Spinal-fluid Gamma-globulin and Multiple Sclerosis*

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It has been known for more than 20 years that the gamma-globulin content of the spinal fluid is often disproportionately increased in multiple sclerosis (Kabat, Landow, and Moore, 1942). This finding has been confirmed by a variety of techniques and is now of established value in the diagnosis of multiple sclerosis. However, the quantitative estimation of spinal-fluid gamma-globulin is not widely available as a routine diagnostic test, presumably because the original electrophoresis and immunochemical techniques are difficult and laborious. Simpler methods for estimating spinal-fluid gamma-globulin were introduced following Kunkel's (1947) demonstration that zinc sulphate precipitates gamma-globulin selectively from dilute serum (Donovan, Foley, and Moloney, 1951; Roboz, Hess, and Forster, 1953; Papadopoulos, Hess, O'Doherty, and McLane, 1959), and it has been claimed that such methods are as accurate as aids to the diagnosis of multiple sclerosis as are electrophoresis and immunochemical methods (Foster and Horn, 1962; Tourtellotte, 1963; Castaigne, Cambier, and Schuller, 1965).

A simple zinc sulphate method for estimating spinal-fluid gamma-globulin has been in routine use in the Royal Victoria Infirmary, Newcastle upon Tyne, for the past five years. The following report outlines the results obtained during this period.

Methods

The method of Papadopoulos *et al.* (1959) was used for gamma-globulin determinations. It was found that thorough mixing was very important when adding the Folin-Ciocalteu reagent used for total protein determinations. Throughout the study the tests were carried out by a junior technician.

Results

A total of 2,313 spinal fluids from all departments in the hospital were examined (Table I). The mean value of the gamma-globulin proportion of the total spinal-fluid protein for the whole series, including blood-stained specimens, was 12.9% (S.D. 9.4). Excluding patients with multiple sclerosis and neurosyphilis, the mean was 12.0% (S.D. 8.5), and the statistically derived figure of 29% (mean plus 2 S.D.) constitutes the upper limit of the spinal-fluid gamma-globulin fraction in "miscellaneous" neurological diseases. Less than 5% of specimens from this group will yield gamma-globulin levels above this figure. From the clinical viewpoint it is more useful to know the proportion of patients with multiple sclerosis who have gamma-globulin levels above 29% than to know the proportion in whom the level is above the upper limit for "normal" spinal fluid, which, depending on the method used, lies between 13% and 16% of the total protein (Foster and Horn, 1962).

TABLE I.—*Spinal-fluid Gamma-globulin Expressed as Percentage of Total Protein*

	Mean	S.D.	Mean + 2 S.D.
Whole series—2,313 spinal fluids ..	12·9	9·4	
Whole series, excluding M.S. and neurosyphilis	12·0	8·5	29
Multiple sclerosis—167 spinal fluids ..	26·8	12·6	
Neurosyphilis—10 spinal fluids ..	36·5	7·6	

After excluding specimens containing more than 20 red blood cells per c.mm. and specimens obtained by ventricular tap, conditions in which gamma-globulin levels above 29% were found in more than 5% of patients fell into four groups (Table II).

TABLE II.—*Diseases with Gamma-globulin Above 29% of Total Protein Occurring in More than 5% of Patients*

	No. of Patients	Above 29%	
		No.	%
Multiple sclerosis	167	74	44
Neurosyphilis	10	9	90
Diseases associated with a high serum globulin	21	9	43
Encephalitis (?Viral)	8	2	
Encephalomyelitis			
After vaccination	1	1	50
„ rubella	1	1	
„ polio inoculation	1	1	
Measles polyradiculitis	1	1	

Multiple Sclerosis

After excluding specimens with more than 20 red blood cells per c.mm. 167 spinal fluids from patients with multiple sclerosis were available for analysis. The mean gamma-globulin was 26.8% (S.D. 12.6). This agrees closely with the mean of 25% (S.D. 5.0) found by Papadopoulos, Hess, O'Doherty, and Wakeman (1963) using the same method. Of the 167 patients, 44% had levels above 29%. In other words, almost half of the patients with multiple sclerosis gave a gamma-globulin level above a figure which was exceeded by less than one in 20 specimens from other diseases. The only exceptions were neurosyphilis, diseases associated with high serum-globulin levels, and encephalitis and encephalomyelitis. No correlation was found between the gamma-globulin level and the duration of the disease, the clinical activity of the disease, and the presence or absence of a paretic Lange curve. This is in agreement with previous reports in which paper electrophoresis and immunochemical methods were used to determine spinal-fluid gamma-globulin (Yahr, Goldensohn, and Kabat, 1954; Ivers, McKenzie, McGuckin, and Goldstein, 1961; Tourtellotte, 1963; Bradshaw, 1964). On the other hand, there was a correlation between the gamma-globulin proportion and the extent of neurological involvement as estimated by clinical evidence and also with the spinal-fluid white-cell count.

The mean gamma-globulin for severely disabled patients was 30.6% and the mean for the slightly disabled was 27.1%. This difference does not reach statistically significant levels, but the trend is in agreement with the findings of Yahr *et al.* (1954), Schapira and Park (1961), and Tourtellotte (1963), and supports the view of Bradshaw (1964) that the test is rather less useful in that situation where laboratory substantiation would be of particular value—that is, in patients with minimal

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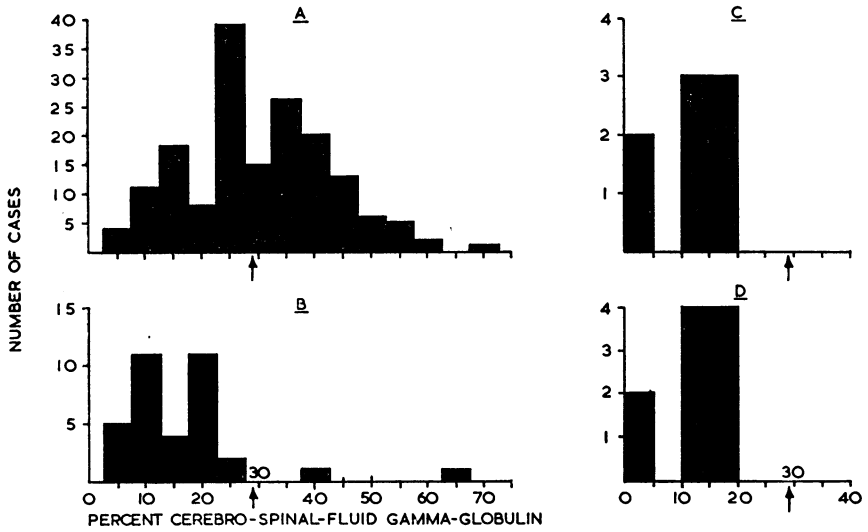
lesions. Patients with a normal spinal-fluid white-cell count (less than 5/c.mm.) had a mean gamma-globulin proportion of 25.1%. The mean gamma-globulin proportion in patients with more than 10 white cells per c.mm. was 30.3%, the difference being statistically significant (P = 0.01 – 0.05). Using a paper-electrophoresis method, Ivers *et al.* (1961) also found a correlation betwen the presence of an excessive number of white cells in the spinal fluid and the gamma-globulin proportion, as did Tourtellotte (1963) and Yahr *et al.* (1954).



Percentage of spinal-fluid gamma-globulin in (A) multiple sclerosis, (B) cervical spondylosis, (C) intraspinal tumours (meningiomas and neurofibromas), and (D) hereditary ataxias and familial spastic paraplegia.

Three conditions which are occasionally difficult to distinguish clinically from multiple sclerosis are represented in the Chart. Over the period of the present study eight patients with spinal meningiomas and neurofibromas and 10 patients with familial spastic paraplegia and hereditary ataxias had non-traumatic lumbar punctures, and 34 specimens of spinal fluid with less than 20 R.B.C./c.mm. were obtained from 31 patients with cervical spondylosis, of whom 22 had had myelography and 11 laminectomy. It can be seen that few patients with these three diseases had gamma-globulin levels above 29%.

Neurosyphilis

After excluding specimens with more than 20 red blood cells per c.mm. 10 patients with neurosyphilis were examined. The mean gamma-globulin proportion was 36.5% (S.D. 7.68). All but one had values above 29%: all had more than 80 mg. of total protein per 100 ml.

Diseases Associated with High Serum-globulin Levels

High levels of serum globulin are a recognized cause of raised spinal-fluid gamma-globulin whether or not there is clinical evidence of neurological involvement (Yahr *et al.*, 1954; Castaigne *et al.*, 1965). Patients with high serum globulins whose spinal fluid was examined during the present study are presented in Table III. All with spinal-fluid gamma-globulin levels above 29% had raised serum globulins. The reasons for spinal tap in these patients are shown in Table III.

TABLE III.—*Spinal-fluid Gamma-globulins in Patients with High Serum Globulins*

	No. of Patients	Gamma-globulin Expressed as % of Total Protein
Collagen diseases:		
Temporal arteritis	3	18, 25, 16
Rheumatoid arthritis (headache)	1	*34*
Polyarteritis nodosa (paraplegia)	1	*30·6*
Haemolytic anaemia ?Collagen disease (confusion)	1	*34*
Multiple myeloma (paraplegia)	1	*32*
Carcinomatous meningitis	5	*39, 31·2*, 23, 25, 22
Wernicke's encephalopathy	2	*50*, 22
Myxoedema (cerebral embolus; meningioma)	7	*30, 29·1*, 25, 5, 3·9, 19, 4·9

Encephalitis and Encephalomyelitis

Twelve patients with encephalitis and encephalomyelitis had non-traumatic spinal taps during the period of the present study (Table II). Two of the eight patients with encephalitis—presumed viral in origin on clinical evidence though without isolation of the virus—had levels above 29%, and all patients diagnosed clinically as suffering from para-infective perivenous encephalomyelitis had high levels. In contrast less than 5% of patients with cerebral abscesses and purulent meningitis had gamma-globulin levels above 29%. Among miscellaneous causes found in the literature for very high gamma-globulin levels post-vaccinial and other types of perivenous encephalomyelitis are recorded, though they have not been singled out for special attention. Ivers *et al.* (1961), in particular, recorded 6 out of 13 patients with "encephalitis" who had high levels of gamma-globulin.

Kabat, Freedman, Murray, and Knaub (1950) recorded one case of post-vaccinial encephalomyelitis associated with a high spinal-fluid gamma-globulin, while Ziegler and Ross (1955) and Castaigne *et al.* (1965) also recorded cases, and Foster and Horn (1962) reported one instance of measles polyradiculopathy associated with a high spinal-fluid gamma-globulin. Further work may show that spinal-fluid gamma-globulin estimation is of value in distinguishing encephalomyelitis of the type thought to be allergic in nature from true virus encephalitis.

Apart from these four conditions a variety of diseases showed occasional spinal fluids with levels above 29% among the 2,313 spinal fluids examined (Table IV). After excluding cases with more than 20 red blood cells per c.mm. and ventricular taps

TABLE IV.—*Miscellaneous Causes of a High Gamma-globulin (More than 29%) in 2,313 Routine Spinal Fluid Examinations*

	No. of Patients	Gamma-globulin (%)
Cerebral infarction	3	33, 30, 30
Glioma	3	34, 32·2, 29
Cerebral abscess	1	34
Meningococcal meningitis	1	41
Lymphocytic meningitis and epilepsy	1	45
Epilepsy ?cause	1	32
Headache ?cause	1	34
Stupor ?cause	1	36
Pneumonia and confusion	1	31, 31 (2 specimens)
Pre-senile dementia	1	38
Obesity ?hypothalamic lesion	1	34
Optic atrophy	1	35
Chronic polyneuropathy	1	29
Guillain–Barré syndrome	1	30
Brachial neuritis	1	30
Sciatica	2	42, 30
Paraplegia ?cause	1	31
Monoplegia ?cause	1	37
Doubtful multiple sclerosis	3	36, 31, 60

26 specimens were found in which the level was above 29%. Some almost certainly have multiple sclerosis. The high gamma-globulin level in one case of pre-senile dementia is of interest, as others have reported this among miscellaneous causes of high levels of gamma-globulin (Yahr *et al.*, 1954). Within the more common disorders shown in Table IV fewer than 5% of patients yielded spinal-fluid gamma-globulin levels above 29%.

Technical Factors

The development of spinal-fluid gamma-globulin estimation as a diagnostic test depends on the consistency of results in any one disease and its independence of variables normally associated with lumbar puncture. A limited investigation of the effects of these variables was undertaken.

1. *Quantity of Spinal Fluid.*—Fourteen patients had gamma-globulin estimated in an initial 3-ml. specimen and in a subsequent 12-ml. specimen removed at the same tap. No significant difference was found in the proportion of gamma-globulin in the two specimens.

2. *Effect of a Recent Lumbar Puncture.*—Although repeated estimations of gamma-globulin in the same specimen of spinal fluid gave consistent results, specimens obtained at second lumbar puncture did not, even when circumstances suggested little change in the patient's clinical state. Five patients had lumbar punctures repeated within 14 days. In all patients the gamma-globulin proportion was lower at the second lumbar puncture, the average fall being 50%. Further lumbar puncture within 12 months of a previous myelogram yielded variable results, the gamma-globulin content being sometimes increased and sometimes reduced. However, only one patient in seven with original gamma-globulin levels below 29% showed a rise above this level after myelography.

3. *Blood Contamination.*—The effect of blood contamination on the spinal-fluid gamma-globulin level was determined by serial dilution of blood with spinal fluid. Contamination by fewer than 650 cells per c.mm. had no effect on the total protein or on the percentage of gamma-globulin present, whether the specimen was examined spun or unspun, and the spinal-fluid gamma-globulin percentage varied by less than 1% in these two series of estimations. In the present study, spinal fluids containing more than 20 red blood cells per c.mm. were excluded to conform with previous studies, but in fact this appears to have been unnecessary.

4. *Ventricular Fluid.*—Seven patients with Parkinson's disease had lumbar punctures carried out 30 minutes before ventriculography. All but one ventricular fluid contained more than 1,000 red blood cells per c.mm. The average total ventricular-fluid protein was 19 mg./100 ml. and the average lumbar-fluid protein was 40 mg./100 ml. The gamma-globulin proportion, however, averaged 36% in the ventricular fluids and 20% in the lumbar fluids.

5. *Effects of Delay in Examining Spinal Fluid.*—Unspun specimens with varying degrees of contamination ranging from 2 to 1,000 red blood cells per c.mm. were examined immediately after lumbar puncture and 24 hours later. The gamma-globulin content did not differ significantly between these two estimations.

Thus it would appear that a recent lumbar puncture, myelography within the preceding 12 months, or the use of ventricular fluid invalidates the diagnostic significance of the spinal-fluid gamma-globulin proportion. On the other hand, specimens need not be examined immediately, nor does the quantity of fluid removed at lumbar puncture appear to affect the result. Contamination with blood, at least to levels at which it is not apparent to the naked eye, can also be disregarded.

Discussion

Spinal-fluid gamma-globulin estimations are generally accepted as the best available laboratory indication of multiple sclerosis. The more commonly used tests are most useful in a negative sense: in a series of 700 patients with multiple sclerosis seen at the National Hospital, Locoge and Cumings (1958) found a total protein of more than 100 mg./100 ml. in one specimen in 25, as few as one or two neutrophils per c.mm. less often than once in every 100 cases, and a total mononuclear count greater than 50/c.mm. once in every 200 specimens. On the other hand, they found that the test most widely used to provide positive evidence of multiple sclerosis—a paretic Lange curve with a negative Wassermann reaction—was found in 12.2% of patients.

In the present study, 44% of patients with multiple sclerosis had gamma-globulin proportions *above the upper limit found in other neurological diseases.* The study of Schapira and Park (1961) on the paper electrophoresis determination of spinal-fluid gamma-globulin in multiple sclerosis is of interest in this connexion since it was carried out in our laboratory. Their figures demonstrated that 52% of patients with multiple sclerosis had gamma-globulin levels above a figure which was exceeded by fewer than 5% of patients with other neurological diseases. Tourtellotte (1963) and Castaigne *et al.* (1965), using zinc-sulphate-precipitation methods, found between 65 and 75% of multiple sclerosis patients with spinal-fluid gamma-globulin above the normal range. This compares favourably with the incidence of values above the normal range found in unselected multiple sclerosis patients by electrophoresis methods —between 40 and 67%—and by immunochemical methods—between 50 and 67% (Yahr *et al.*, 1954 ; Ivers *et al.*, 1961 ; Bradshaw, 1964). Hass and Hochwald's (1961) demonstration that even small increases in the proportion of gamma-globulin cause some albumin and some alpha-1 and alpha-2 globulin to be precipitated may explain why zinc sulphate methods have sometimes shown a higher incidence of "abnormality" in multiple sclerosis than has been obtained by other techniques.

Summary

A simple zinc sulphate precipitation method for estimating spinal-fluid gamma-globulin has been used in the routine examination of 2,313 spinal fluids. Of the patients with multiple sclerosis, 44% had values above 29% of the total protein. Except for neurosyphilis, diseases associated with high serum-globulin levels, and certain encephalitides, this figure was exceeded in fewer than 5% of patients with other neurological diseases. In multiple sclerosis the test is rather less useful in the mild case. Gamma-globulin proportions determined by this method do not correlate with clinical activity or duration of the disease or with the Lange result, but are related to the spinal-fluid white-cell count and to the apparent extent of pathological involvement of the nervous system. A recent lumbar puncture or myelogram invalidates the significance of the test. It is suggested that the simplicity, reliability, and value of zinc-sulphate-precipitation methods of estimating spinal-fluid gamma-globulin commend this test for routine diagnostic use.

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