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GAMMA GLOBULIN STUDIES IN MULTIPLE SCLEROSIS AND THEIR APPLICATION TO THE PROBLEM OF DIAGNOSIS

BY

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The demonstration of the association between a positive colloidal gold reaction and an increase in the γ-globulin in the cerebrospinal fluid (Kabat, Landow, and Moore, 1942) has stimulated further study of this protein fraction. An increase in this moiety in the spinal fluid of patients with multiple sclerosis has been reported by workers using free-solution and paper electrophoresis, as well as immunochemical methods. It has been shown (Yahr, Goldensohn, and Kabat, 1954) that the serum γ-globulin remains unaltered, although changes have been reported in the serum albumin and α- and β-globulin concentrations (Volk, Saifer, and Rabiner, 1954; Press, 1956). The present study is primarily concerned with the γ-globulin fraction of the cerebrospinal fluid as determined by filter paper electrophoresis. The application and limitations of this estimation in the diagnosis of multiple sclerosis are discussed.

Material

The cerebrospinal fluids of 156 patients together with the sera in some of these cases were examined by means of paper electrophoresis. This series of patients consisted of three groups:—

Group 1: 81 Patients with Multiple Sclerosis.—In 13 patients the spinal fluid was examined during the first episode of the disease, and in 12 patients during an acute exacerbation characterized by significant worsening of existing symptoms or clinical evidence of fresh lesions of less than two weeks' duration. In the remaining 56 patients the disease was considered to be chronic: there were permanent symptoms (often with severe disability) which were slowly progressive or in some cases static. The diagnosis in all instances was unequivocal; doubtful cases were excluded from the study.

Group II: 50 Patients with Other Neurological Disorders.—These are listed in Table I.

Group III: 25 Control Cases.—These were regarded as "normal" for the purpose of this study, and consisted largely of patients with psychiatric illnesses, in whom clinical examination failed to reveal any evidence of organic nervous disease, and in whom spinal fluid examination by routine methods (protein, cells, Lange colloidal gold curve) gave results within the normal range. The clinical diagnoses in this group were depression (12 cases), hysteria (three), headache without abnormal physical signs and negative ancillary findings (three), anxiety state (two), and single cases of cough syncope, schizophrenia, otosclerosis, frontal sinusitis, and cerebral arteriosclerosis.

Method

Gamma globulin concentrations in cerebrospinal fluid and sera were estimated by quantitative filter paper electrophoresis. The procedure evolved in this study was designed for the analysis of small amounts of cerebrospinal fluid. In most cases about 5 ml. of spinal fluid was required, the precise volume depending upon the initial total protein concentration.

The total protein content was estimated by the method of Lowry, Rosebrough, Farr, and Randall (1951) which is a simple but sensitive colorimetric technique requiring only 0·1 or 0·2 ml. of spinal fluid. The cerebrospinal fluid was then concentrated to a protein content suitable for electrophoresis. This was achieved by means of ultrafiltration against negative pressure through Visking dialysis tubing. A convenient and reliable means of concentration is a most important feature of the analysis. Ultrafiltration was allowed to proceed until a protein concentration of approximately 2 g. per 100 ml. was obtained. The protein content of the sera was adjusted to similar concentration by dilution with saline.

Electrophoresis was carried out in the apparatus designed by Latner (1952) using Whatman No. 100 filter paper strips (46 cm. $\times$ 2 cm.) and barbitone buffer,

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TABLE I

DIAGNOSTIC ANALYSIS OF "OTHER NEUROLOGICAL CONDITIONS" WITH γ GLOBULIN VALUES

Condition	No. of Cases	Spinal Fluid γ Globulin as % of Total Protein
Epilepsy	10	13·3, 12·0, 11·6, 10·8, 10·5, 9·8, 7·8, 6·5, 5·8 4·6
Cerebral arterial diseases	7	19·0, 16·4, 15·4, 13·4, 11·6, 8·2, 4·7
Hereditary degenerative disorders	7	19·5, 18·5, 15·2, 14·0, 8·6, 7·7, 7·5
Cervical spondylosis	5	20·4, 14·4, 12·6, 12·4, 10·8
Migraine	3	13·7, 11·3, 10·0
Prolapsed intervertebral disc	2	12·3, 8·6
Motor neurone disease	2	12·7, 12·5
Spinal cord tumours	2	14·7, 12·0
Neurosyphilis	2	20·0, 15·8
Polyneuritis	2	25·5, 11·5
Brain tumour (metastases)	1	19·7
Muscular dystrophy	1	14·0
Subacute combined degeneration	1	11·0
Myalgia paraesthetica and Paget's disease	1	18·6
Polymyositis	1	17·5
Ménière's syndrome	1	7·4

TABLE II

MEAN VALUES AND STANDARD DEVIATIONS FOR γ GLOBULIN, ALBUMIN, A/G RATIO, AND TOTAL PROTEIN IN SPINAL FLUID

	No. of Cases	γ Globulin (as % of total protein)		Albumin (absolute amount) (mg./100 ml.)		Albumin: γ Globulin Ratio		Total Protein (mg./100 ml.)	
		Mean %	S.D.	Mean %	S.D.	Mean %	S.D.	Mean %	S.D.
Controls	25	11·1	±3·18	31·5	±9·32	6·6	±2·32	46·2	±12·36
Multiple sclerosis	81	22·4	±9·47	35·6	±9·74	3·5	±1·98	56·4	±13·42
Other neurological conditions	50	13·0	±4·57	38·0	±13·89	6·4	±3·20	58·6	±31·39

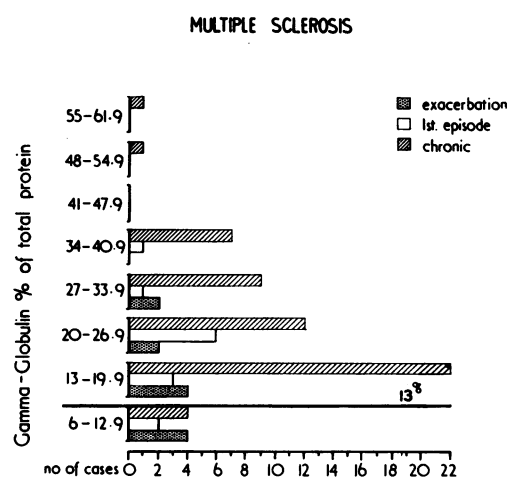
*p*H 8·6, μ0·05. A current of 0·4 mA per paper strip was applied for 16 hours. The strips were dried in a hot air oven and stained with Lissamine green. A double-beam automatic recording reflectance densitometer (Joyce, Loebel & Co., Ltd., Newcastle upon Tyne) was used to scan the stained strips. The method (Park, 1959) of analysing the diagrams obtained and the precautions taken will be published in detail elsewhere (Latner and Park, in preparation). Proof of the validity and reliability of the method was obtained by analysing protein mixtures containing albumin, β-, and γ-globulin in known proportions.

Results

The mean values and standard deviations of the albumin and γ-globulin fractions in the cerebrospinal fluid are given in Table II. This shows a highly significant increase of the γ-globulin fraction in multiple sclerosis when compared with the control group ($P<0·001$). There is also a highly significant increase of γ-globulin in multiple sclerosis when compared with the group of other neurological conditions ($P \ll 0·001$).

That this is due to an absolute increase in γ-globulin is demonstrated by the fact that the albumin/γ-globulin ratios show similar significant differences, whereas there is no significant difference when these groups are compared with respect to their absolute albumin content.

Applying a 99% upper confidence limit to the mean of the control group gives a value of 13% for the spinal fluid γ-globulin (expressed as a percentage of the total protein). Figure 1 shows that this value is exceeded in 88% of patients with multiple sclerosis, in 83% of patients in the first episode, in 67% of



Distribution of Spinal fluid gamma-globulin (expressed as % of t.p.)

FIG. 1

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TABLE III

MEAN VALUES AND STANDARD DEVIATIONS FOR γ GLOBULIN, ALBUMIN, A/G RATIO, AND TOTAL PROTEIN IN SERUM

	No. of Cases	γ Globulin (as % of total protein)		Albumin (absolute amount) (g./100 ml.)		Albumin: γ Globulin Ratio		Total Protein (g./100 ml.)	
		Mean %	S.D.	Mean %	S.D.	Mean %	S.D.	Mean %	S.D.
Controls	16	21·5	±6·01	4·5	±0·64	3·1	±1·06	7·4	±0·80
Multiple sclerosis	27	20·6	±4·06	4·2	±0·66	3·1	±1·02	7·1	±0·61
Other neurological conditions	22	18·6	±4·29	4·8	±0·66	4·3	±2·27	7·6	±0·76

patients during exacerbations, and in 93% of chronic established cases.

Table III gives the mean values and standard deviations of the albumin and γ-globulin fractions in the sera examined. It is seen that the mean γ-globulin concentration in the multiple sclerosis group shows no significant difference from the control cases ($P>0·5$), or from the group of other neurological conditions ($P>0·6$).

In both Tables II and III we have expressed the γ-globulin fraction as a percentage of the total protein, since this value is then independent of changes in the total protein.

Discussion

In applying these results to the problem of diagnosis in multiple sclerosis, three aspects require particular consideration.

Does this method provide a greater aid in diagnosis than the usual routine laboratory examination of the spinal fluid? Secondly, in what percentage of multiple sclerosis cases is the γ-globulin content of the spinal fluid raised, both in the cases as a whole, and in various phases of the disease? This latter distinction we consider important, since an aid in diagnosis would be particularly valuable during the initial episode of the disease. Thirdly, and perhaps most important of all, does the estimation of γ-globulin help in the differentiation of multiple sclerosis from other neurological conditions with which it is likely to be confused clinically?

Figure 1 shows that a raised spinal fluid γ-globulin (>13% of total protein) was found in 88% of all cases of multiple sclerosis; conventional laboratory examination, however, revealed abnormalities in only 38% of these cases. It is apparent, therefore, that although the spinal fluid γ-globulin is not raised in every instance of multiple sclerosis, the high proportion of abnormal results yielded by this estimation is of clinical value. The pathogenetic significance of this finding is still far from clear and the assumption that it is a manifestation of an immunological disturbance has recently been questioned (Field and Ridley, 1960).

The γ-globulin levels in the various phases of the disease are graphically represented in Figure 1: an increase occurs in 93% of chronic established cases, in 83% of patients in the first episode, and in 67% of patients during exacerbations. The highest incidence of a raised γ-globulin level occurs in the spinal fluid of patients in whom the disease is well established, due to multiple lesions, and usually associated with marked disability, a finding which is in agreement with that of other workers (Yahr *et al.*, 1954). Although the clinical diagnosis usually presents little difficulty in this group of patients, we have included in our series five patients presenting with a featureless chronic paraplegia. Such cases are often presumptively regarded as instances of multiple sclerosis when myelography and other special investigations have failed to provide an alternative diagnosis. It has been our experience that routine spinal fluid examination has rarely furnished information of diagnostic value in this connexion, and it is of some interest, therefore, that the spinal fluid γ-globulin was raised in three of these five patients.

During the initial episode of the disease when diagnosis is important and often difficult, the γ-globulin level was raised in 10 out of 12 patients, whereas routine spinal fluid examination revealed abnormalities in only half of these cases.

A further analysis of conditions likely to be confused clinically with multiple sclerosis is of interest. Thus, the γ-globulin content was within normal limits in three out of five cases of cervical spondylosis, in both cases of prolapsed intervertebral disc, in three out of seven cases of hereditary degenerative disorders. Although the above constitute only a small number of cases, the diagnostic help which may be obtained from the estimation of gamma globulin is well illustrated in the following case:—

Mrs. D. B., a 27-year-old housewife, was first admitted under the care of Dr. Henry Miller in October, 1959, with a history of progressive weakness of both legs associated with some unsteadiness of 16 months' duration. The symptoms began quite abruptly while the patient was at a dance, and were followed soon after by some weakness

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and clumsiness of both hands. There was no fluctuation of the severity of the complaints apart from the fact that the patient felt that her symptoms were less marked towards the end of the day.

The only other complaints were those of burning pains between her shoulder blades radiating down the spine, which followed a fall six months previously, and an occasional sensation of "pins and needles" in both calves.

Direct questioning revealed no other significant neurological symptoms, and her general health had been good apart from some weight loss (8 lb. in three months).

Her past history and family history revealed no significant features.

There were no abnormal physical signs in the cranial nerves. The arms were slightly spastic, but there was no wasting of the hands or fasciculation of muscles. Increased tone was very evident in both legs, and these features were associated with some weakness, marked increase of tendon reflexes, bilateral ankle clonus, and extensor plantar responses. There was no sensory loss, but some incoordination of both arms (R > L) could be demonstrated. The abdominal reflexes were retained. Full blood investigations, including Wassermann reaction and radiological studies of the skull, chest, and cervical spine, were normal. On lumbar puncture the pressure was 55 mm. of spinal fluid with normal manometrics. A clear colourless fluid was obtained with less than 1 cell per c.mm.; W.R. negative, Lange: 0000000000. The γ globulin content of spinal fluid was 3·8 mg. %, *i.e.*, 7% of total protein.

In spite of some unusual features, *i.e.*, retained abdominal reflexes and the absence of any sensory loss, a diagnosis of multiple sclerosis was considered to be the most likely. The low spinal fluid γ globulin content, however, was very much against this diagnosis, particularly since by this time symptoms were well established and the disease was progressing relentlessly.

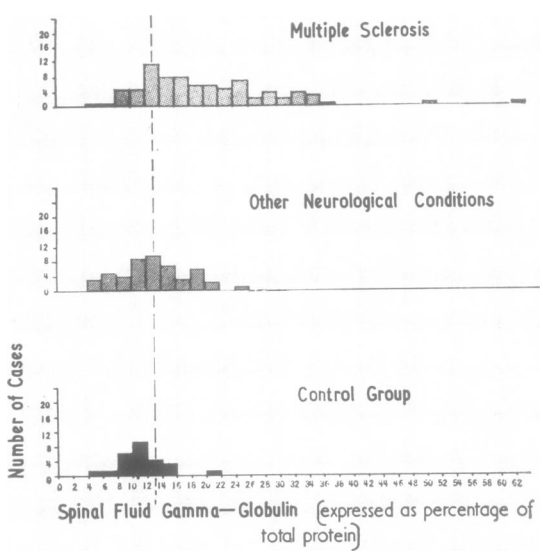

FIG. 2

The patient was seen at regular intervals in the outpatient department when in October, 1960, the correct diagnosis of motor neurone disease became obvious by the development of fasciculation of the tongue, and gross wasting of both arms. The patient had recently complained of marked difficulty in speaking and swallowing.

That an elevated γ-globulin content of the cerebrospinal fluid is not pathognomonic of multiple sclerosis and occurs also in some cases of a heterogeneous group of neurological disorders is illustrated in Fig. 2. A statistical comparison of the two groups, however, shows a highly significant difference ($P \ll 0{\cdot}001$). It may also be seen from Fig. 2 that a figure of over 18% γ-globulin (expressed as % of total protein) is very suggestive of multiple sclerosis, no fewer than 62% of cases of multiple sclerosis gave figures above that level, compared with only 16% of the other neurological conditions, and 4% of the controls.

Summary

Gamma-globulin levels in cerebrospinal fluid and serum have been estimated by means of quantitative paper electrophoresis.

Examination of the serum revealed no significant changes of this fraction in patients with multiple sclerosis.

However, a significant increase of γ-globulin was found in the cerebrospinal fluid of patients with multiple sclerosis when compared with the control group, this method yielding a much higher proportion of abnormal results than conventional laboratory examination.

The spinal fluid γ-globulin levels found in multiple sclerosis were also significantly higher than those found in a group of miscellaneous neurological disorders.

The estimation of γ-globulin in the cerebrospinal fluid is considered to be of help in the clinical diagnosis of multiple sclerosis.

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