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ALTERED RESPONSE TO SCRAPIE TISSUES IN NEUROLOGICAL DISEASE

Possible Evidence for an Antigen Associated with Reactive Astrocytes

BY

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When scrapie mouse brain is injected into guinea-pigs the immunological response is different from that produced by normal mouse brain (Field and Shenton, 1972). The guinea-pig lymphocytes become more sensitive to scrapie than normal brain and the difference is greater when scrapie brain has been injected into a guinea-pig than when normal brain has been injected. The same scrapie-normal antigen difference in lymphocyte response is found when guinea-pigs are tested with scrapie and normal spleen.

Since it has been reported that a scrapie-like condition emerged in sheep in Iceland after inoculation of brain material from a patient who died in an acute stage of multiple sclerosis (MS) (Pálsson, Pattison and Field, 1965), it seemed worth finding out if patients with multiple sclerosis (and other destructive neurological diseases) present the same sort of sensitization as has been found when an animal has been injected with scrapie material (Field and Shenton, 1972). The same scrapie-normal difference was found in MS, but also in neurosyphilis (GPI) and to a lesser degree in other (less destructive) neurological diseases. The scrapie-normal difference appears to be associated with the development of gliosis and raises the question whether a new antigenic situation arises under such conditions.

Materials and Methods

Altogether 24 MS patients (3 clinically acute; 21 quiescent); 14 patients with general paralysis of the insane (neurosyphilis (GPI)); 20 patients with miscellaneous other nervous diseases (OND); and a group of 21 normal subjects together with some cancer patients have been studied. In addition, two chimpanzees with experimental kuru and one with Jakob-Creutzfeldt disease transmitted in this Unit, have also been studied. In each case the response of blood lymphocytes to scrapie mouse brain suspension, normal mouse brain, scrapie mouse spleen and normal spleen has been measured. Since scrapie has emerged unexpectedly in Icelandic sheep inoculated with multiple sclerosis (Pálsson *et al.*, 1965) and since sheep either inoculated experimentally with scrapie or naturally infected show unusual lymphocytic reactivity to scrapie as opposed to normal mouse tissues (Field and Shenton, 1973) our primary interest was to test MS lymphocyte reaction to these tissues and determine if it resembled that of scrapie sheep lymphocytes. The other groups studied served as controls for the MS patients.

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Blood lymphocytes were prepared from 10–15 ml of venous blood by the carbonyl-iron-methyl cellulose method of Coulson and Chalmers (1967) as modified by Hughes and Caspary (1970). Scrapie and normal brain and spleen test materials were made from Webster-Swiss mice, and were identical with those used as antigens for immunizing guinea-pigs in the diagnostic test for scrapie (Field and Shenton, 1972). These materials to which lymphocyte sensitization was tested will be referred to, therefore, as antigens. Animals were inoculated intracerebrally with 0·05 ml of a 10^{-1} suspension (1,000 g for ten minutes clearing) of scrapie mouse brain which had been repeatedly passaged in the Unit since receipt from Dr. R. L. Chandler some years ago. Such animals were killed when there was clear evidence of scrapie—about five months later—and the brain and spleen removed sterile. As controls similar animals which had been injected with normal mouse brain were used as source of antigens. Brain and spleen suspensions were made up in the usual way in sterile polyfusor saline and cleared by centrifugation at 1,000 g for ten minutes. In the case of the spleens, however, either the whole organ or the made-up suspension was subjected to 5,000 r irradiation from a cobalt bomb in order to eliminate a two-way reaction with the human cells under test. All antigens were aliquoted and kept frozen at −20°C until used. In addition, the lymphocytes have been tested for sensitization to encephalitogenic factor (EF) (Caspary and Field, 1965). This is a basic protein isolated from normal human brain and capable of producing experimental allergic encephalomyelitis (EAE) in guinea-pigs in doses of 1 μg when injected in a suspension of Freund's complete adjuvant and may be regarded as the putative antigen if MS is thought of as an "auto-immune" disease.

Lymphocyte sensitization was estimated by the MEM (macrophage electrophoretic mobility method described by Field and Caspary (1970) and presented with full experimental protocol by Caspary and Field (1971). In principle the method depends upon the observation that when lymphocytes are brought into contact with their sensitizing antigen some material is produced (protein in nature (Caspary, 1971, 1972)) with the property of causing normal guinea-pig macrophages to travel more slowly in an electric field. This material (macrophage slowing factor—MSF) may well be identical with macrophage inhibitory factor (MIF) which inhibits active macrophage amœboid movement. The behaviour of normal guinea-pig peritoneal macrophages in an electric field is thus used as an indicator system for lymphocyte-antigen recognition and interaction.

Results have been expressed as percentage slowing. Thus if t_c=time of macrophage migration when no antigen is present (control time); t_e=time of migration when antigen is present; then $t_e > t_c$ and $\frac{t_e - t_c}{t_c} \times 100$ is a measure of lymphocyte sensitization. It is these percentage slowings which are presented in the tables. In order to rule out direct effect of antigen on macrophages which might cause them to travel more slowly in an electric field, each antigen batch is routinely tested for any direct slowing effect in the absence of lymphocytes. None was found.

In carrying out a test $0{\cdot}5 \times 10^6$ lymphocytes are mixed with 0·1 ml antigen suspension (10 per cent brain or spleen or 33 μg/ml EF) and 10^7 irradiated normal guinea-pig macrophages. The mixture is incubated at 20°C for ninety minutes and the time of macrophage migration in each sample measured "blind" on randomly scrambled specimens.

Results

Multiple Sclerosis (MS)

As previously reported (Caspary and Field, 1970) all patients with MS showed a well-marked response to EF except for one, patient No. 13, who had been given prednisone (7·5 mg daily) for ten years before test. This accords with our experience of the depressant effect of the drug on lymphocyte-antigen interaction in other conditions, too. The level of sensitization against scrapie brain always exceeded that measured against normal brain, and the same holds true when scrapie and normal spleen were used as antigens. The difference with scrapie as compared with

TABLE I.—PATIENTS WITH MULTIPLE SCLEROSIS

Patient	Age	Sex	EF	Scrapie brain	Normal brain	Scrapie spleen	Normal spleen	Brain SND	Spleen SND
1	32	F	13·2	15·4	9·8	5·9	1·8	5·6	4·1
2	42	F	16·3	20·7	15·1	7·4	1·3	5·6	6·1
3	39	F	15·1	13·3	7·6	11·3	9·2	5·7	2·1
4	19	F	13·7	11·4	5·6	9·6	3·5	5·8	6·1
5	32	F	14·5	17·7	13·3	8·6	1·0	4·4	7·6
6	23	M	15·5	20·1	14·9	6·0	0·3	5·2	5·7
7	23	F	15·0	19·1	13·4	6·5	1·3	5·7	5·2
8	27	M	14·4	20·6	13·0	7·3	1·3	7·6	6·0
9	32	F	15·4	20·0	14·3	6 2	1·0	5·7	5·2
10	21	F	17·1	22·6	14·5	6·9	1·0	8 1	5·9
11	51	F	14·2	15·9	10·8	11·3	6·3	5·1	5·0
12	46	F	15·9	16·3	10·7	11·1	5·9	5·6	5·2
13	55	F	7·8	10·9	6·2	6·0	0·8	4 7	5·2
14	47	M	14·5	19·6	12·3	9·7	1·8	7·3	7·9
15	34	F	16·6	16·6	12·2	7·7	2·4	4·4	5·3
16	57	F	15·0	19·0	12·8	8·3	0·8	6·2	7·5
17	24	M	14·5	19·0	13·9	7·0	1·3	5·1	5·7
18	55	F	13·3	17·5	12·5	5·9	0·9	5·0	5·0
19	49	F	13·2	16·6	11·9	7·3	1·4	4·7	5·9
20	45	M	13·6	15·5	10·1	6·1	1·0	5·4	5·1
21	56	F	12·5	16·8	12·0	7·3	1·6	4·8	5·7
22	32	M	15·6	17·2	11·8	6·7	0·7	5·4	6·0
23	43	F	14·0	16·6	11·9	6·9	0·5	4·7	6·4
24	61	M	15·9	18·9	13·7	5·9	1·2	5·2	4·7

normal tissue (scrapie-normal difference—SND) was higher in the MS group than in the normal-miscellaneous group (Table IV) and inspection shows that this is in large part due to the high figure recorded with scrapie tissue. No difference was apparent between active and quiescent multiple sclerosis.

Other Neurological Diseases (Excluding GPI)

In these patients, too, EF sensitization was well marked as previously reported (Caspary and Field, 1970). The SNDs both with respect to brain and spleen antigens were clearly less than in MS ($t=11{\cdot}4086$, $df=42$, $P<{\cdot}001$; and $7{\cdot}5479$, 42, $P<{\cdot}001$ respectively) (Table II).

TABLE II.—OTHER NEUROLOGICAL DISEASES (OND) EXCLUDING GPI (GENERAL PARALYSIS OF THE INSANE) AND GLIOMA

Patient	Age	Sex	EF	Scrapie brain	Normal brain	Scrapie spleen	Normal spleen	Brain SND	Spleen SND	
1	68	M	10·6	—	—	3·9	0·8	—	3·1	Lumbar disc lesion
2	16	M	13·4	12·2	11·2	2·8	1·8	1·0	1·0	No diagnosis
3	55	M	13·5	15·5	13·5	4·7	0 3	2·0	4·4	Encephalitis lethargica
4	39	F	11·0	—	—	4·0	0·0	—	4·0	Tuberculous meningitis
5	41	M	14·9	17·0	13·5	3·4	2·3	3·5	1·1	L. pariet. tumour
6	50	M	14·0	6·7	5·1	5·7	4·5	1·6	1·2	CNS secondaries
7	41	M	12·9	15·5	13·4	3·9	1·3	2·2	2·6	Subarach. haemorrhage
8	54	F	15·6	11·8	8·2	6·4	2·0	3·6	4·4	Herpes encephalitis
9	54	F	13 6	14 9	12·0	6·7	2·6	2 9	4 1	Zoster
10	55	M	13·7	15·1	12·9	6·4	6·3	2·6	0·1	Syringomyelia
11	36	F	12·3	12·2	11·0	4·4	1·8	1·2	2·6	Stroke
12	52	M	—	3 7	2·9	2·8	3·3	0·8	−0·5	? encephalitis
13	58	M	15 6	15 4	13·9	3·4	2·4	1·5	1·0	Cerebral tumour
14	49	F	13·8	15·3	13·1	4·0	1·0	2·2	3·0	Motor neuron disease
15	64	M	10·7	6·0	3·9	5·8	4·0	2·1	1·8	Motor neuron disease
16	65	F	13·3	14·7	12·1	4·0	1·0	2·6	3·0	Stroke
17	62	F	14·3	15·0	12·8	4·6	1·8	2·2	2·8	Presenile dementia
18	34	M	13·5	15·4	12·8	4·4	1·8	2·6	2·6	Motor neuron disease
19	16	M	13·5	12·4	11·4	1·7	0·9	1·0	0·8	No diagnosis
20	71	F	13·9	16·2	12 2	5·7	0 8	4 0	4 9	Oligodendroglioma

TABLE III.—GENERAL PARALYSIS OF THE INSANE

Patient	Age	Sex	EF	Scrapie brain	Normal brain	Scrapie spleen	Normal spleen	Brain SND	Spleen SND
1	77	M	16·0	20·8	13·3	7·3	1·3	7·5	6·0
2	75	M	15·9	20·1	13·4	7·0	1·1	6·7	5·9
3	52	M	—	20·3	13·1	7·3	1·5	7·2	5·8
4	69	M	16·2	20·4	14·0	8·0	2·3	6·4	5·7
5	76	M	15·8	19·1	12·3	6·7	0·4	6·8	6·3
6	49	M	15·2	19·7	12·3	7·9	1·2	7·4	6·7
7	66	M	14·3	18·5	12·6	6·7	0·7	5·9	6·0
8	76	M	16·1	19·9	16·6	6·7	1·8	3·3	4·9
9	65	F	—	19·2	12·7	8·1	1·6	6·5	6·5
10	73	M	16·1	17·4	12·2	5·2	1·5	5·2	3·7
11	69	F	—	19·2	12·3	7·6	2·1	6·9	5·5
12	77	M	16·2	20·7	13·5	9·0	1·2	7·2	7·8
13	58	F	16·4	18·1	12·8	7·4	1·3	5·3	6·1
14	66	M	16·5	18·2	11·9	7·5	1·7	6·3	5·8

General Paralysis of the Insane (GPI: Neurosyphilis)

As expected EF sensitization was well marked. Unexpected, however, was a well-marked SND—indeed often greater than in MS itself (Table III).

TABLE IV

Patient	*Age*	*Sex*	*EF*	*Scrapie brain*	*Normal brain*	*Scrapie spleen*	*Normal spleen*	*Brain SND*	*Spleen SND*	
						(*a*) NORMALS				
1	28	F	0·8	1·8	−0·3	2·3	0·3	2·1	2·0	
2	23	M	—	—	—	2·9	1·0	—	1·9	
3	23	F	1·5	—	—	3·7	0·8	—	2·9	
4	75	M	2·1	2·4	0·8	3·2	2·2	1·6	1·0	
5	27	M	—	2·9	0·8	3·0	2·2	2·1	0·8	
6	23	F	—	3·2	0·7	3·5	2·5	2·5	1·0	
7	23	F	—	1·4	1·3	1·8	0·8	0·1	1·0	
8	21	F	—	2·5	1·7	3·9	2·0	0·8	1·9	
9	64	M	—	1·0	0·3	0·6	0·3	0·7	0·3	
10	20	F	1·2	2·6	0·1	2·2	0·8	2·5	1·4	
11	59	M	—	2·1	0·6	1·3	0·8	1·5	0·5	
12	35	M	2·2	2·2	1·5	2·9	1·5	0·7	1·4	
13	32	M	—	0·4	0·3	0·6	0·4	0·1	0·2	
14	29	M	2·7	3·0	0·5	3·2	2·0	2·5	1·2	
15	61	F	—	1·3	0·5	1·1	0·8	0·8	0·3	
16	57	M	4·4	4·7	3·5	3·9	3·4	1·2	0·5	
17	66	M	2·7	4·4	2·2	—	—	2·2	—	
18	28	M	4·7	6·2	2·9	2·1	1·4	3·3	0·7	
19	60	M	—	4·8	2·7	4·9	2·9	2·1	2·0	
20	67	M	1·1	1·1	1·2	0·2	0·3	−0·1	−0·1	
21	78	F	0·9	2·0	1·2	1·2	0·1	0·8	1·1	
						(*b*) MISCELLANEOUS DISEASES				
22	45	F	—	10·9	10·1	3·1	2·3	0·8	0·8	Carcinoma breast
23	80	F	—	11·1	10·1	2·4	1·4	1·0	1·0	Carcinoma uterus
24	13	M	12·2	10·3	9·2	3·6	2·6	1·1	1·0	Osteosarc. humerus
25	68	F	9·2	2·4	3·8	2·9	2·5	−1·4	0·4	? carcinoma gut
26	75	F	11·3	4·8	4·4	4·4	4·7	0·4	−0·3	? carcinoma lung
27	52	M	12·8	6·4	4·2	7·6	6·4	2·2	1·2	Carcinoma uterus
28	63	M	12·7	—	—	2·7	−0·1	—	2·8	Carcinoma bladder
29	58	M	15·6	15·4	13·9	3·4	2·4	1·5	1·0	Meningioma
30	55	F	16·4	8·4	7·0	5·2	3·0	1·4	2·2	Carcinoma œsophagus
31	56	M	10·9	10·9	8·2	7·9	5·6	2·7	2·3	? sarcoidosis ? generalized carc.
32	21	F	0·8	3·8	3·4	2·5	1·8	0·4	0·7	Diabetes
33	24	F	—	1·5	1·3	1·8	1·6	0·2	0·2	? granulomatous disease
34	40	F	12·9	12·8	11·0	1·7	1·3	1·8	0·4	Carcinoma breast
35	69	F	10·9	0·8	1·3	0·3	0·3	−0·5	—	Melanoma
36	68	F	1·1	0·5	0·4	1·0	0·6	0·1	0·4	Ameloblastoma
37	70	F	14·0	11·2	9·3	12·0	10·6	1·9	1·4	Sarcoidosis
38	80	F	1·8	1·6	0·9	1·5	0·4	0·7	1·1	Leukæmia
39	72	F	—	2·0	0·9	1·1	0·7	1·1	0·4	Chronic constipation
40	60	M	8·4*	1·5	0·7	1·7	0·4	0·8	1·3	Myasthenia gravis
41	30	M	—	1·4	1·0	1·3	0·8	0·4	0·5	Psoriasis
42	56	M	—	2·1	1·6	1·2	0·9	0·5	0·3	Myasthenia gravis
43	23	F	0·0	1·4	1·3	1·8	0·8	0·1	1·0	Pigmented nævus
44	20	F	0·4	0·4	0·3	—	—	0·1	—	Infectious mononucleosis
45	70	F	14·0	11·2	9·3	12·0	10·6	1·9	1·4	Sarcoidosis
46	68	F	—	0·5	0·4	1·0	0·6	0·1	0·4	Abscess of gum
47	32	M	0·0	0·4	0·3	0·6	0·4	0·1	0·2	Saphenous thromb.
48	64	M	—	1·0	0·3	0·6	0·3	0·7	0·3	Pigmented mole
49	68	F	9·2	3·8	2·4	2·9	2·5	1·4	0·4	Parathyroid adenoma
						(*c*) GLIOMAS				
50	39	M	16·6	21·5	13·3	9·8	0·9	8·2	8·9	
51	61	F	—	17·9	11·5	6·7	1·1	6·4	5·6	
52	54	F	16·1	19·1	13·9	7·7	2·5	5·5	5·2	
53	3	M	—	19·4	14·3	7·6	2·4	5·1	5·2	
54	39	M	16·3	20·9	14·9	7·6	2·4	6·0	5·2	
						(*d*) KURU CHIMPANZEES				
55	3	F	9·0	13·7	8·3	13·5	1·4	5·4	13·1	
56	3	F	7·3	11·9	7·5	12·2	1·7	4·4	11·5	
						(*e*) JAKOB-CREUTZFELDT				
57	2	M	8·0	11·7	5·2	8·0	0·1	6·5	7·9	Experimental chimpanzee
58	53	M	16·2	18·7	13·7	5·9	1·3	5·0	4·6	

* There is an elevation of sensitization to EF in this disease.

Normal Subjects and those with Other Diseases

Apart from patients with cancer (Field and Caspary, 1970) EF sensitization did not rise above 5 per cent (the limit of normal in all our control series). SND both in respect of brain and spleen antigen was clearly much less than in the MS and GPI groups and, in lesser degree, that in OND. The statistical significances between these groups is summarized in Table V.

TABLE V

	SND	
	Brain	*Spleen*
OND *v.* MS	< ·001	< ·001
OND *v.* GPI	< ·001	< ·001
OND *v.* Normal	·02–·01*	·005–·001
MS *v.* GPI	·05–·025†	·5–·4

*Accepted as significant. † Not accepted as significant. MS and GPI both highly significantly different from normals.

DISCUSSION

Our first results with MS suggested that such patients possessed the same SND as was produced in guinea-pigs by inoculation of scrapie material (Field and Shenton, 1972) and thus appeared to offer support for the association found by Pálsson *et al.* (1965) between multiple sclerosis and scrapie. However, subsequent study of the GPI and OND control groups showed that the large SND was not limited to MS and that some common factor must be sought. It would seem that in multiple sclerosis and GPI, and to a lesser degree in other neurological diseases, lymphocytes are especially sensitized to an antigen present in scrapie mouse brain and spleen but not in the corresponding normal tissues. This special antigenicity results in a large SND when guinea-pigs are injected with scrapie mouse material and this may be used as a rapid means of titrating scrapie activity (Field and Shenton, 1972). Since, however, a similar large SND may be induced by inoculating brain of a mouse which had not been infected with scrapie but has been fed with cuprizone which induces marked enlargement of astrocytes (Field and Shenton, 1972; Pattison and Jebbett, 1971), the effect is not specific to scrapie infection but may perhaps be linked to the occurrence of astrocytic hypertrophy. There is evidence both for and against this hypothesis which it is difficult at this stage to reconcile.

If one asks what is common to MS, GPI, kuru and scrapie, and cuprizone intoxication of mice, astrocytic hypertrophy comes to mind, so that sensitization to a new antigen associated with astrocytes might be detectable in all these conditions. This possibility is reinforced when the high difference in glioma and in chimpanzees with kuru and Jakob-Creutzfeldt disease is taken into account (Table IV). In these diseases astroglial overgrowth in grey matter is striking and seemingly precocious (figs. 1, 2, Plate XLIX). This interpretation derives further support from the study of mice fed on cuprizone (Pattison and Jebbett, 1971). The brain of such an animal which shows striking astroglial hypertrophy when injected into guinea-pigs, induces well-marked scrapie-normal differences. There is, however, some difficulty in

accommodating this attractive explanation with certain further observations. Thus, astroglia is, of course, not present in spleen, though this tissue from a scrapie mouse produces a marked scrapie-normal difference when injected into guinea-pigs (Field and Shenton, 1972). Splenic tissue is rich in scrapie agent and it might therefore be supposed that the high SND was associated with the presence of scrapie agent; but here, too, there is a difficulty. After intracerebral injection of scrapie agent into mice, the special scrapie antigenicity and SND takes about fifty days to develop even though agent is demonstrable all along with the exception of a brief "eclipse period" in some cases (Field, Joyce and Keith, 1971). Hence SND is not bound solely to the presence of scrapie agent. A possible explanation could be that scrapie agent induces biochemical or biophysical changes in surface membranes of cells (Hunter, 1972) and this endows them with new antigenicity which takes place, however, over some weeks during which time the agent is present though the special antigenicity is not yet manifest. From a serial study which correlates the development of special antigenicity with histological change in the injected material it is clear that the former antecedes astrocytic hypertrophy and aurophilia in the Cajal stain. It is tempting, therefore, to regard the new morphological and staining characters of the astrocytes as associated with altered surface membrane. Since direct experiment has shown that other tissues, e.g. liver, kidney, muscle, which remain morphologically unaltered, also show the new antigenicity seen in scrapie brain, it is possible that widespread membrane change at the molecular biological level is not necessarily linked to visible structural change.

The results in the group of other neurological diseases (OND) which showed results intermediate between normal and MS-GPI-glioma group probably reflects the varying degree of astrocytosis present in these conditions. This brings out an important point, often inadequately considered, as to what are the proper controls for studies of MS. Conditions should be chosen in which there is approximately the same degree of nervous parenchyma destruction, and GPI is one such. Comparison of MS with other neurological diseases such as epilepsy, "headaches" and even "stroke" is probably not valid.

Specially instructive is patient No. 4, a 19-year-old nurse who suffered her first attack of MS ten months previously and who was studied in an acute attack, interestingly enough occurring in the left leg within one month of surgical treatment of a left hammer toe. Despite full working capacity with minimal neurological disturbance this patient showed a very high scrapie-normal difference. This may indicate that at this early stage of the disease there is precocious astroglial change as claimed by Charcot (1872–73); Müller (1904); Anton and Wohlwill (1912); Jacob (1969) and reviewed by Field (1967). Clearly there is no evidence from this work of a specific association between MS (or GPI) and scrapie though immunological relations certainly exist. Moreover, there is evidence that hypertrophied astrocytes (from whatever cause) develop a new and common antigen to which lymphocytes in the blood become sensitized. These conclusions are borne out by studies of sensitization in guinea-pigs inoculated with samples of human pathological brain to be described elsewhere.

Finally, it should be noted that, if the interpretation offered is correct, then mouse astrocytic antigen is capable of stimulating lymphocytes sensitized against human astrocytic antigen(s). The antigen(s) would appear to be non-species specific, a situation already well recognized in the case of encephalitogenic factor and certain other antigens implicated in "auto-immune" disease.

Summary

Lymphocytes from patients with multiple sclerosis, general paralysis of the insane and glioma show greater "sensitization" to scrapie mouse brain than do lymphocytes from normal people, from those with other non-neurological diseases; and from those with neurological disease in which there is no reason to suppose that astroglial overgrowth has been marked.

Whilst this together with other data suggests that sensitization to some constituent of scrapie material is linked with astrogliosis, this cannot be a unique association since injection of non-nervous scrapie material results in the same type of sensitization in animals. The relation of the observations to the ætiopathogenesis of multiple sclerosis is briefly discussed.

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LEGENDS FOR PLATE

PLATE XLIX

FIG. 1.—Parietal cortex of chimpanzee (Susie) inoculated eighteen months previously, intramuscularly, with 1 ml 10^{-1} brain material of first passage chimpanzee kuru and killed at onset of disease. Cajal stain. Note enlargement of astrocytes. ×224.

FIG. 2.—Parietal cortex of chimpanzee (Cedric) intracerebrally inoculated eighteen months previously with 10^{-1} brain suspension of patient with Creutzfeldt-Jakob disease. Note enlarged astrocytes. Cajal stain. ×224.

PLATE XLIX

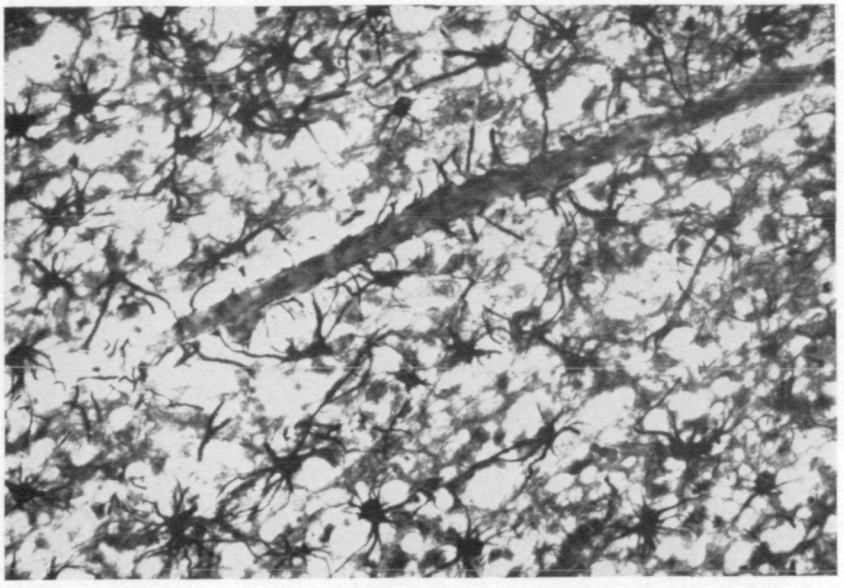

FIG. 1.

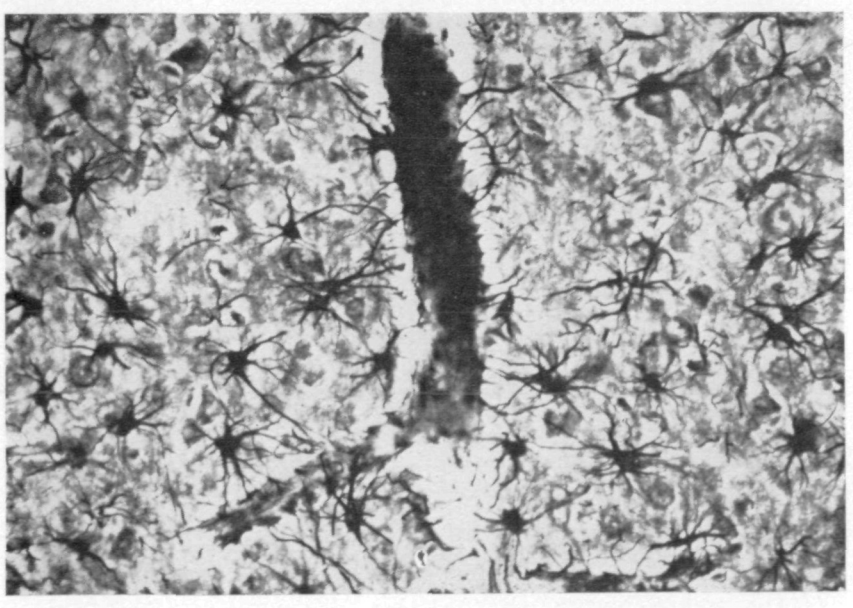

FIG. 2.

To illustrate article by E. J. Field and B. K. Shenton.