Infective Agents and Multiple Sclerosis

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Although the pathogenesis of multiple sclerosis remains obscure, much pathological and clinical evidence suggests an infective agent as a possible aetiological factor. If such an agent is responsible for initiating the disease it seems probable that there is a latent period of several years before the development of clinical features of multiple sclerosis. By that time it might be impossible to obtain laboratory evidence of the incriminating organism. However, if the agent remained latent in the central nervous system it might provide a constant antigenic stimulus manifesting itself in high antibody levels.

In a previous study (Ross, 1962) sera from 40 cases of multiple sclerosis were examined by complement-fixation (C.F.) technique for antibodies to several viral and rickettsial antigens. Mainly negative findings were obtained for lymphocytic choriomeningitis, louping-ill, Q fever, murine typhus, and rickettsialpox. Antibodies were detected for herpes in 33 (82%), for mumps in 29 (72%), and for the psittacosis-lymphogranuloma group (P-LGV) in 4 (10%). However, the absence of controls in this investigation meant that no significance could be placed on the positive findings. Thus a further investigation was required to compare antibody levels in a series of cases of multiple sclerosis with those obtained in a series of carefully matched controls.

Present Investigation

In the initial part of the present study the sera were tested for antibodies to herpes, mumps, P-LGV, and Q fever (*Rickettsia burneti*) for the reasons given below. An attempt was also made to obtain some epidemiological information relevant to infection with these agents.

Herpes Simplex.—This virus is noted for its latent behaviour in cells. It seemed possible that if primary herpes infection occurred in the central nervous system reactivation of latent virus might give the picture typical of the relapses and remissions of multiple sclerosis.

Mumps.—This causes a generalized systemic infection in which there is frequent involvement of the meninges and occasional involvement of the brain and spinal cord; pathological findings in patients who have died of mumps encephalitis have shown perivascular areas of demyelinization of white matter (Donohue, 1941). It has been reported by Soule *et al.* (1959) that, *in vitro*, mumps virus produces a lipolytic enzyme which can attack sphingomyelin of fowl erythrocytes although human erythrocytes are not affected. In the present study tests were carried out for both soluble (S) and viral (V) antibodies; the former disappear within a few weeks of mumps infection whereas the latter remain in high titre for many years.

P-LGV.—Several workers have emphasized a possible relationship between multiple sclerosis and agricultural or rural exposure (Campbell *et al.*, 1947; Sutherland and Wilson, 1951); this exposure might involve a liability to contract P-LGV infections which commonly affect birds and animals.

Q Fever.—The negative findings for Q fever in our previous study were not in keeping with those of Le Gac *et al.* (1960), who reported that, using a microagglutination technique, rickettsial or neorickettsial antibodies were present in sera from 23 out of 27 cases of multiple sclerosis, the commonest being antibodies to Q fever (10 cases). Since it has been reported that microagglutination tests are more sensitive than C.F. for the detection of Q fever antibodies, and that after recovery from illness agglutinating antibodies persist much longer than C.F. antibodies (Babudieri, 1958), tests for Q fever were carried out in the present study by both C.F. and microagglutination methods. In addition, microagglutination tests for *R. conori* (boutonneuse fever), *R. mooseri* (murine typhus), and a neorickettsial antigen "Q-18" were also included.

Later in the study tests for measles, varicella-zoster (chickenpox-shingles), poliovirus types 1 and 2, and *Mycoplasma pneumoniae* (Eaton's agent) were also carried out for reasons given below; at this later period, however, it was not possible to contact many of the control cases to obtain epidemiological information relevant to these antigens.

Measles.—Adams and Imagawa (1962) investigated measles antibody levels by C.F. and neutralization techniques in over 100 patients with multiple sclerosis and in a control series of cases. They found significantly greater levels of measles antibody in serum and in cerebrospinal fluid from cases of multiple sclerosis than in controls.

Varicella-Zoster.—After infection with varicella it is generally believed that the virus spreads up nerves to involve posterior-root ganglia, and there remains latent. In later life the virus may be activated to give the typical distribution of the lesions of zoster. That neurological involvement by zoster is not limited to posterior-root ganglia is evident not only from clinical reports of motor lesions (Taterka and O'Sullivan, 1943) but also from pathological changes reported by Greenfield *et al.* (1958), who found that the inflammatory changes were not confined to posterior-root ganglia but frequently extended into posterior, lateral, and anterior horns of grey matter. Reactivation of virus in the spinal cord might result in the clinical relapse of multiple sclerosis.

Poliovirus.—Poskanzer *et al.* (1963) have suggested that there are many epidemiological similarities between multiple sclerosis and poliomyelitis, and that an enteric-viral infection is a possible causal factor in multiple sclerosis. They suggested that the greater incidence of multiple sclerosis in countries of increasing geographic latitude might be a reflection of improved sanitation with less likelihood of infection by enteroviruses in infancy. In the present study it was impracticable to test for all the complex range of enteroviruses, but tests were C.F. antibodies to poliovirus type 1 and type 2 were carried out in order to obtain a comparison of the incidence of these enteroviral infections in the two groups.

Mycoplasma pneumoniae.—Little is yet known about the diseases associated with this organism, apart from atypical pneumonia (Marmion and Goodburn, 1961) and Stevens–Johnson syndrome (Ludlam *et al.*, 1964).

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Materials and Methods

The series comprised 96 cases of multiple sclerosis with a similar number of controls. The majority of these—80 cases and 80 controls—were resident in the Dundee and Perth areas, and were personally examined by one of us (J. A. R. L.). Of the remainder, 10 were from Edinburgh and 6 from Glasgow. The multiple sclerosis cases comprised 60 females and 36 males; their ages ranged from 13 to 67 years (15 were under 30, 59 were 30–50, and 22 were over 50); no case in which the subsequent course of the disease made the diagnosis of multiple sclerosis doubtful was included. Controls were mainly patients without neurological disease, except for two who had a neurological illness unrelated to multiple sclerosis. The control was of the same sex and same age (±5 years, except for 10 pairs where the difference between case and control was 6–9 years). In nearly every case the control was attending the same hospital as the patient with multiple sclerosis, and sera were generally collected from both on the same day. It was not possible during the course of the study to match for social class according to the Registrar-General's classification, or for other social factors such as size of house or number of children at home.

Epidemiology

A form was completed whenever possible—from 76 cases and 76 matched controls—stating if the patient had had mumps, and at what age; if he suffered from recurrent herpes labialis, and approximate frequency of attacks. Evidence of contact with potential sources of P-LGV infection was obtained by inquiring if, during the period of five years prior to first symptoms, the patient was a close contact of birds (budgerigars, pigeons, or fowls) or animals (sheep, cows, cats, dogs, rats, mice). In the later part of the investigation inquiry was also made about past history of measles, chickenpox, and shingles; however, by that time it was impossible to contact most of the controls used in the earlier part of the study.

Serology

Sera were stored at −20° C.; prior to testing by C.F. or microagglutination they were inactivated at 56° C. for 30 minutes. Serum from each case was tested at the same time as the corresponding control serum.

C.F. Tests.—Antigens for the test were prepared from infected tissues as follows: herpes from chorioallantoic membrane extracts of embryonated eggs (Ross and Stevenson, 1961) or from baby-hamster kidney cells, BHK21-C13 (Ross *et al.*, 1964); mumps S and V from chorioallantoic membrane and allantoic fluid respectively; P-LGV from yolk-sac; varicella-zoster from the cellular phase of human embryonic lung fibroblast cell cultures by homogenization with 1/50 the original volume of distilled water followed by centrifugation to remove cell debris, the supernate constituting the antigen; poliovirus type 1 (Brunenders) from monkey-kidney cell cultures by the method of Hare and Warren (1958); *Mycoplasma pneumoniae* by the method of Chanock *et al.* (1962). Q fever (phase 2) and poliovirus type 2 (M.E.F.1) antigens were obtained from the Standards laboratory, Central Public Health Laboratory, Colindale, and measles antigen from Wellcome Research Laboratories. The optimal titre of each antigen was determined by two-dimensional titrations against human convalescent serum. Control antigens prepared from uninfected culture material were used at the same dilutions as the specific antigens. C.F. tests were carried out in plastic plates with 0.1-ml. volumes each of serum dilution, complement (four 50% haemolytic doses), and antigen dilution. Dilutions of all reagents were made in barbitone-buffered saline (Bradstreet and Taylor, 1962). After standing at 4° C. overnight the plates were kept at 37° C. for 20 minutes, and then 0.1 ml. of 2% optimally sensitized sheep cells were added to each cup. The plates were left for a further 40 minutes at 37° C. and were shaken at 15, 30, and 40 minutes and then allowed to settle at room temperature before reading. The serum titre was the dilution of serum giving 75% or greater fixation. All titres in this paper are expressed as reciprocals.

Microagglutination Tests for Rickettsiae.—These were carried out using the slide-agglutination technique described by Giroud and Giroud (1944) and Giroud *et al.* (1961). The antigen suspensions for the tests—*R. burneti* (Q fever), *R. conori* (boutonneuse fever), *R. mooseri* (murine typhus), and neorickettsial antigen Q-18—were kindly supplied by Dr. P. Giroud, Pasteur Institute, Paris. The lowest serum dilution in the serum-antigen mixtures were those recommended by Giroud—namely, 1 in 20 for *R. burneti*, 1 in 160 for *R. conori*, 1 in 160 for *R. mooseri*, and 1 in 20 for Q-18. Agglutination was read as follows: +++ if in large groups; ++ if in groups of 10 to 20 particles; + if in groups of fewer than 10 particles; − if no agglutination.

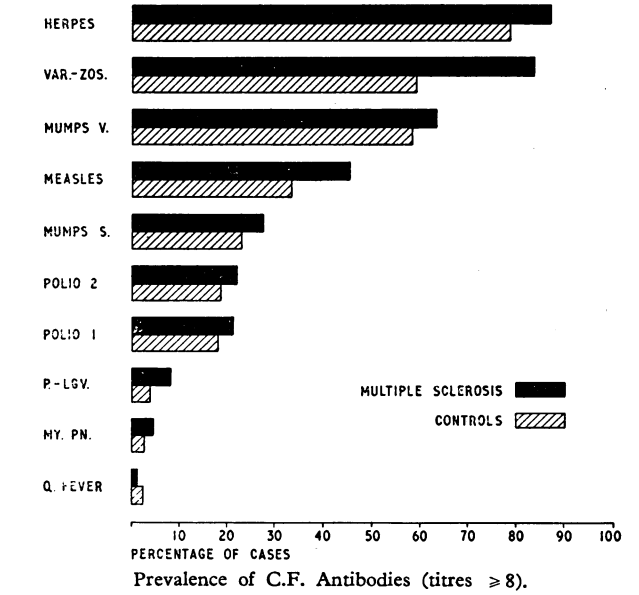
Results

C.F. Tests

The range of serological titres for the various antigens in the cases and controls is shown in Table I. Although sera from 96 cases and 96 controls were examined, a few gave non-specific reactions against individual antigens; these, along with the corresponding results for the matched sera, were excluded from the totals. In the latter part of the study the total number of sera available for testing were fewer in number because many had been completely used up in earlier tests. It will be seen that there was no significant difference between cases and controls in the range of titres for herpes, mumps (both S and

TABLE I.—*Complement-fixation Titres in Cases of Multiple Sclerosis (MS) and Controls (C)*

Antigen	<8		8–32		64–128		256 or Greater		Totals	
	MS	C	MS	C	MS	C	MS	C	MS	C
Herpes	12	19	47	39	32	34	2	1	93	93
Mumps S	65	69	25	21	0	0	0	0	90	90
Mumps V	33	38	48	46	9	7	1	0	91	91
Psittacosis-LGV	87	91	5	1	3	3	0	0	95	95
Q fever	84	83	1	2	0	0	0	0	85	85
Measles	44	54	28	22	8	5	1	0	81	81
Varicella-zoster	12	29	49	40	11	3	0	0	72	72
Polio type 1	59	61	15	14	1	0	0	0	75	75
Polio type 2	42	44	11	8	1	2	0	0	54	54
Mycoplasma pneumoniae	64	65	3	2	0	0	0	0	67	67



Prevalence of C.F. Antibodies (titres ≥ 8).

V), P-LGV, poliovirus types 1 and 2, and Q fever. Although the figures for measles suggested a tendency towards higher antibody levels in the multiple sclerosis than in the control group the differences were not statistically significant. Varicella-zoster was the only antigen which showed a statistically significant difference: of 72 sera from both groups, antibody to varicella-zoster was detected (titres ≥ 8) in 60 (83.3%) cases compared with 43 (59.7%) controls: χ^2, $0.01>P>0.001$. Comparison of the two groups in relation to high titres (≥ 64) of varicella-zoster antibody gave 11 (15.2%) cases compared with 3 (4.2%) controls; the difference is again statistically significant: χ^2, $0.05>P>0.02$.

Arrangement of the infective agents in order of percentage prevalence of antibodies (titres ≥ 8) in the control group is shown in the Chart, herpes having the highest prevalence (79.6%) and Q fever the lowest (2.4%). For all the agents except Q fever, antibodies were detected in a higher proportion of the cases than the controls.

Microagglutination Tests

The results of microagglutination tests on sera from 30 cases and matched controls are shown in Table II. No marked

TABLE II.—*Microagglutination Tests (30 Cases with Matched Controls)*

Degree of Agglutination	*R. burneti*		*R. conori*		*R. mooseri*		Q-18	
	MS	C	MS	C	MS	C	MS	C
+++	0	0	0	0	0	0	0	0
++	1	1	0	0	0	0	1	0
+	1	4	0	1	2	1	1	1
−	28	25	30	29	28	29	28	29

MS = multiple sclerosis cases. C = controls.

agglutination reaction (+++) was obtained for the rickettsial and neorickettsial agents; some agglutination (++ or +) was obtained with a few sera, but there was no significant difference between cases and controls. Because of these negative findings and also because of shortage of rickettsial antigens no further sera were tested by microagglutination.

Epidemiological

From Table III it will be seen that there was no appreciable difference between the cases and controls in clinical histories

TABLE III.—*Epidemiological Histories*

	Total No. with Positive History		Total No. of Matched Cases
	MS	C	
Herpes	27	31	71
Mumps	28	36	67
Animal contact	32	28	63
Bird contact	23	23	59
Measles	9	10	14
Chickenpox (varicella)	6	6	12
Shingles (zoster)	1	0	9

of previous infection with mumps or herpes simplex, or in histories of close contact with birds or animals. For measles, chickenpox, and shingles there was again no difference between the two groups, but here the numbers where information was available were too small for analysis.

Discussion

The present study has shown that, with the exception of varicella-zoster, the range of antibody levels to a wide variety of infective agents was similar in the multiple sclerosis and control groups. Despite this similarity, C.F. antibody for each of the agents except Q fever was consistently present in a greater number of cases than controls, although a statistically significant difference was obtained only with varicella-zoster. Two possible explanations for this general trend are (*a*) that the control group was unsatisfactory, or (*b*) that there was a difference between cases and controls in immunological response to infective agents. Controls were matched with cases according to what we considered were the most important requirements—namely, age, sex, same hospital population, and concurrent collection of sera. However, it was not found possible also to match for social class or for factors such as population density at home or in the surrounding community. An imbalance in these factors could explain the consistent difference in antibody levels between the two groups. A difference in immunological response to infective agents might also play a part, as Saifer *et al.* (1953) have reported quantitative and qualitative abnormalities of serum albumin and globulin fractions in patients with multiple sclerosis. However, as the range of titres for all the antigens except varicella-zoster were so similar in both groups it would appear that these serum protein abnormalities do not in general affect C.F. antibody responses.

The finding of a significantly greater number of cases in the multiple sclerosis than in the control group both with detectable antibody to varicella-zoster (titres ≥ 8) and with high antibody (titres ≥ 64) might also be partially or wholly due to an unsatisfactory control group or to a difference in immunological response. On the other hand, the consistent results obtained with the other antigens serve as an internal control for the divergent varicella-zoster findings. It would therefore appear either that patients with multiple sclerosis may be particularly susceptible to infection with varicella-zoster or that infection with this virus may be related to the aetiology of multiple sclerosis.

Several factors might be thought to favour susceptibility to infection with varicella-zoster. Patients with multiple sclerosis tend to spend longer periods in hospital than patients of the same age without neurological disease, and it might be argued that cross-infection is more common in hospital than at home. However, cross-infection is rare from shingles (zoster) and common from chickenpox (varicella). Thus in an adult ward there would probably be no greater liability to cross-infection with varicella-zoster than at home. Another factor might be that, although the initial damage of multiple sclerosis is caused by some other agent, this damaged nervous system is more susceptible to infection with varicella-zoster. However, one might expect both these factors to apply also to infection with herpes simplex, and in the present study the two groups showed no significant difference in herpes antibody levels. On the other hand, the damaged central nervous system of multiple sclerosis might have a specific susceptibility to infection with varicella-zoster.

Concerning direct association of varicella-zoster in the aetiology of multiple sclerosis it might be argued that after infections with varicella in childhood or adolescence the virus becomes latent not only in posterior-root ganglia but in other cells of the central nervous system. Then, after a period of several years, when antibody has waned, the virus may become reactivated: if latent virus were present only in posterior-root ganglia this would produce lesions typical of zoster, but if present in other nerve cells reactivation of the virus might result in the clinical and pathological picture of multiple sclerosis. To investigate this possibility serial varicella-zoster antibody levels could be correlated in each case of multiple sclerosis with clinical relapses and remissions. It might also be relevant to correlate clinical evidence of shingles in cases of multiple sclerosis with relapses and remissions; however, this is not likely to prove rewarding, as any close correlation would probably have been noted by other observers.

Attempts to isolate varicella-zoster virus from necropsy specimens of brain and spinal cord might also prove unrewarding, since it has been shown that within tissue-culture cells the virus seems to be inactivated with the death of the cell

(Weller, 1953). Again, even if varicella-zoster were isolated from necropsy material this would have little significance, as it might result from direct or indirect association with the disease or might even be found in "normal" brain and spinal cord. Tests for varicella-zoster antigen might be carried out on necropsy specimens of central nervous system in a series of cases of multiple sclerosis along with similar specimens from a control series; for this purpose autoradiography, using radioactively labelled antiserum, would probably be more sensitive than fluorescent antibody tests.

Many studies in Europe and North America (cited by Allison, 1961) have shown that multiple sclerosis is essentially a disease of cold and temperate climates. In the southern hemisphere information is incomplete, but multiple sclerosis seems to be of uniformly low prevalence in South Africa (Dean, 1949) and in Australia in latitudes approaching the Equator (Sutherland *et al.*, 1962). In these areas of low prevalence there may be universal infection in early life with viruses such as varicella; for example, it is now recognized that, in areas where hygiene is poor, infection with poliovirus is acquired early in life and there is less risk of paralytic disease. It thus seems possible that the age of primary infection with viruses such as varicella may be relevant to the aetiology of multiple sclerosis.

That varicella-zoster may not be the only incriminating infective agent is suggested by the report of Adams and Imagawa (1962) of statistically significant higher measles antibody levels in cases of multiple sclerosis than in controls. However, their paper did not specify the criteria used for their controls; this is of considerable importance, since age and social factors such as population density affect measles antibody levels (Black, 1962). In a more recent study of measles antibody levels in multiple sclerosis (Reed *et al.*, 1964) the findings were inconclusive—a result similar to that obtained in the present study.

Using both microagglutination and C.F. techniques, we have been unable to detect any difference in rickettsial antibodies between the two groups. Thus we have been unable to confirm the findings of Le Gac *et al.* (1960) even by the use of the same technique employed by him and also by the use of the same antigens supplied to us by his collaborator, Dr. P. Giroud. As there were no control cases in Le Gac's series the high incidence of rickettsial infections in his cases of multiple sclerosis might have been simply a reflection of the greater prevalence of these infections in some areas of France than in Scotland.

Summary

Sera from 96 cases of multiple sclerosis and from 96 matched controls were examined for complement-fixation antibodies to herpes, mumps, the psittacosis-lymphogranuloma group, measles, varicella-zoster, poliovirus type 1 and type 2, and *Mycoplasma pneumoniae*. C.F. antibody for each of the agents except Q fever was consistently present in a greater number of cases than controls, although a statistically significant difference was obtained only with varicella-zoster. Several possible explanations for these findings are discussed.

Sera from 30 of the cases with matched controls were also examined for microagglutinating antibodies to several rickettsial agents. No appreciable differences in microagglutinating antibodies were detected.

Clinical histories of previous infection with mumps and herpes, and histories of close contact with birds or animals, were equally prevalent in the multiple sclerosis and control groups; for measles, chickenpox, and shingles clinical information was insufficient for analysis.

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References

Adams, J. M., and Imagawa, D. T. (1962). *Proc. Soc. exp. Biol. (N.Y.)*, **111**, 562.
Allison, R. S. (1961). *Proc. roy. Soc. Med.*, **54**, 1.
Babudieri, B. (1958). *Bull. Wld Hlth Org.*, **19**, 981.
Black, F. L. (1962). *Amer. J. Dis. Child.*, **103**, 242.
Bradstreet, C. M. P., and Taylor, C. E. D. (1962). *Mth. Bull. Minist. Hlth Lab. Serv.*, **21**, 96.
Campbell, A. M. G., Daniel, P., Porter, R. J., Russell, W. R., Smith, H. V., and Innes, J. R. M. (1947). *Brain*, **70**, 50.
Chanock, R. M., James, W. D., Fox, H. H., Turner, H. C., Mufson, M. A., and Hayflick, L. (1962). *Proc. Soc. exp. Biol. (N.Y.)*, **110**, 884.
Dean, G. (1949). *Brit. med. J.*, **1**, 842.
Donohue, W. L. (1941). *J. Pediat.* **19**, 42.
Giroud, P., and Giroud, M.-L. (1944). *Bull. Soc. Path. exot.*, **37**, 84.
—— Capponi, M., and Dumas, N. (1961). *Ann. Biol. clin.*, **19**, 203.
Greenfield, J. G., Blackwood, W., McMenemy, W. H., Meyer, A., and Norman, R. M. (1958). *Neuropathology*. Arnold, London.
Hare, J. D., and Warren, J. (1958). *Amer. J. Hyg.*, **67**, 350.
Le Gac, P., Giroud, P., and Dumas, N. (1960). *C. R. Acad. Sci. (Paris)*, **250**, 1937.
Ludlam, G. B., Bridges, J. B., and Benn, E. C. (1964). *Lancet*, **1**, 958.
Marmion, B. P., and Goodburn, G. M. (1961). *Nature (Lond.)*, **189**, 247.
Poskanzer, D. C., Schapira, K., and Miller, H. (1963). *Lancet*, **2**, 917.
Reed, D., Sever, J., Kurtzke, J., and Kurland, L. (1964). *Arch. Neurol. (Chic.)*, **10**, 402.
Ross, C. A. C. (1962). *Brit. med. J.*, **1**, 1523.
—— Russell, W. C., and Wildy, P. (1964). *Arch. ges. Virusforsch.*, **15**, 58.
—— and Stevenson, J. (1961). *Lancet*, **2**, 682.
Saifer, A., Rabiner, A. M., Oreskes, I., and Volk, B. W. (1953). *Amer. J. med. Sci.*, **225**, 287.
Soule, D. W., Marinetti, G. V., and Morgan, H. R. (1959). *J. exp. Med.*, **110**, 93.
Sutherland, J. M., Tyrer, J. H., and Eadie, M. J. (1962). *Brain*, **85**, 149.
—— and Wilson, D. R. (1951). *Glasg. med. J.*, **32**, 302.
Taterka, J. H., and O'Sullivan, M. E. (1943). *J. Amer. med. Ass.*, **122**, 737.
Weller, T. H. (1953). *Proc. Soc. exp. Biol. (N.Y.)*, **83**, 340.