FIELD
B. K. SHENTON

Medical Research Council,
Demyelinating Diseases Unit,
Newcastle General Hospital,
Westgate Road,
Newcastle upon Tyne NE4 6BE

Received July 25, 1972.

1 Chandler, R. L., *Lancet*, ii, 1378 (1961).
2 Field, E. J., *Int. Rev. Exp. Path.*, **8**, 129 (1969).
3 Field, E. J., and Caspary, E. A., *Lancet*, ii, 1337 (1970).
4 Caspary, E. A., and Field, E. J., *Brit. Med. J.*, **2**, 613 (1971).
5 Coulson, A. S., and Chalmers, D. G., *Immunology*, **12**, 417 (1967).
6 Hughes, D., and Caspary, E. A., *Int. Arch. Allergy*, **37**, 506 (1970).
7 Gardiner, A. C., *Res. Vet. Sci.*, **7**, 190 (1966).
8 Chandler, R. L., *Vet. Rec.*, **71**, 58 (1959).
9 Narang, H. K., Shenton, B., Giorgi, P. P., and Field, E. J., *Nature*, **240**, 106 (1972).

Scrapie Agent and Neurones

THE nature of the scrapie agent[1,2] is of considerable interest especially as to whether it is a virus in the strict sense, or some replicating agent devoid of nucleic acid[3,4], or indeed whether scrapie disease represents a transmissible progressive biochemical transformation of cellular membranes[5,6]. Thorough electron microscope studies have been carried out in the disease and, although a number of particles have (not unexpectedly) been described[7,8], their role in scrapie is unconfirmed, especially as none has the special features associated with virions or has the size or other characters currently attributed to the agent.

During careful search in the brain of the scrapie rat granular inclusion bodies were seen in the cytoplasm of apparently normal neurones and their axons in the sites studied; the cortex, Ammon's horn and thalamus. At high magnification these uninteresting neuronal inclusions comprised very large numbers of minute elongated particles about 60 nm long and 20 nm wide, with a dense linear core about 4 nm wide (Figs. 1 and 2). When these particles had once been recognized they were also quickly found in blocks of scrapie rat brain which had been examined years ago.

A thorough and realistic search of comparable normal rat brain has not revealed similar particles though the vagaries of electron microscope sampling must constantly be borne in mind. The size of the particles is about that deduced from modern infectivity filtration experiments[9]. Moreover the size of central rod accords well with that of the target nucleic acid estimate made by Alper *et al.*[3,4] and by Latarjet *et al.*[10].

If the particles described are indeed scrapie agent, it is surprising to find them in neurones rather than glial cells since there is widespread agreement that the first element in the nervous system to react in the scrapie process is the astroglia[11–13] and the working assumption has been made that colonization of astroglial cells by the agent is responsible for their precocious hypertrophy. Indeed we have tended to concentrate search on glial cells, even though unusual neurological reactions may be detected early in the incubation period[14]. Simultaneously with the electron microscope findings here reported, unexpected collateral evidence has emerged that the agent may in fact proliferate in neurones rather than glial cells.

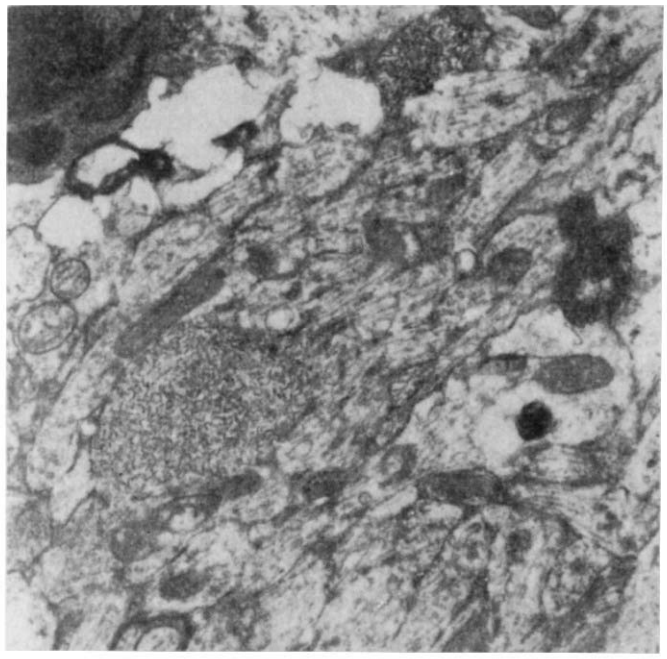

Fig. 1 Scrapie rat thalamus. Axon close to a nerve cell body showing a colony-like accumulation of small elongated particles not bounded by membrane. The neurotubular and other constituents of the axon are normal. ×20,625.

Table 1 Immunological Titration of Scrapie Activity

	Neuronal compartment				Glial compartment			
Antigen	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Normal mouse brain	9.1	9.9	8.5	6.7	8.6	7.7	7.1	6.9
Scrapie mouse brain	14.6	13.9	11.3	9.2	11.3	8.1	8.4	8.1
Brain difference	5.5	4.0	2.8	2.5*	2.7	0.4	1.3	1.2
Normal mouse spleen	6.0	5.9	5.7	4.9	6.2	6.2	5.6	4.4
Scrapie mouse spleen	9.7	9.4	9.1	8.2	9.1	8.2	6.9	6.5
Spleen difference	3.7	3.5	3.4	3.3	2.9	2.0	1.3	2.1

* With the degree of scatter in our readings a difference of 2.5% corresponds to $P < 0.01$.

Guinea-pigs inoculated intracutaneously in right foot with 10^{-1} to 10^{-4} preparations of neurones and glial cells from mouse scrapie brain (0.1 ml. in each case; equivalent to 300 μg to 0.3 μg protein). Sensitization of the guinea-pig lymphocytes with respect to 10^{-1} scrapie brain and spleen and also to normal brain and spleen is measured by the macrophage electrophoretic slowing method and expressed as percentage slowing of macrophages[17].

Preparations of neuronal and glial perikarya have been obtained from scrapie mouse brain using a modification (Giorgi, unpublished work) of the method of Sellinger *et al.*[15]. To these preparations we have applied the macrophage electrophoretic method of titration of scrapie agent[16] in a study of the apportionment of scrapie activity in infected brain as between neuronal and glial compartments. The method briefly consists in immunizing guinea-pigs with successive dilutions of the material under test and measuring the degree of sensitization developed by the lymphocytes to scrapie brain (or spleen) and to normal brain (or spleen) as antigen. To our surprise there was clearly more scrapie activity associated with the neurone enriched fraction than with the glial compartment (Table 1). It might well be that the scrapie agent (or process) affects, or at least reaches greater development within, neurones