# Preliminary Communications

## Virus Antibodies in Subacute Sclerosing Panencephalitis: a Study of 22 Patients*

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Subacute sclerosing panencephalitis was the term suggested by Greenfield (1950) to cover the subacute inclusion-body encephalitis described by Dawson (1933) and the subacute sclerosing leucoencephalitis of Van Bogaert (1945), as well as mixed forms. This has been accepted to some extent in the English literature (*B.M.J.*, 1965), but the earlier terms are still in use (Ulrich and Kidd, 1966; Dayan *et al.*, 1967). The histological appearances of the condition certainly differ from case to case, but they may also vary during the evolution of the disease; in an example reported by Schiøtt (1959) inclusion bodies were found in brain biopsy material obtained six months from the onset of symptoms, but not in post-mortem material two months later. At present, therefore, the condition cannot be clearly subdivided on histological grounds.

The inclusion bodies suggest that the disease might have a viral origin, but until recently there was no evidence to incriminate any particular virus. However, in 1965 Bouteille *et al.* published electronmicrographs of biopsy material, and the appearances of the inclusions were strikingly similar to those of measles virus grown in dog-kidney-tissue culture (Tawara, 1965). Tellez-Nagel and Harter (1966) and Dayan *et al.* (1967) have published similar pictures from other cases. Connolly *et al.* (1967) have described the presence of measles complement-fixing and haemagglutination-inhibiting antibodies in the serum and C.S.F. of three cases, and have demonstrated fluorescence in post-mortem brain material treated with fluorescein-labelled measles antiserum.

As part of a virological investigation into encephalitis we have examined sera from patients with subacute sclerosing panencephalitis for the presence of viral antibodies. Measles antibody was present in all cases, often in high titre. This report compares the results of serological tests for measles and other viral antibodies in patients and controls.

### Patients and Methods

We were able to study 22 patients (16 males and 6 females) referred to this department for investigation. One of them was also studied by Connolly *et al.* (1967). Their ages at the onset of neurological symptoms ranged from 5 to 18 years (Table I). Some of them were included in the clinical report of Metz *et al.* (1964); all were observed to suffer from typical repetitive movements, and all had at least two of the following features: convulsions, intellectual deterioration, behavioural disorder, and impaired motor function. The C.S.F. Lange test gave a paretic rise in all but two cases, in one of which it was not performed. All but one had the characteristic E.E.G. changes (Cobb and Hill, 1950). Confirmation of the diagnosis by pathological examination of biopsy or post-mortem material, or both, was obtained in 14 cases, including one with an uncharacteristic E.E.G. and the one in which the Lange test was not done; inclusion bodies were found in eight. Serum was available from all 22 patients, and C.S.F. from nine of them.

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Serum was also available from 23 control subjects (13 males and 10 females) of the same age range as the patients (Table I). None suffered from any sort of progressive neurological disease, and they were referred to this department for investigation of childhood infectious diseases, such as gastroenteritis or aseptic meningitis, or for screening for rubella antibodies. None was known to suffer from immunological deficiency.

TABLE I.—*Age and Sex Distribution of 22 Patients and 23 Controls*

| | | Age in Years | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Patients | M | 1 | | | 1 | 1 | 3 | 3 | 1 | 1 | | 2 | | 2 | 1 |
| | F | | 2 | | | | 1 | 1 | 1 | | | | 1 | | |
| Controls | M | 2 | 1 | 1 | | 1 | 3 | 2 | 2 | | | 1 | | | |
| | F | | 2 | 1 | 1 | | 1 | 2 | 1 | 1 | | | | | 1 |

Before examination sera and C.S.F. specimens were stored at −20° C. for periods of up to six years.

### Complement-fixation Test for Measles and Other Viral Antibodies

The sera were diluted 1:10 in veronal-buffered saline and inactivated at 56° C. for 30 minutes; two of the patients' specimens proved anticomplementary and required a further 30 minutes' heating. Serial twofold dilutions were then made from 1:10 to 1:2,560. The C.S.F. specimens were not inactivated; they were tested undiluted and in serial twofold dilutions up to 1:32. Antigens and control antisera were kindly provided by the Standards Reference Laboratory at Colindale, except for the herpes antigen, which was made in this department from a strain of herpes simplex cultured in a continuous line of rabbit-kidney cells (RK-13). The test was performed by means of a microtechnique on perspex plates, four units of antigen and two units of complement being used. Fixation was allowed to take place overnight at 4° C., and the following day two volumes of 0.4% sensitized sheep red cells were added. The endpoint of the titration was taken as the highest dilution showing complete or almost complete fixation of complement.

Complement-fixation tests on patients' sera and control sera were performed on different days; a positive antiserum and three of the patients' sera were tested on both occasions, the results agreeing to within one dilution. Serum and C.S.F. pairs were titrated together in five cases and separately in four with identical antiserum results.

### Haemagglutination-inhibition Test for Measles

The sera were diluted 1:2 in normal saline and were then absorbed with 25% kaolin for 20 minutes at room temperature, and with 50% Patas red cells overnight at 4° C. The dilution of the specimens after treatment was taken as 1:4 and serial twofold dilutions were prepared from 1:8 to 1:2,048. Four sera were available only at a dilution of 1:10, at which they had been stored for three weeks; they were absorbed in a similar way and diluted from 1:20 onwards. The C.S.F. specimens were not absorbed; they were serially diluted up to 1:32. Haemagglutinating antigen was prepared in this laboratory from an Edmonston B strain of measles virus kindly supplied by Glaxo Laboratories Limited, and was treated with Tween 80 and ether before use. Eight haemagglutinating doses were used, and the test was carried out by a microtechnique in Takatsy plates. Specimens of serum and C.S.F. were

allowed to react with antigen at room temperature for two hours; one volume of 1% Patas cells was then added and the plates were incubated at 37° C. until the cells had settled—that is, for about 45 minutes. The endpoint of the titration was taken as the highest showing complete or almost complete inhibition of agglutination.

All save one of the patients' sera and all the control sera were tested for haemagglutination-inhibiting antibody together on the same day. Five of the serum/C.S.F. pairs were titrated together, and two were done separately; there was insufficient C.S.F. for testing from the remaining two.

## Results

The measles complement-fixing and haemagglutination-inhibiting antibody titres in patients and controls are shown in Tables II and III. Where more than one serum was available from a patient the first specimen has been recorded, except for one haemagglutination-inhibition result, where the second specimen has been recorded because the first had been stored at 1:10 dilution; this can cause a fall in titre. The antibody levels in the patients are much higher than in the controls, and the differences are highly significant: grouping the complement-fixing antibody levels into those of 80 and above and those below 80, $\chi^2=30.35$, $P<0.01$; similarly, grouping the haemagglutination-inhibiting antibody levels into those of 64 and above and those less than 64, $\chi^2=9.79$, $P<0.01$. The patients' levels are comparable to those we have found in convalescent measles patients when using the same technique.

TABLE II.—*Reciprocal of Measles Complement-fixing Antibody Titre in 22 Patients and 23 Controls*

| | Reciprocal of Titre | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | <10 | 10 | 20 | 40 | 80 | 160 | 320 | 640 |
| No. of patients | 7 | 5 | 7 | 2 | 6 | 1 | 10 | 3 |
| „ „ controls | | | | 2 | 2 | | | |

TABLE III.—*Reciprocal of Measles Haemagglutination-inhibiting Antibody Titre in 22 Patients and 23 Controls*

| | Reciprocal of Titre | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | <8 | 8 | 16 | 24 | 32 | 48 | 64 | 96 | 128 | 256 | 512 |
| No. of patients | 2 | 4 | 2 | 1 | 2 | 1 | 3 | 2 | 6 | 2 | 3 |
| „ „ controls | | | 7 | | 4 | | 6 | | | | |

Because patients and controls are not precisely matched for age and sex, the results have been examined for correlation with both these variables for patients and controls, and there is no correlation with either.

Tables IV and V give the antibody levels of patients in whom a neuropathological examination was made, divided into those with inclusion bodies and those without. There is no difference in complement-fixing antibody levels between these groups, and, though haemagglutination-inhibiting antibody levels are higher in the patients with inclusions, the numbers are small and the difference falls short of statistical significance (by Yates's method $\chi^2=3.22$, $0.1>P>0.05$).

TABLE IV.—*Measles Complement-fixing Antibody*

| | Reciprocal of Titres | | | | |
|---|---|---|---|---|---|
| | 40 | 80 | 160 | 320 | 640 |
| Patients without inclusion bodies | 1 | 2 | | 4 | |
| „ with „ „ .. | 1 | 1 | 1 | 3 | 1 |

TABLE V.—*Measles Haemagglutination-inhibiting Antibody*

| | Reciprocal of Titres | | | | | |
|---|---|---|---|---|---|---|
| | 16 | 32 | 64 | 128 | 256 | 512 |
| Patients without inclusion bodies | 1 | 3 | 1 | 1 | 1 | |
| „ with „ „ | | | 1 | 5 | 1 | 1 |

Figs. 1 and 2 show complement-fixing and haemagglutination-inhibiting antibody titres in relation to the duration of neurological symptoms. No correlation is seen and there is no significant rise in titre in the serial specimens available.

The results of complement-fixation tests against other virus antigens are shown in Table VI. Herpes antibody is present with significantly greater frequency in patients than in controls, but 11 patients had no demonstrable herpes antibody. There is no difference in the incidence or titre of other antibodies.

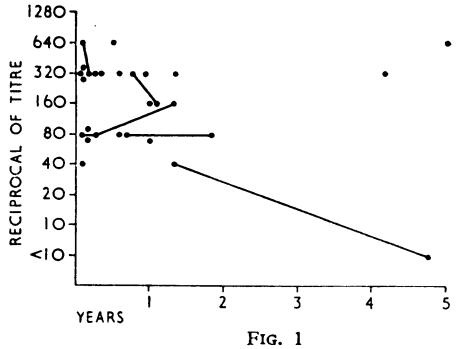


FIG. 1

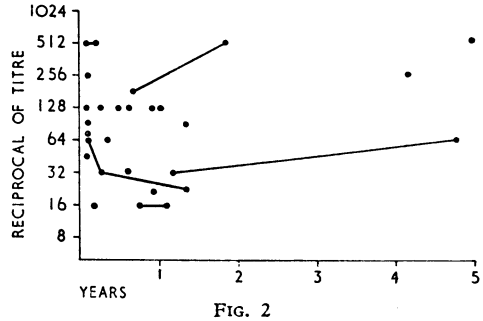


FIG. 2

FIG. 1.—Reciprocal of measles complement-fixing antibody titre related to duration of neurological disease in years in 22 patients. FIG. 2.—Reciprocal of measles haemagglutination-inhibiting antibody titre related to duration of neurological disease in years in 22 patients.

Measles complement-fixing antibody was demonstrated in the C.S.F. in five out of nine patients tested and haemagglutination-inhibiting antibody in seven out of seven. Herpes complement-fixing antibody could be found in only two out of nine. The serum complement-fixing antibody levels obtained in the simultaneous serum and C.S.F. tests were considerably lower than on several previous estimations, owing to deterioration with storage, so an accurate C.S.F./serum ratio cannot be calculated.

TABLE VI.—*Reciprocal Complement-fixing Antibody Titres to Viral Antigens in 22 Patients and 23 Controls*

| | | Reciprocal of Titre | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | <10 | 10 | 20 | 40 | 80 | 160 | 320 |
| Mumps S | Patients | 18 | 2 | 2 | | | | |
| | Controls | 19 | 3 | 1 | | | | |
| „ V | Patients | 13 | 3 | 3 | 1 | 2 | | |
| | Controls | 7 | 4 | 7 | 3 | 1 | | 1 |
| Herpes | Patients | 8 | 5 | 3 | 6 | | | |
| | Controls | 19 | | 1 | 1 | 1 | 1 | |
| LCM | Patients | 21 | 1 | | | | | |
| | Controls | 23 | | | | | | |
| Flu A | Patients | 21 | 1 | | | | | |
| | Controls | 22 | | 1 | | | | |
| „ B | Patients | 20 | 1 | | | 1 | | |
| | Controls | 21 | | 1 | | | 1 | |
| C | Patients | 12 | | 9 | 1 | | | |
| | Controls | 7 | 4 | 8 | 2 | 2 | | |
| Adeno | Patients | 12 | 6 | 3 | 1 | | | |
| | Controls | 13 | 1 | 7 | 1 | | | 1 |

## Discussion

These investigations have revealed abnormally high titres of both complement-fixing and haemagglutination-inhibiting measles antibodies in the serum of 22 patients with subacute sclerosing panencephalitis. Such titres are usually found only in the convalescent phase of measles, after which complement-fixing antibody declines rapidly and haemagglutination-inhibiting antibody more slowly (Krugman *et al.*, 1965). In one of our patients the neurological illness may have begun with an attack of measles which occurred at the age of 8 months and was complicated by corneal ulceration ; her legs became spastic soon afterwards and her subsequent development was retarded, but the full picture of subacute sclerosing panencephalitis was not seen until five years later. However, the remaining 16 patients from whom a history was available had had uncomplicated attacks of measles which preceded their encephalitis by intervals of 3 to 14 years.

Abnormally high titres of measles antibody have also been found in the serum of patients with multiple sclerosis (Adams and Imagawa, 1962 ; Reed *et al.*, 1964 ; Clarke *et al.*, 1965), and this has not yet been explained. In subacute sclerosing encephalitis, however, the supporting evidence of electron-microscopy and immunofluorescence justifies the suggestion that measles, or an antigenically related virus, is the cause of the disease. If this is so three possible mechanisms can be considered.

Firstly, measles virus might have entered the central nervous system at the time of the original attack and remained latent there for some years before being reactivated and producing disease. This would be analogous to the generally accepted pathogenesis of varicella-zoster infection in man, and to the deferred encephalitis which can be produced experimentally in rabbits infected with herpes simplex (Good and Campbell, 1948 ; Schmidt and Rasmussen, 1960). There is a certain amount of evidence that the central nervous system can be involved in natural measles infection. In uncomplicated cases Ojala (1947) found pleocytosis in the C.S.F. in some instances, and Pampiglione (1964) discovered E.E.G. abnormalities in all cases tested. Adams *et al.* (1966) described measles-type inclusions in the brains of patients who had died of acute measles encephalitis. Thus the virus often affects the nervous system in some way, and may gain entry to it in certain circumstances. There is support for the concept of latency in the work of Enders-Ruckle (1965), who reported the isolation of measles virus from lymph nodes and spleen some years after measles infection. At present, however, there is no other recorded example of latent infection with measles or with any other ribonucleic acid virus.

Secondly, the disease may be caused by reinfection with measles in an already immune or partially immune person. It is generally accepted that immunity to measles is long-lasting, but this is based on the fact that second attacks of measles with a rash are exceedingly rare, and the sustained immunity may itself be the result of repeated subclinical infection (Krugman *et al.*, 1965). We have not found any history of exposure to reinfection in our cases.

Thirdly, the disease may be due to a virus related to measles, such as canine distemper virus, which can certainly produce a subacute as well as an acute neurological disease in dogs. At present these two viruses cannot be clearly differentiated on the basis of serum antibodies, immunofluorescence, or electron-microscopy. One of our patients had a pet ferret which died just before his encephalitis began, but we have found no other history of specific contact with animals likely to have been suffering from distemper virus infection.

With any of these mechanisms one would expect the antibody titre to rise in the early stages of the neurological disease, unless latent measles infection had provided a continuing stimulus to antibody production ever since the original attack of measles. We do not know whether these patients' antibody levels were normal in the interval between their original attack and the onset of their encephalitis, and we have not demonstrated a significant rise in titre with progress of the disease. However, in only one case was a specimen of serum obtained in the first month of illness, so that an early rise could have been missed. We have not found any parallel to the case described by Connolly *et al.* (1967) in which the antibody titres rose markedly over a period of 10 months. The mortality rate in this condition is of course high, but in three of our patients the disease has become arrested, and in one there has been a good recovery after about a year's illness. Four years after recovery this patient's complement-fixing antibody level has fallen to an undetectable level, but haemagglutination-inhibiting antibody has not altered significantly.

There is now evidence that measles-like virus particles and virus antigen are present in the brain in this disease, but despite many virological experiments the virus has never been isolated from brain tissue. This situation may be analogous to the experimental work of Rustigian (1962), who described a chronic measles infection in HeLa cells ; the infection could be demonstrated by the presence of typical inclusion bodies, specific immunofluorescence, and complement-fixing antigen, but the virus could not be passaged to other tissue cultures.

## Summary

Measles complement-fixing and haemagglutination-inhibiting antibodies have been found in the serum of 22 patients with subacute sclerosing panencephalitis in significantly higher titre than in controls. These findings support the results of electron-microscopy and immunofluorescence in suggesting that the disease is due to a measles-like virus. The evidence does not at present enable us to differentiate between a latent measles infection which has become reactivated, a reinfection with measles, and a primary infection with a related agent such as canine distemper virus.

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