[6] Follett, E. A. C., and Goldman, R. D., *Exp. Cell Res.*, **59**, 124 (1970).
[7] Wartiovaara, J., Lehtonen, E., Nordling, S., and Saxén, L., *Nature*, **238**, 407 (1972).
[8] Abercrombie, M., Heaysman, J. E., and Pegrum, S. M., *Exp. Cell Res.*, **67**, 359 (1971).
[9] Huxley, H., *Nature*, **243**, 445 (1973).
[10] Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Ludeña, M. A., Taylor, E. L., Wrenn, J. T., and Yamada, K. M., *Science*, **171**, 135 (1971).
[11] Jahn, T. L., and Bovee, E. C., *Physiol. Rev.*, **49**, 793 (1969).
[12] Bettex-Galland, M., and Lüscher, E. F., *Adv. Protein Chem.*, **20**, 1 (Academic, NY and London, 1965).
[13] Huxley, H., *Biochim. Biophys. Acta*, **12**, 387 (1953).
[14] Small, J. V., and Squire, F. M., *J. Mol. Biol.*, **67**, 117 (1972).
[15] Harris, A., *Locomotion of Tissue Cells*, CIBA Symposium (in the press).
[16] Berl, S., Puszkin, S., and Nicklas, W. J., *Science*, **179**, 441 (1973).
[17] Bunge, M., *Anat. Rec.*, **175**, 280 (1973).
[18] Cornell, R., *Exp. Cell Res.*, **57**, 86 (1969).
[19] Graham, R. C., and Karnovsky, M. J., *J. Histochem. Cytochem.*, **14**, 291 (1966).
[20] Abercrombie, M., Heaysman, J. E. M., and Pegrum, S. M., *Exp. Cell Res.*, **62**, 389 (1970).
[21] Bray, D., *Proc. US Nat. Acad. Sci.*, **65**, 905 (1970).

Rapid Immunological Method for Diagnosis of Natural Scrapie in Sheep

SCRAPIE may be diagnosed rapidly in the experimental mouse by inoculating some tissue (brain, spleen, muscle, for example) into a guinea-pig and measuring the degree to which the guinea-pig lymphocytes become sensitized to scrapie mouse brain (or spleen) as compared with normal mouse brain (or spleen)[1]. Lymphocyte sensitization is measured by the macrophage electrophoresis migration (MEM) test[2–4]. This in principle depends on the observation that when lymphocytes are brought into contact with specific antigen they elaborate some material (involving protein synthesis[5]) which causes normal guinea-pig peritoneal macrophages to travel more slowly in an electric field. Thus normal guinea-pig macrophages are used as an indicator system for lymphocyte-antigen interaction. The percentage slowings induced by such interactions are calculated and a high percentage indicates lymphocyte sensitization to the test antigen.

As the guinea-pig injected experimentally with scrapie

Table 1 Macrophage Electrophoresis Migration*

	Normal	Scrapie	OND†	Clinical details
Newcastle (Longbenton)—Veterinary Investigation Centre (January 11, 1973)				
1 Normal brain	2.8			3-yr-old normal
Scrapie brain	3.4			
Normal spleen	2.4			
Scrapie spleen	3.2			
S-ND‡ (brain)	0.6			
S-ND (spleen)	0.8			
2 Normal brain		15.6		3-yr-old natural scrapie
Scrapie brain		23.3		
Normal spleen		4.5		
Scrapie spleen		12.4		
S-ND (brain)		7.7		
S-ND (spleen)		7.9		
Moredun Institute, Edinburgh (January 30, 1973)				
3 Normal brain	2.9			Born April 16, 1970; 34 months not affected by scrapie
Scrapie brain	3.6			
Normal spleen	2.9			
Scrapie spleen	3.7			
S-ND (brain)	0.7			
S-ND (spleen)	0.8			
4 Normal brain	2.6			Born April 7, 1970; 34 months not affected by scrapie
Scrapie brain	3.3			
Normal spleen	2.6			
Scrapie spleen	3.3			
EF	1.5			
S-ND (brain)	0.9			
S-ND (spleen)	0.7			
5 Normal brain	2.7			Born April 3, 1971; 22 months not affected by scrapie
Scrapie brain	3.4			
Normal spleen	2.5			
Scrapie spleen	3.3			
S-ND (brain)	0.7			
S-ND (spleen)	0.8			
6 Normal brain		14.2		Born April 22, 1971; 22 months; natural clinical scrapie October 1972
Scrapie brain		23.4		
Normal spleen		4.2		
Scrapie spleen		12.6		
EF		17.6		
S-ND (brain)		9.2		
S-ND (spleen)		8.4		
7 Normal brain		15.3		Born April 10, 1971, 22 months; natural clinical scrapie; December 1972
Scrapie brain		23.1		
Normal spleen		4.5		
Scrapie spleen		12.8		
S-ND (brain)		7.8		
S-ND (spleen)		8.3		
8 Normal brain		14.1		Born April 15, 1971; 22 months; natural clinical scrapie, January 1972
Scrapie brain		23.2		
Normal spleen		4.5		
Scrapie spleen		13.0		
S-ND (brain)		9.1		
S-ND (spleen)		8.5		
Moredun February 7, 1973				
9 Normal brain	2.6			Old ram; born 1966; injected scrapie 1967. Nil
Scrapie brain	5.3			
Normal spleen	2.1			
Scrapie spleen	4.7			
S-ND (brain)	2.7			
S-ND (spleen)	2.6			
10 Normal brain	3.4			Senile ram; born 1964; injected scrapie 1964. Nil
Scrapie brain	4.9			
Normal spleen	2.9			
Scrapie spleen	4.8			
EF	4.6? ageing			
S-ND (brain)	1.5			
S-ND (spleen)	1.9			
11 Normal brain		14.9		Born 1969; injected scrapie November 10, 1971; now clinical scrapie
Scrapie brain		22.9		
Normal spleen		3.1		
Scrapie spleen		12.2		
EF		18.7		
S-ND (brain)		8.0		
S-ND (spleen)		9.1		
12 Normal brain			16.3	Natural border disease; born 1970
Scrapie brain			17.7	
Normal spleen			2.8	
Scrapie spleen			4.7	
EF			16.3	
S-ND (brain)			1.4	
S-ND (spleen)			1.9	
13 Normal brain			16.2	Experimental border disease; born 1970
Scrapie brain			17.4	
Normal spleen			2.6	
Scrapie spleen			4.4	
S-ND (brain)			1.2	
S-ND (spleen)			1.8	
14 Normal brain			9.5	Cerebrocortical necrosis recovered by January 20, 1973; born 1968
Scrapie brain			12.3	
Normal spleen			2.0	
Scrapie spleen			5.3	
EF			11.0	
S-ND (brain)			2.7	
S-ND (spleen)			3.3	

* Figures are percentage macrophage slowing in MEM test[2,4], calculated as $(t_e - t_c/t_c) \times 100$ where t_e = migration time of macrophages when antigen present; t_c = migration time of macrophages when no antigen present (control).
† OND, other neurological diseases.
‡ S-ND, scrapie-normal difference.

material shows increased reactivity of its lymphocytes to scrapie as opposed to normal tissues, the same might be true of a sheep affected with natural scrapie. Experiments were set up with lymphocytes prepared from the blood of normal sheep, scrapie sheep with both the natural and experimental forms and sheep with such other neurological diseases (OND) as were available for study. These comprised two cases of border disease and one of cerebro-cortical necrosis. In addition some old sheep injected with scrapie material but which had not shown any clinical illness were also studied. Results are given in Table 1. In normal animals the difference in lymphocyte response to scrapie compared with normal tissue (scrapie-normal difference: S-ND) does not exceed 2.7%. When either brain or splenic tissue is used as antigen, S-ND is much higher in both natural and experimental scrapie (animals numbers 1, 6, 7, 8, 11). In those with border disease, either natural or experimental, and in the animal with recovered cerebro-cortical necrosis it was within the normal range. These preliminary results suggest that determination of the S-ND in response to mouse scrapie and normal brain or splenic tissue may be used to make a diagnosis of scrapie in the sheep.

A study of human neurological disease including multiple sclerosis, neurosyphilis, kuru, Jakob-Creutzfeldt disease and miscellaneous disease not especially associated with gliosis, indicated that a high S-ND may be associated with astroglial overgrowth in the brain[6]. The same high S-ND may be induced in guinea-pig lymphocytes, however, by the injection of scrapie non-nervous tissue (for example, muscle) in which there are, of course, no astroglial cells. It may well be, therefore, that astrogliosis is only the cerebral morphological manifestation of some change induced in cell membranes (associated with altered antigenicity) by the scrapie agent[6-8].

Border disease in sheep is not especially characterized by astrogliosis. The degree of astrocyte hypertrophy in scrapie may depend upon the "strain" of scrapie agent used and perhaps also on the breed of sheep employed. The MEM test is highly discriminatory and might be of value in distinguishing different "strains" of scrapie agent.

In a few cases sensitization to encephalitogenic factor (EF)[9] has been tested. As expected, normal sheep showed very little, but animals with scrapie, border disease or cerebro-cortical necrosis showed the sensitization which would be expected as a consequence of brain destruction[10].

Clearly a more extensive study of sheep with a variety of neurological diseases needs to be carried out, especially any which is characterized by astroglial proliferation, as these too may be expected to show a high S-ND[6]. Such study may indeed establish the test described as a rapid and simple means of excluding scrapie without the need to kill the animal and prolonged bioassay; as it might prove of considerable economic importance, further independent study on a larger scale seems desirable.

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1 Field, E. J., and Shenton, B. K., *Nature*, **240**, 104 (1972).
2 Field, E. J., and Caspary, E. A., *Lancet*, ii, 1337 (1970).
3 Field, E. J., and Caspary, E. A., *J. clin. Path.*, **24**, 179 (1971).
4 Caspary, E. A., and Field, E. J., *Br. med. J.*, **2**, 613 (1971).
5 Caspary, E. A., *Nature new Biology*, **231**, 24 (1971).
6 Field, E. J., and Shenton, B. K., *Brain* (in the press).
7 Gibbons, R. A., and Hunter, G. D., *Nature*, **215**, 1041 (1967).
8 Hunter, G. D., *J. infect. Dis.*, **125**, 427 (1972).
9 Caspary, E. A., and Field, E. J., *Ann. NY Acad. Sci.*, **122**, 182 (1965).
10 Caspary, E. A., and Field, E. J., *Eur. Neurol.*, **4**, 257 (1970).

Embryonic and Adult Chymotrypsinogens of Chicken Pancreas

BEEF and pig pancreas contain chymotrypsinogens A, B and C, homologous proteins whose activation products have different substrate specificities[1-4]. Chymotrypsinogens of the A and B type have been found in several vertebrate species[5] whereas the C type chymotrypsinogen seemed to be restricted to the pig and cow[2-4]. An investigation in this laboratory[6] showed that chick pancreas secretory granules contain three chymotrypsinogens, called chymotrypsinogens 1, 2 and 3. The specificities of the activation products of chymotrypsinogens 1 and 2 resembled those of bovine chymotrypsinogens B and A respectively[2,6,7]. Because of the small relative amounts of chymotrypsinogen 3 in the extracts, the specificity of its activation product could not be determined with certainty, but some similarities to mammalian chymotrypsin C were detected[6]. As the data of a later investigation[8] suggested that embryonic chick pancreas might contain relatively large amounts of chymotrypsinogen 3, the chymotrypsinogens in the developing chick pancreas were further studied. This report shows that the specificity of chick embryo chymotrypsin 3 is similar to that of mammalian chymotrypsin C. We also show that chymotrypsinogen 3 is the major chymotrypsinogen species during embryonic development but becomes a minor species after hatching, when chymotrypsinogens 1 and 2 become prominent.

Table 1 Specificity of Chymotrypsin 3 from Embryonic Chick Pancreas

Substrate	Methanol concentration (% v/v)	Relative activity
ATEE	0	100
BLEE	0	156
BTEE	2.5	255
ATryEE	30	3

Relative activity = (rate of hydrolysis of substrate/rate of hydrolysis of ATEE) × 100. Chymotrypsinogen 3 was separated from other chymotrypsinogens of 19-d-old embryo pancreas as described in the legend to Fig. 1. Activities of the peak fraction of chymotrypsinogen 3 were measured after activation with trypsin. For other details see Fig. 1 and ref. 6.

Chromatography on DEAE cellulose of the pancreatic secretory proteins of chick embryos and newly hatched chicks shows that, as in the case of older chickens[6], there are three species of chymotrypsinogen present (Fig. 1). The activities of the activation products of the three chymotrypsinogens were tested on N-acetyl-L-tyrosine ethyl ester (ATEE), on N-acetyl-L-tryptophan ethyl ester (ATryEE) in 20% methanol and on N-benzoyl-L-leucine ethyl ester (BLEE). The specificities of the activation products of chymotrypsinogens in the first two peaks corresponded respectively with the specificities of chymotrypsin 1 (type B) and chymotrypsin 2 (type A) described previously[6]. Table 1 shows that the specificity of the third chymotrypsin(ogen) peak resembles that of mammalian chymotrypsin(ogen) C, which has a high relative activity on BLEE and a low relative activity on ATryEE[2,6]. The activities of chymotrypsin 3 on BLEE and N-benzoyl-L-tyrosine ethyl