
INTRATHECAL TUBERCULIN IN DISSEMINATED SCLEROSIS: THE IMMUNOLOGICAL ASPECTS

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Four years ago a preliminary report was published on the intrathecal injection of the purified protein derivative of tuberculin (P.P.D.) in disseminated sclerosis (Smith, Espir, Whitty, and Russell, 1957). In someone sensitized to tuberculin, whether as a result of natural infection or of vaccination, these injections provoke a wave of meningitis which is the expression of a true, specific allergic reaction (Switchenbank, Smith, and Vollum, 1953). It has been suggested that, in disseminated sclerosis, an alteration in formation and flow of the cerebrospinal (C.S.F.) and of the blood-cerebrospinal fluid barrier, such as occurs in meningitis, might influence the course of the disease for the better (Bourdillon, Fischer-Williams, Smith, and Taylor, 1957). The intrathecal injection of P.P.D. is the least objectionable way of setting up a meningitis, since the reactions are sterile and self-limiting, and experience gained in treating tuberculous meningitis has shown that it is possible to evolve a scheme of dosage which minimizes the dangers (Smith, Vollum, Taylor, and Taylor, 1956). Further, the reactions markedly and constantly increase the permeability of the blood-C.S.F. barrier (Smith, Taylor, L. M., and Hunter, 1955). Further, these reactions offer a direct method for testing once again the well-known hypothesis that hyperaemia of the neuraxia would be beneficial, since animal experiment has shown that the inflammation is not limited to the leptomeninges but also involves the perforating vessels of the brain-stem (Bosanquet, Daniel, and Vollum, 1953). For these reasons volunteers for this hitherto untried form of treatment were sought from amongst our most severely affected patients.

In the event this method of treatment proved to have yet another advantage. Ever since tuberculin was first prepared (Koch, 1890) its antigenic properties have been studied intensively, both in the field and in the laboratory, and its name has been given to one main kind of allergy, the "delayed" or "tuberculin type" of sensitivity. Its intradermal and intrathecal use in disseminated sclerosis has therefore provided data for a study of the "delayed" type of immune response in this disease.

In our preliminary report we described the intrathecal reactions in disseminated sclerosis and showed that they differed markedly and consistently in pattern from those seen in cases of different kinds of psychosis. Reasons were given for accepting the latter as exemplifying the normal, and it was concluded that the distortion of pattern seen in disseminated sclerosis was evidence of an immunological disturbance in that disease. This conclusion was supported by the finding that certain tuberculin-negative patients proved surprisingly refractory to vaccination with B.C.G. or Wells' vole bacillus vaccine (W.V.V.). Finally, the hope that these reactions might have a therapeutic value was encouraged by the fact that, at the time when our report was published, no patient had relapsed or continued to grow worse since treatment (Smith et al., 1957).

Many patients did, indeed, improve. Without making any claims to a causal relationship between treatment and improvement, it was at least clear that the reactions neither prevented nor delayed remission. This being so, we steadily expanded this study until, at the present time, over 280 patients have been treated. The immunological aspects of this work are considered here and later, in a separate clinical paper, an attempt will be made to evaluate the therapeutic possibilities.

Clinical Material

Patients were recruited from among those attending the Department of Neurology of the United Oxford Hospitals, or were referred to us from elsewhere for the purpose of this study. Care was always taken to explain that this attempt at treatment was a research project and that, as such, its therapeutic value was not established; that it was not free from risk; and that its aim was to delay

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further deterioration or relapse rather than to restore function.

The present study is limited to 248 cases in which, on clinical grounds, the diagnosis was reasonably certain. Cases are excluded in which some complicating factor, such as cervical spondylosis, was suspected, or in which the laboratory data are incomplete. The basis on which cases were selected for treatment, and on which the diagnosis was accepted, is discussed in the clinical paper.

Sex Incidence.—One hundred and five patients were males and 143 females. This ratio of 1 : 1.38 is of the same order as in other published series (McAlpine, Compston, and Lumsden, 1955; McAlpine, 1961) and almost certainly reflects a true excess of women, at least in this country. This conclusion is strengthened by the fact that, while we have approximately equal hospital accommodation for either sex, we have, from the beginning of this study, laboured under a heavy waiting list for women while that for men has never been a problem.

Age Incidence.—The distribution of patients according to age at time of treatment is shown in Table I. The peak incidence, with 45.5% of cases, is in the fourth decade of life. The comparative rarity of patients over 50 years old or under 20 is because, on the one hand, we are reluctant to submit the older patient to such a strenuous form of treatment, while on the other, we insist that there should have been at least two well-marked episodes in the illness before admitting anyone for treatment.

The men tended to be slightly older than the women but the difference is not significant. The mean age for men is 35.9 years and the range from 16 to 60, while for women the mean is 35.3 years with a range of from 14 to 57. For both sexes there is a well marked peak in the third decade (Fig. 1).

Duration of the Illness.—Table II shows the duration of the illness at the time of treatment for both sexes. There are rather more than the expected number of males among those in whom the duration exceeded seven years. The difference just falls short of conventional significance at the 5% level. When, however, the cases of natural conversion (vide infra) are considered alone, the difference achieves significance.

Clinical Classification.—It is characteristic of disseminated sclerosis that the pattern of the illness should vary widely from case to case. In an attempt to meet some of the difficulties that this imposes we have used the following classification, derived from published statistical studies of the disease, notably that of Müller (1949).

Cases are divided into five groups, according to the pattern of the illness at the time of treatment, thus:

A (i) Relapsing-Remitting.—This group is made up of those cases in which the illness is running a relapsing, remitting course, and in which the duration
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does not exceed five years. For inclusion no sign or symptom must have grown worse for more than one year. It follows that all cases in which the total duration of the illness is less than one year fall automatically into this group.

A (ii) Relapsing-Remitting, Duration More than Five Years.—This group consists of those cases in which relapses and remissions are still occurring although the duration of the illness exceeds five years. As in A (i) progression of any symptom for more than one year excludes.

B (i) Progressive.—For inclusion in this group the duration of the illness must exceed one year and there must have been no sustained improvement of any sign or symptom at any time. Even a stationary phase for as long as three months excludes.

B (ii) Progressive Except for Remission of a Retrobulbar Neuritis.—The criteria are the same as for B (i), except that the history includes an attack of retrobulbar neuritis with subsequent improvement in vision.

C Relapsing-Progressive.—This group is made up of the cases in which the earlier pattern of relapses and remissions has given place to one of steady progression. For inclusion at least one sign or symptom must have grown steadily worse for more than one year.

The arbitrary choice of one year for use in these definitions is based on Müller's finding that spontaneous remission, and in this he includes a stationary phase, occurs in only 6% of cases in which symptoms have progressed for one year. Since he and others have shown that the interval between relapse increases with the duration of the illness, especially after this has reached five years, it follows that the expectation of relapse is highest in group A (i) and the expectation of remission lowest in group B (i) and (ii). This classification is further discussed in the clinical section.

Table III shows the cases in the present series

<table>
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<tr>
<td>CLASSIFICATION OF PRESENT SERIES</td>
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<tr>
<td>Number of Cases</td>
</tr>
<tr>
<td>A (i) Relapsing-remitting</td>
</tr>
<tr>
<td>A (ii) Relapsing-remitting duration over 5 years</td>
</tr>
<tr>
<td>B (i) Progressive</td>
</tr>
<tr>
<td>B (ii) Progressive except for retrobulbar neuritis</td>
</tr>
<tr>
<td>C Relapsing-progressive</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

classified in this way. The relative frequencies of the different groups are, almost certainly, not representative of the natural distribution since, when selecting patients for treatment, we have given preference to those belonging to groups A (i) and B (i) and (ii). Nevertheless group C, the relapsing-progressive, remains the largest, reflecting the well-marked tendency for the disease, sooner or later, to become progressive.

Methods of Study

The material for this study was derived from the results of the Mantoux tests; from the results of vaccination where the Mantoux test was negative; from a study of the C.S.F. during the reaction; and from the progress of the illness during the following year.

The Mantoux Test.—Serial Mantoux tests were done in the usual way using 1/10,000, 1/1,000, and 1/100 dilutions of old tuberculin. Sometimes the 1/10,000 dilution was accidentally omitted. The site of injection was inspected at 24 hours and the test read at 48 hours. The minimal requirement for a positive result was an area of induration measuring not less than 10 mm. in diameter.

Vaccination.—When the Mantoux test was negative to old tuberculin 1/100 the patient was vaccinated either with Wells’ voile bacillus vaccine (W.V.V.), or B.C.G., or first one and then the other.

Vaccination with W.V.V. was carried out by Dr. R. V. Volumn by the multiple puncture technique. If conversion was not obtained within a reasonable time this was repeated. Certain patients, who proved highly resistant, were vaccinated a third time with 0.1 mg. given by intradermal injection. Both fresh and freeze-dried vaccines were used.

B.C.G. vaccination was carried out at the chest clinic most conveniently situated for the patient. Again both fresh and freeze-dried vaccine were used.

Following vaccination the Mantoux test to old tuberculin 1/100 was carried out as often as was practicable. If conversion was not obtained in two or three months the patient was re-vaccinated. As soon as possible after conversion the patient was admitted for treatment.

Examination of the C.S.F.—The normal intrathecal tuberculin reaction, when fully developed, is diphasic. The cell counts show two well-marked peaks, one occurring 24 hours, and the other five to eight days after the injection. The protein content tends to follow the same pattern as does the increase in permeability of the blood-C.S.F. barrier as shown by the bromide test (Swithinbank et al., 1953; Smith et al., 1955). The C.S.F. examinations were accordingly timed to cover both phases of the reaction.

Soon after admission a diagnostic tap was performed. Cell counts and protein estimations were done and often the Lange curve was determined. In some cases, especially towards the latter part of this study, the protein of the C.S.F., and sometimes of the serum, was fractionated with particular reference to the globulin fraction. At first the immunological method of Kabat, Glusman, and Knaub (1948) was used but later this was superseded by electrophoresis (Sheff and Mynors, in preparation).
At least two days before the first injection of P.P.D. 2 g. sodium bromide was given by mouth. Lumbar puncture was repeated on the first and either the fifth or sixth day after intrathecal injection of P.P.D., and in some cases more often. After the definitive reaction the C.S.F. was examined at intervals until the patients' discharge from hospital. When possible 5 to 10 ml. C.S.F. was collected at each tap in two separate portions. From 5 to 10 ml. of blood was collected in a dry tube at the same time as each spinal tap. Total and differential white cell counts were done. The protein content was estimated nephelometrically and in duplicate, once in the clinical laboratory of the hospital where the patient was, and again by one of us (G.H.). Cases in which there was a significant discrepancy between the results of the estimations have been excluded from this study. The red cells were also counted in each specimen, since contamination with blood is a potent source of error in C.S.F. protein determination and in cell count. The bromide content of both serum and C.S.F. was estimated (Hunter, 1955) and the results expressed as the bromide ratio, i.e., C.S.F. bromide: serum bromide.

Intrathecal Dosage of P.P.D.—The hazards of intrathecal P.P.D. and the clinical events accompanying the reaction will be described in the clinical section. The aim of treatment is to produce the maximal changes in the C.S.F. compatible with safety. Experience has shown that if the injection of P.P.D. is repeated before the effects of the previous injection have subsided, that is to say, during the second phase of the previous reaction, the reaction to the second injection is often greatly enhanced even though the dose is kept the same. This helps to increase the margin of safety. The result of the Mantoux test is no guide to dosage since, by contrast with the findings in tuberculous meningitis and in the psychotonic patients, there is no apparent correlation in cases of disseminated sclerosis between the intensity of the intradermal and that of the intrathecal reactions. Vaccinated patients have, however, proved much more tolerant to intrathecal P.P.D. than those in whom the Mantoux test converted naturally.

The following scheme of dosage has proved practicable. The P.P.D. is obtained from the Ministry of Agriculture Research Laboratories, Weybridge, and diluted with normal saline to a solution containing 7-5 μg. per ml. This is known as the “standard” (ST) solution. This is then diluted tenfold to form the ST/10 solution. Except in a few early cases higher dilutions than this have not been used. The first dose given is 0.5 ml. ST/10, i.e., 0.375 μg. The size of the second dose depends on the severity of the reaction to the first, but does not exceed 2.5 ml. ST/10 (1.875 μg.). Rarely, a third dose of 0.5 ml. ST (3.75 μg.) is given. In vaccinated cases the initial dose is usually 2.5 ml. ST/10 (1.875 μg.), and 0.5 ml. ST (3.75 μg.) and even 1.0 ml. ST (7.5 μg.) may be given as the second and third doses.

Results of the Mantoux Test

Table IV shows the results of the Mantoux test in 248 cases. In 103 cases, or 41.9% of the total, the test was positive to O.T. 1/1,000. This incidence is almost certainly a little too high, and that of 15-4% for positive reactions to O.T. 1/10,000 a little too low, as not everyone who proved positive to the lower dilution was also tested with the higher.

In 55 cases, 22-4% of the series, the test was negative to O.T. 1/100. How this incidence compares with that of the population as a whole is not known. In this series there is considerably variation as to age and social status; it includes both urban and rural dwellers. This variation has made it impossible to find a comparable series among the many published tuberculin surveys. Unquestionably, a fair number of tuberculin-negative cases is to be expected, since disseminated sclerosis affects young people. But, as Table IV shows, our Mantoux-negative cases, though relatively more common among those in the second and third decades of life, are by no means confined to this age group. Thirty-one of the patients, or well over one half, were over 30 years old and nine were over 40. In these 55 cases tuberculin sensitivity was conferred artificially. They are, therefore, in many respects most conveniently treated as a separate group. In this study they are called the “vaccinated” to distinguish them from the “natural conversions”.

Effect of Sex on Intradermal Sensitivity.—In Great Britain, the older the individual, the more likely he is to be infected with M. tuberculosis and so to become tuberculin positive. The incidence of tuberculin sensitivity in any given population is, therefore, largely determined by the age distribution within that population. Further, for any given age group, the incidence of tuberculin sensitivity, and therefore presumably, the risk of exposure is about the same for the two sexes. Nor is the intensity of the reaction normally affected by the sex of the patient as shown by Griep and Bleiker (1957) in their careful quantitative study of the intradermal tuberculin test. Our experience with cases of tuberculous meningitis is the same. In this disease tuberculin sensitivity is commonly depressed and may even be abolished (Taylor, L. M., Smith, and Vollum,
1953). Table V shows the results of serial Mantoux tests carried out in 50 adolescents and adults with tuberculous meningitis. There is clearly no difference whatever between the sexes.

<table>
<thead>
<tr>
<th>Mantoux Test Positive to O.T.</th>
<th>Total No. of Cases</th>
<th>No. of Males</th>
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<tbody>
<tr>
<td>1/10,000</td>
<td>10</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>1/1,000</td>
<td>24</td>
<td>12 (50%)</td>
</tr>
<tr>
<td>1/100</td>
<td>13</td>
<td>6 (46%)</td>
</tr>
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Mantoux Test Negative

| 1/100                         | 3                  | 2 (67%)      |
| Total                         | 50                 | 25 (50%)     |

In our cases of disseminated sclerosis, however, the position is very different. The mean age and age distribution for the two sexes is much the same and therefore the proportion of Mantoux-negative cases should also be the same. But, in point of fact, 42, or 30%, of the women were Mantoux negative, and only 13, or 12.5%, of the men. This difference is significant at the 1% level.

Further, when the cases are divided into four groups according to the result of the Mantoux test, and the distribution of the sexes studied within each group, the proportion of males is seen to decline progressively with the decrease in sensitivity (Fig. 2 and Table VI). Thus, at the two extremes, of the 38 in whom the test was positive to O.T. 1/10,000, 23, or 60.5%, were males; while in the 55 in whom the test was negative only 13, or 23.6%, were males. This difference is also significant. (The value for \( \chi^2 \) was calculated, allowing for continuity, \( \chi^2 = 8.426 \) (d.f. = 1); 0.01 > \( P > 0.001 \).)

In this series, therefore, the males are significantly more sensitive to tuberculin than are the females.

**The Response to Vaccination**

The observation made in our earlier paper (Smith et al., 1957) that some tuberculin-negative patients were surprisingly refractory to vaccination, has now received ample confirmation. Unfortunately, in a fair number of cases the full history of Mantoux conversion, and possible reversion, is not known as the patients lived far away: they were vaccinated near their homes and after discharge from hospital were not available for re-testing. However, we have been able to make enough observations for it to be abundantly clear that the response to vaccination was abnormal in a considerable proportion of cases.

The chief abnormalities noted have been delayed or incomplete conversion, the patients not infrequently requiring re-vaccination; and a high reversion rate. In addition, the degree of sensitivity conferred has been low in that in the great majority of cases the Mantoux test has only become positive to O.T. 1/100.

**Conversion Time.**—This is best studied in those patients vaccinated with W.V.V. As a research tool W.V.V. has the advantage of being a natural strain of murine tubercle bacilli not attenuated artificially. Further, the conversion rate is very high, the reversion rate low, and conversion takes
place rapidly. Thus Hall and Wylie (1952) obtained 100% conversion within four weeks in a group of 33 children attending a special school.

Of the 27 patients vaccinated with W.V.V., the conversion time is known in 20, in 12 it was significantly prolonged beyond four weeks, six had to be re-vaccinated, and in three full conversion was not obtained in spite of repeated vaccinations.

Such information as we have about the time of conversion in our patients after B.C.G. vaccination indicates that delay was equally common; certainly re-vaccination was frequently required.

**Reversion of the Mantoux Test.**—Unfortunately, only nine patients have been retested after conversion to W.V.V. Four of these were found to be again tuberculin negative. Once again, the reversion appears to be at least as common after vaccination with B.C.G. as with W.V.V.

Finally, reversion to the negative state after natural conversion has been seen in three, possibly four cases; and in another two, in which the Mantoux test was negative, a radiograph of the chest showed calcification typical of an old tuberculous primary complex.

Since these figures really give little idea of the bizarre response to vaccination, short notes of some representative cases are appended.

**Case 1 (No. 236): Slow Conversion with Rapid Reversion.**—A woman of 37 had been ill for four and a half years during which time her symptoms had progressed slowly but without remission (clinical group B (i)). Her general condition was excellent and her disability mild. Eight weeks after vaccination with W.V.V. the Mantoux test was still negative and she was accordingly re-vaccinated. After a further eight weeks the test was still negative nor was either vaccination site detectable. She was accordingly re-vaccinated with 0.1 mg. of lyophilized vaccine given by a single intradermal injection. Three months later she had a small ulcer at the third vaccination site and the Mantoux test was positive. She was then treated with intrathecal P.P.D. Nine months later the ulcer was firmly healed and the Mantoux test was once again negative. Her neurological condition was unchanged. All Mantoux tests were done with O.T. 1/100.

**Case 2 (No. 88): Normal Conversion with Reversion.**—Slow conversion after vaccination was followed again by reversion.

A trained nurse of 24 years had been vaccinated with B.C.G. when she entered her training school six years earlier. The Mantoux test had converted rapidly and was still positive to O.T. 1/100 when she was first seen by us. She had by then been ill for one and a half years during which time the disease had run an increasingly rapid, progressive course without remission (clinical group B (i)) to the point where she was badly disabled by a spastic atactic paraparesis and severe frequency of micturition.

Following treatment with intrathecal P.P.D. she had an excellent remission and returned to nursing. Eighteen months later she had a severe relapse and was admitted for re-treatment. The Mantoux test was now found to be negative. She was then vaccinated with W.V.V. and, as conversion was not obtained, was vaccinated with 0.1 mg. lyophilized vaccine. This time the Mantoux test did convert and accordingly she was re-treated. Her recovery from the reaction was delayed by an attack of lobar pneumonia but at the present time, one year later, she is again in remission, though with a moderate residual paraparesis, and the Mantoux test has once again become negative.

**Case 3 (No. 171): Reversion after Natural Conversion.—Failure to reconvert after vaccination.**

A woman of 42, who had been ill for seven years and in whom the disease had at first remitted and then entered the progressive phase (clinical group C), was severely disabled by generalized ataxia, a moderate spastic paraparesis, and gross dementia. The Mantoux test was positive to O.T. 1/100. The reactions obtained to even very large intrathecal doses of P.P.D. were most disappointing and accordingly she was vaccinated with W.V.V. in an attempt to increase the sensitivity to tuberculin. One week later the Mantoux test was found to be negative and it remained so in spite of a further vaccination. Following treatment she improved a little, both physically and mentally, but relapsed nine months later. Her present Mantoux status is unknown.

What it is in these patients that prevents tuberculin sensitivity of the skin from developing in the normal way is not known. The low conversion and high reversion rates are reminiscent of the findings in sarcoidosis but, by contrast with Scadding's findings in that condition (Citron and Scadding, 1957), injecting cortisone with the tuberculin had no detectable effect on the result.

Close scrutiny of the site of the intradermal injection often gives the impression that the reaction begins to develop normally and is then aborted. Thus it is not uncommon to find a well-marked flare, and even a little early induration, 24 hours after the injection; but then, when the test is read at 48 hours, all signs of a reaction have disappeared. In a few patients, in whom the intrathecal response was negligible, the C.S.F. was examined at 12 as well as at 24 hours after the injection of P.P.D., with rather similar results. The 12-hour specimens have contained many hundreds of cells per c.mm., the great majority of which have disappeared from the 24-hourly specimen.

The relationship between the development of this immunological abnormality and the evolution of the disease is also obscure. Such information as we have, however, suggests that, so far from ante-dating it, the abnormality develops as the disease advances. Thus, in Case 3 Mantoux conversion before the development of disseminated sclerosis
appears to have been perfectly normal, and in several of our cases the first vaccination produced prompt and satisfactory conversion to be followed a few years later by reversion and by marked resistance to vaccination. Nor have we found any obvious relationship between reversion of the Mantoux test and the onset of relapse. It is true that many of those patients in whom the test was found to have reverted were in relapse, but it was the relapse that led to their re-admission, and therefore gave us the opportunity of re-testing them, and we know of at least one patient in whom the Mantoux test has reverted and in whom, nevertheless, the progressive phase of the disease has been interrupted by an excellent remission.

An attempt is made below to determine whether or not this abnormality can be related to any particular clinical feature of the illness.

Clinical Differences between Vaccinated and Natural Conversions.—It follows from what has been said that, in all probability, the vaccinated must form a mixed group made up, on the one hand, of those who have never been infected with M. tuberculosis and, on the other, of those who, having been infected, were either not sensitized or else lost their sensitivity. As Table IV shows, the proportion of young patients is rather larger among the vaccinated than in those with natural conversions. For the rest, the only difference we have found is in the distribution of the clinical groups.

Table VII shows the natural conversions and the vaccinated divided into the different clinical groups. The total number of the natural conversions divided by that of the vaccinated gives a ratio of 3.5. If the distribution of the groups were uniform then this should be the ratio in each separate group. In fact, this is so for the two classical groups, that is to say, for the young relapsing-remitting cases (A (i)) and the relapsing progressives (C). For the rest, the ratio for the long-term relapsing cases (A (ii)) is too small while for the steadily progressives (B (i) and (ii)) it is too large. This finding suggests that among the vaccinated there is a greater tendency to remit than among the natural conversions, in that rather more patients have retained the capacity to remit for more than five years while in rather fewer the disease had run a relentlessly progressive course from the outset.

The Intrathecal Reaction

In our earlier paper it was shown that the major departure of the pattern of the reaction in disseminated sclerosis from that of the normal was that the first phase of the reaction failed to develop properly, largely because the expected number of polymorphonuclear leucocytes did not appear in the C.S.F. In addition, taking the reaction as a whole, the rise in the protein content was remarkably high, particularly in conjunction with the low cell counts. Both these anomalies may now be examined in more detail.

The First Phase of the Reaction

For the purposes of this study the first phase of the reaction is defined as the changes in the C.S.F. 24 hours after an injection of P.P.D.

Cell Counts.—In the psychotic cases the most constant feature of the reaction was a well-marked pleocytosis, with a high percentage of polymorphonuclear leucocytes, 24 hours after the injection of P.P.D. when counts of 1,000 or more cells per c.mm. were common (Swithenbank et al., 1953). In cases of tuberculous meningitis a similar sharp rise in the cell count is the rule. Fig. 3 shows the distribution of the cell counts 24 hours after P.P.D. in 50 cases of tuberculous meningitis.

All patients were adolescent or adult. When several doses of P.P.D. were given the reaction showing the highest cell count at 24 hours was chosen. The dose of P.P.D. varied widely from case to case but, as in the cases of disseminated sclerosis, the aim was to produce the greatest changes in the C.S.F. compatible with safety. The tendency towards high cell counts is obvious, and in 29 cases, or well over one half, 1,000 or more cells per c.mm. were present.

In Fig. 4 the 24-hourly cell counts in the 248 cases of disseminated sclerosis are set out in the same way.

The findings in the natural conversions are shown above the line and in the vaccinated below. As in the cases of tuberculous meningitis, when several doses of P.P.D. were given the most severe reaction was chosen. Among these natural conversions, where the cell count was 100 or less per c.mm., are several early cases in which very small doses of P.P.D. were given. For the rest, the principle of dosage was to produce the briskest reaction compatible with safety.
FIG. 4.-Similar histogram to that in Fig. 3, but for the cases of tuberculous meningitis (children excluded) according to the cell count in the C.S.F. 24 hours after P.P.D. The incidence rises smoothly to a peak at 1,500 cells or more per c.mm.

The distribution of the cell counts contrasts sharply with that in the cases of tuberculous meningitis. Among the natural conversions the incidence shows a sharp peak at the 100 to 249 range and then declines smoothly to the range of from 700 to 849 where there are only two cases. At this point, however, the decline stops and the incidence again increases to a second, smaller peak at the 1,000 to 1,500 range.

A very similar distribution is seen among the vaccinated. Once again, the incidence declines from a well-marked peak to zero, and once again this decline is reversed by a second group of cases in which the C.S.F. contained 1,000 cells or more per c.mm. On the whole, the cell counts among the vaccinated tend to be higher than among the natural conversions. The peak incidence lies to the right of that for the natural conversions while the percentage of cases with cells of 1,000 or more per c.mm. is higher (20% as compared with 9-2%). This difference, however, falls short of conventional significance.

Thus in 18, or 9-2%, of the natural conversions, and in 11, or 20% of the vaccinated, that is to say, in 11-7% of all cases, the C.S.F. contained 1,000 or more cells per c.mm. Differential cell counts show that the high total counts are largely due to the numbers of polymorphonuclear leucocytes present. These cases are too numerous, and their occurrence among the natural conversions and the vaccinated too constant, for them to represent merely a chance fluctuation on the distribution curve. Scrutiny of the histories and clinical findings in these cases leaves little doubt that the diagnosis was correct; certainly the criteria for diagnosis were applied at least as stringently as in the rest.

It appears, therefore, that this series is not homogeneous but consists of two separate groups: one, the large majority, in which the cellular component of the first phase fails to develop properly; and the other, a small minority, in which it approximates to the normal. This conclusion is also supported by study of the individual case. When, as has sometimes happened, two or even three separate courses of P.P.D. have been given, the pattern of the reaction has proved remarkably constant for the individual.

Taking 1,000 cells per c.mm. as the dividing mark, we find it convenient to speak of the majority as the "low-cell group" and of the minority as the "high-cell" group.

**Specificity of Findings for Disseminated Sclerosis.**—To what extent these findings are specific to disseminated sclerosis is not yet established. With very few exceptions our own experience with P.P.D. is limited to psychoses of various kinds, to tuberculous meningitis, and to disseminated sclerosis itself. Marshall and O'Grady (1959), however, have published data which go some way towards filling this
The Protein Content.—Comparison of the protein content of the C.S.F. of the first phase with the cell counts provides additional evidence that the "high-cell" cases form a distinct group within the series as a whole. Fig. 6 shows the distribution of cases according to the range into which the protein content falls. (As in Fig. 4, the natural conversions are shown above the line and the vaccinated below.)

gap. These derive from 16 cases of disseminated sclerosis and 10 control cases made up of five cases of motoneurone disease, three of Huntington's chorea, and two of Parkinson's disease. From a simple comparison of the means of the cell counts 24 hours after injection in the two groups, they conclude that there is no significant difference between them. If, however, the distribution of the cell counts is considered (Fig. 5) the fallacy of this conclusion becomes obvious. Allowing for the difference in the total numbers, the distribution of their cases of disseminated sclerosis—all natural conversions—is remarkably like that in Fig. 4. All the cell counts but one were less than 600 per c.mm., that is to say, they belong to the "low-cell" group, while in the remaining two the cells were over 1,500 per c.mm., typical of the "high-cell" group.

Of the control group, in four the reaction, as judged by both the cells and the protein, was negligible, while in the remaining six the cell counts were all above 600 per c.mm. Even with so few cases in each group this difference in distribution is highly significant (P = < 0.01). More observations on the important question of specificity are badly needed; but such data as there are suggest that this distortion of the first phase of the reaction is not simply a function of chronic neurological disease as such.

The above data of Marshall and O'Grady were analysed by the use of the non-parametric Wald-Wolfowitz runs test. This test is sensitive to simple differences of any type whatever—central tendency, variance, skewness, or distribution. The scores from the independent samples are combined and ranked in order of magnitude, and the number of runs in the series determined. (A run is a sequence of scores from the same sample.) If the samples were identical in every way the scores would be distributed randomly, like well-shuffled cards. Difference in any parameter will produce aggregates of the two scores (clumping) and clumping will reduce the number of runs. The fewer the runs the less likely that the samples would be drawn from a common population. In this case a total of five runs was obtained and from the published table, and allowing for the small numbers, a total of seven runs or less will occur only once in 20 trials. Thus a total of five is significant at a level of better than 1%.

The Protein Content.—Comparison of the protein content of the C.S.F. of the first phase with the cell counts provides additional evidence that the "high-cell" cases form a distinct group within the series as a whole. Fig. 6 shows the distribution of cases according to the range into which the protein content falls. (As in Fig. 4, the natural conversions are shown above the line and the vaccinated below.)
By contrast with the cell counts, there is no sign of a second peak on the distribution curve for either group.

Comparison between the natural conversions and the vaccinated, however, shows that, though the shape of the distribution curve is much the same, the protein contents tend to be lower among the latter.

The peak incidence for the vaccinated lies to the left of that for the natural conversions and the percentages of cases in which the protein was less than 250 mg. per 100 ml. is twice as large among the natural conversions. At the other end of the scale, in no less than 78 of the natural conversions, i.e., in 39-4 %, the protein content reached 550 mg. per 100 ml. or more, as compared with eight, or only 14-6 % of the vaccinated. This difference is significant at the 0-1 % level (χ² = 11-53, d.f. = 1, P < 0-001). Finally, in 17 of the “low-cell” cases, but only one of the “high-cell”, 1 g. or more protein per 100 ml. was present.

Considering, then, Figs. 4 and 6 together, it is seen that, on the whole, the vaccinated tend to have higher cell counts and lower protein contents than the natural conversions, as well as having a much higher tolerance to P.P.D.

It is interesting to compare the protein contents for the cases in the several cell ranges since, if the height of the cell count is merely the expression of the intensity of the reaction, then the protein content should vary directly with the cell count.

Table VIII shows the range and mean value of the protein contents for the cases in each cell range for natural conversions, together with the number of cases in which the protein rose to 550 mg. per 100 ml. The natural conversions are separated from the vaccinated since their protein values are not comparable.

The Natural Conversions.—The 32 cases in which the cell counts are less than 100 per c.mm. are not strictly comparable with the rest, since they include those early cases which were admittedly under-treated. Among the rest, the mean protein is higher for those cases in which the cell counts were between 100 and 249, and which are also the most numerous. Thereafter, as the cell counts rise, so the mean protein content slowly declines, and the mean for the 18 “high-cell” cases is only 540 mg. per 100 ml. compared with 602 mg. per 100 ml. for the 58 cases with cell counts of between 100 and 249 per c.mm. This difference between the means is due to the scarcity among the “high-cell” group of cases in which the protein content rose to 550 mg. or more per 100 ml. As Table VIII shows, the proportion of cases with the high protein remains steady at about 46 % of cases until the “high-cell” group is reached when it falls abruptly to 27-7 %.

So far, then, from the protein contents rising with the cell count they appear to vary independently, and the “high cell” group of cases differs from the majority in that there is not the same tendency to produce the very high protein contents. In this respect too, then, as well as in the cell counts, these cases approximate more closely to the normal than do the rest.

The Vaccinated.—In Table VIII the protein contents in the vaccinated are set out according to the different cell ranges. In no case did the protein content rise to 1 g. or more per 100 ml. and in only eight did it reach 550 mg. As with the natural

| Table VIII |
|-----------------|-----------------|-----------------|-----------------|
| **Cell Range** | **No. of Cases** | **Protein** | **Protein 550 g.** |
| Natural Conversions | | | and Over |
| <100 | 32 | 74-980 | 312 | 8 (22-6 %) |
| 100-249 | 55 | 109-1,790 | 602 | 26 (46-5 %) |
| 250-399 | 40 | 180-1,860 | 580 | 18 (44-4 %) |
| 400-549 | 24 | 134-1,240 | 538 | 11 (46-0 %) |
| 550-999 | 24 | 110-1,225 | 529 | 11 (46-0 %) |
| 1,000 and over | 18 | 201-1,770 | 540 | 3 (27-7 %) |
| Total | 193 | | 76 (39-4 %) | |
| Vaccinated | | | 18 (9-3 %) |
| <100 | 2 | 161-775 | 468 | 1 |
| 100-249 | 9 | 114-905 | 378 | 1 (11-1 %) |
| 250-399 | 17 | 70-804 | 354 | 2 (11-8 %) |
| 400-549 | 8 | 180-701 | 336 | 1 (12-5 %) |
| 550-999 | 8 | 127-946 | 284 | 1 (12-5 %) |
| 1,000 and over | 11 | 250-617 | 403 | 2 (18-1 %) |
| Total | 55 | | 8 (14-6 %) |
conversions, among the "low-cell" cases the mean protein value declines as the cell counts rise from 378 mg. for those with cell counts of 100 to 249, to 284 mg. for those in the cell range of 550 to 999 per c.mm. For the "high-cell" group, however, the mean is rather higher, at 403 mg. per ml. Cell range for cell range, there is less difference between the vaccinated and natural conversion "high-cell" cases than between the "low-cell" groups. Indeed, excluding the solitary natural conversion in which both the cell count and the protein content exceeded 1,000, the mean protein value for the high-cell, natural conversions is 463 mg. per 100 ml., which is not much higher than the mean of 403 mg. for the vaccinated high-cell group.

Thus, among both the natural conversions and the vaccinated, the cell counts and protein contents vary independently so far as the low-cell cases are concerned. The high-cell cases among the vaccinated closely resemble the high-cell cases among natural conversions, and it is this minority group that, with regard to both the cell counts and protein contents, the first phase of the reaction approximates most closely to the normal.

Other Differences between High- and Low-cell Groups.—Granted, then, that in this series the cases can be divided into two groups according as to whether or not the first phase of the reaction develops normally, it remains to show whether these two groups differ in other respects. They have been compared for age, duration and pattern of disease, Mantoux status, and dosage without any outstanding difference, though the high-cell cases may possibly be rather the more tolerant of P.P.D. Our studies of the different protein fractions in the C.S.F. are not yet sufficiently advanced to show whether or not the behaviour of any fraction or fractions can be correlated with the development of the first phase. Simple quantitative estimation of the γ-globulin component of the resting C.S.F. has, however, shown no difference between the high- and low-cell cases, though confirming the finding that, in disseminated sclerosis, it is usually elevated.

So far, the only other differences found between the low- and high-cell cases are the sex distribution and the immediate prognosis.

Sex Distribution in Relation to Cell Counts.—As has already been shown, the incidence of males among the vaccinated is unexpectedly low. In order,

<table>
<thead>
<tr>
<th>Cell Range</th>
<th>No. of Cases</th>
<th>No. of Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>32</td>
<td>18 (56.25%)</td>
</tr>
<tr>
<td>100-249</td>
<td>55</td>
<td>29 (52.7%)</td>
</tr>
<tr>
<td>250-399</td>
<td>40</td>
<td>19 (47.5%)</td>
</tr>
<tr>
<td>400-549</td>
<td>24</td>
<td>9 (37.5%)</td>
</tr>
<tr>
<td>550-699</td>
<td>15</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>700-999</td>
<td>9</td>
<td>2 (22.2%)</td>
</tr>
<tr>
<td>1,000 and over</td>
<td>18</td>
<td>10 (55.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>193</td>
<td>92 (47.6%)</td>
</tr>
</tbody>
</table>

Fig. 7.—The number of cases in each cell range, together with the percentage of males in each group for the natural conversions (a) and the whole series (b). The horizontal lines show the expected percentage of males, i.e., the percentage of males in the natural conversions as a whole and in the total series.

In (a) the percentage of males declines steadily as the cell counts rise until the high-cell group is reached when it rises sharply. In (b) the same relationship is preserved in spite of the inclusion of the vaccinated.
therefore, to avoid the possible introduction of a second and complicating factor, consideration of the relation between the sex distribution and the cell counts is, in the first instance, limited to the natural conversions. In Table IX these cases are set out according to the cell counts. For each cell range the total number of cases is shown, together with the number and percentage of males it includes. Throughout the low-cell cases the percentage of males falls steadily and significantly as the cell counts increase. When, however, the high-cell cases are reached this inverse relationship no longer holds: on the contrary, these cases again include a high percentage of males. This relationship, and the aberrant behaviour of the high-cell cases, is shown diagrammatically in Fig. 7(a) in which the total number of cases in each cell range, and the percentage of these cases that are males, are plotted beside one another. The horizontal line shows the expected percentage of males, that is to say, the percentage among the natural conversions as a whole.

In Fig. 7(b) the total number of cases and the percentage of males for each cell range are similarly set out, but for the series as a whole. It is clear that the inclusion of the vaccinated does not alter the shape and relationship of the curves, in spite of the paucity of males among them.

It appears, therefore, that among the low-cell cases there is a marked tendency for the men to produce the lower cell counts and the women the higher. This effect of sex is, however, not operative among the high-cell cases, where the percentage of males approximates to that for the series as a whole.

Sex Distribution in Relation to Protein Contents.—Table X shows the natural conversions set out according to the protein content. For simplicity's sake four protein ranges have been taken as measured in mg. per 100 ml.: less than 350, 350 to 549, 550 to 999, and 1,000 and over. The number of cases falling into each range is tabulated, together with the number and percentage of males it contains. Here, the percentage of males increases with the increase in protein content, a relationship which is clearly shown in Fig. 8(a), when the total number of cases and the percentage of males within each group are set out and where, as in Fig. 7, the horizontal line marks the expected percentage.

Fig. 8(b) shows the same quantities as Fig. 8(a),

![Graph](http://www.jnnp.bmj.com/)

**FIG. 8.**—As in Fig. 7 but for the protein content; (a) natural conversions; (b) whole series. The percentage of men rises progressively with the rise in protein content. This is not affected by the inclusion of the vaccinated in spite of the increase in the number of cases with a low protein content.

<table>
<thead>
<tr>
<th>Protein Range</th>
<th>No. of Cases</th>
<th>No. of Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;350</td>
<td>60</td>
<td>25 (42.0%)</td>
</tr>
<tr>
<td>350-549</td>
<td>58</td>
<td>26 (45.0%)</td>
</tr>
<tr>
<td>550-999</td>
<td>55</td>
<td>28 (55.0%)</td>
</tr>
<tr>
<td>1,000 and over</td>
<td>20</td>
<td>13 (65.0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>193</strong></td>
<td><strong>92 (47.6%)</strong></td>
</tr>
</tbody>
</table>

**TABLE X**

SEX DISTRIBUTION RELATED TO PROTEIN CONTENT IN NATURAL CONVERSIONS

The percentage of men rises progressively with the rise in protein content. This is not affected by the inclusion of the vaccinated in spite of the increase in the number of cases with a low protein content.
but for the series as a whole. Here the inclusion of the vaccinated, with their tendency towards a low protein content, causes the curve for the total number of cases to decline more regularly. The sustained increase in the percentage of males with the increase in protein, and its sharp departure from the expected level, is even more obvious.

In summary then, among the low-cell cases the reaction in males tends to produce fewer cells and more protein than in females. This relationship, however, does not hold for the high-cell cases, which include a relatively large number of men in spite of the high-cell counts and comparatively low protein contents. In other words, in those cases in which the first phase of the reaction develops normally, it does so irrespective of sex: for the rest, the males show a greater departure from the normal than do the females.

Immediate Prognosis in Relation to Cell Counts.—
By immediate prognosis is meant the course of the illness during the first year after treatment. Disregarding all remissions we have assessed this simply from the relapse rate. The criteria by which relapse is diagnosed are discussed in the clinical paper. For present purposes steady deterioration after treatment is equated with relapse. Of the 248 patients in the present series, 230 were followed for six months, and during this time 199, or 86.5%, did not relapse. Two hundred of them also completed a second six-month period of observation; of these 142, or 71%, were free from relapse throughout the year.

When, however, the cases are divided according to the cell counts, it is seen that the relapse rate is significantly higher among the high-cell cases than among the rest.

Table XI shows the cases set out according to the different ranges of cell counts. (Those in which the counts fall between 550 and 999 per c.mm. have been combined in order to make the numbers in the different groups more comparable.) Throughout the different ranges comprising the low-cell group as a whole the percentage of those free from relapse, either at the end of six months or at the end of the year, remains fairly constant. When, however, the high-cell group is reached there is a sharp drop: this is already apparent at the six months follow-up, and is obvious at the end of the year (Fig. 9).

Thus, of the 203 low-cell cases followed for six months, 88% survived without relapse compared with 74% of the 27 high-cell cases. At the end of one year 74% of the 182 low-cell cases have survived compared with only 39% of the 18 high-cell cases. In spite of the disparity between the total numbers in the two groups the difference at one year is significant (χ² = 8.27, d.f. = 1; 0.01 > P > 0.001).

In summary, then, the immediate prognosis has proved to be considerably worse for the high- than for the low-cell cases. This finding, together with the fact that, within the limits of the low-cell group as a whole, the immediate prognosis does not vary with the cell count, provides further support for the view that the high-cell cases differ qualitatively from the rest.

Immediate Prognosis in Relation to Protein Content.—During the course of this study evidence has accumulated suggesting that the freedom from relapse after treatment is related to the intensity of the reaction as gauged from the rise in the protein content. Comparison between the protein content 24 hours after the injection and the subsequent relapse rate, however, revealed no correlation between any.

- Table XI

<table>
<thead>
<tr>
<th>Cell Range</th>
<th>No. Followed</th>
<th>No. without Relapse</th>
<th>% without Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mth. 1 yr.</td>
<td>6 mth. 1 yr.</td>
<td>6 mth. 1 yr.</td>
</tr>
<tr>
<td>&lt;100</td>
<td>31</td>
<td>29</td>
<td>94</td>
</tr>
<tr>
<td>100-249</td>
<td>50</td>
<td>45</td>
<td>87</td>
</tr>
<tr>
<td>250-399</td>
<td>36</td>
<td>34</td>
<td>85</td>
</tr>
<tr>
<td>400-549</td>
<td>29</td>
<td>26</td>
<td>90</td>
</tr>
<tr>
<td>550-999</td>
<td>27</td>
<td>24</td>
<td>89</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>88</td>
</tr>
<tr>
<td>&lt;1,000</td>
<td>203</td>
<td>179</td>
<td>88</td>
</tr>
<tr>
<td>1,000 and over</td>
<td>27</td>
<td>20</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>230</td>
<td>199</td>
<td>86.5</td>
</tr>
</tbody>
</table>

- Fig. 9: The percentage of cases without relapse, both at the end of six months and at the end of one year, is plotted against the cell range. The sharp decrease in cases without relapse among those in which cell counts of 1,000 or more were obtained is detectable at the end of six months and obvious at the end of one year.
between the two except when the protein content rose to 550 mg. or more per 100 ml. Then the relapse rate at six months, and still more at one year, showed a significant fall (Table XII).

### Table XII

**PROGNOSIS RELATED TO PROTEIN CONTENT**

<table>
<thead>
<tr>
<th>Protein Range (mg./100 ml.)</th>
<th>No. Followed</th>
<th>No. without Relapse</th>
<th>% without Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mth.</td>
<td>1 yr.</td>
<td>6 mth.</td>
</tr>
<tr>
<td>&lt;550</td>
<td>153</td>
<td>132</td>
<td>127</td>
</tr>
<tr>
<td>250 and over</td>
<td>77</td>
<td>68</td>
<td>72</td>
</tr>
</tbody>
</table>

It was shown above that one of the ways in which the high-cell cases differ from the low is that the concomitant rise in the protein content tends to be smaller. In case this is the factor that determines the poor prognosis in this group the series has been broken down according to both the cell counts and the protein content (Table XIII). The number of high-cell cases in which the protein rose to 550 mg. or more is too small to allow of a definite conclusion on this point, but certainly there is no striking difference between those with a high protein content and those with a low. Among the low-cell cases, on the other hand, the difference is significant at one year ($\chi^2=5.41$, d.f. = 1; $0.005 > P > 0.01$).

In conclusion, then, it may be said that, from the results obtained in this series, the first phase of the reaction in disseminated sclerosis is abnormal in approximately 90% of cases in that the cell counts are much too low. Further, these cases differ quantitatively from the remainder in regard to the protein content during the first phase, the sex distribution, and the immediate prognosis.

### The Protein Content throughout the Reaction

The other major anomaly of the reaction noted in our preliminary report was the surprisingly high protein content in conjunction with the low cell count. The bromide test has provided further evidence that in disseminated sclerosis the reaction does tend to elevate the protein content unduly.

In the normal, and at equilibrium, the bromide ratio varies between about 0.25 and 0.5; in other words, the serum contains from about two to three times as much bromide as does the C.S.F. When the permeability of the blood-C.S.F. barrier is increased, the ratio rises. Given accurate methods of estimation this has proved an extremely sensitive test, and indeed provides the best method of measuring the permeability of the blood-C.S.F. barrier at present available for clinical use (Hunter, Smith, and Taylor, 1954). When the efficiency of the barrier is impaired protein tends to pass from the blood to the C.S.F. By estimating, therefore, both the protein content of the C.S.F. and the bromide ratio a measure is gained of the degree of increased permeability required to bring about any given elevation of the protein.

During the intrathecal tuberculin reaction, even in patients in whom the meninges are otherwise normal, the bromide ratio rises steeply, often to the order of unity or beyond. An identical effect is found in tuberculous meningitis (Smith et al., 1955): indeed there is now a considerable body of evidence to show that the changes in the C.S.F. in that disease are simply the expression of repeated, spontaneous, intrathecal tuberculin reactions (Taylor, Smith, and Vollum, 1955). It is in order to measure the increase in permeability of the barrier that, throughout this study, the bromide ratio has been estimated as a routine.

In the present series data on the bromide ratio are available for comparison with the protein contents in 242 cases. Whereas in previous sections study of the protein contents has been limited to the values found 24 hours after the injection of P.P.D., here it is the highest values for both protein content and bromide ratio found at any time during the reactions that are considered.

Fig. 10 shows the relationship found between the protein contents and the bromide ratio in these 242 cases, in 100 cases of tuberculous meningitis in which the estimations were made immediately after the patients' admission to hospital, and in eight intrathecal reactions in psychotic patients.

### Table XIII

**PROGNOSIS RELATED TO CELL COUNT AND PROTEIN CONTENT**

<table>
<thead>
<tr>
<th></th>
<th>550 and Over</th>
<th>&lt;550</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1,000 and over</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mth.</td>
<td>7</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td>1 yr.</td>
<td>6</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>6 mth.</td>
<td>70</td>
<td>66</td>
<td>94</td>
</tr>
<tr>
<td>1 yr.</td>
<td>62</td>
<td>53</td>
<td>86</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>68</td>
<td>93-5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>&lt;1,000</th>
<th>6 mth.</th>
<th>1 yr.</th>
<th>6 mth.</th>
<th>1 yr.</th>
<th>6 mth.</th>
<th>1 yr.</th>
<th>6 mth.</th>
<th>1 yr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>6 mth.</td>
<td>1 yr.</td>
<td>6 mth.</td>
<td>1 yr.</td>
<td>6 mth.</td>
<td>1 yr.</td>
<td>6 mth.</td>
<td>1 yr.</td>
</tr>
<tr>
<td>1,000</td>
<td>68</td>
<td>55</td>
<td>80-9</td>
<td>72</td>
<td>93-5</td>
<td>83</td>
<td>132</td>
<td>87</td>
</tr>
<tr>
<td>Total</td>
<td>230</td>
<td>199</td>
<td>86-5</td>
<td>200</td>
<td>142</td>
<td>71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
INTRATECHAL TUBERCULIN IN DISSEMINATED SCLEROSIS

Fig. 10.—This illustrates the relation between the rise in protein content and in the bromide ratio during the intrathecal tuberculin reaction in both disseminated sclerosis and tuberculous meningitis.

Each protein range is plotted against the mean bromide ratio of the cases falling within that range. The figures in brackets show the number of cases from which the mean bromide ratio was calculated.

The curve for disseminated sclerosis lies to the left and above that for tuberculous meningitis, indicating that in that disease any given increase in the permeability of the blood-C.S.F. barrier, as measured by the bromide test, is accompanied by an excess of protein.

(For further explanation, and for evidence that the findings in tuberculous meningitis represent the normal, see text.)

The cases were divided according to the protein content into serial ranges of 100 mg. The mean value for the bromide ratios for the cases in each protein range was then calculated and plotted against the protein range. The cases of tuberculous meningitis were treated in the same way, except that as in only three cases did the protein content exceed 400 mg. per 100 ml.; for these three the means of both the protein contents and the bromide ratio were calculated and plotted against each other. Finally, of the eight psychotic cases, in four the maximal protein lay between 100 and 200 mg. per 100 ml., and in four between 400 and 600 mg. per 100 ml. The mean values for protein contents and bromide ratios in each set of four were calculated and plotted against each other.

It is at once apparent that the curve for disseminated sclerosis lies well to the left of that for tuberculous meningitis. In other words, the same increase in the bromide ratio is accompanied by a much greater increase in protein content in the former disease than in the latter. This difference is seen very clearly when the distribution of cases throughout the different protein ranges is studied. In spite of the uniformly higher bromide ratios among the cases of tuberculous meningitis, in only 3% did the protein content exceed 400 mg. per 100 ml., while in 79% it did not exceed 200 mg. per 100 ml.

Among the cases of disseminated sclerosis, on the other hand, in 60% the protein content was more than 400 mg. per 100 ml., and in only just over 6% did it fail to exceed 200 mg. per 100 ml.

The question then arises as to which curve represents the normal. Here the findings in the cases of psychosis are important. Few as they are, it is obvious that they approximate closely to the curve for tuberculous meningitis, which strongly suggests that it is this one that exemplifies the normal. It is also interesting to note that in those cases of tuberculous meningitis treated with intrathecal P.P.D. in which the bromide ratio was estimated at the height of the reactions, the points for protein content and bromide ratio lie on the same curve as that for untreated tuberculous meningitis although rather higher up it.

If, then, the bromide ratio is accepted as a valid measure of permeability of the blood-C.S.F. barrier, and the evidence for this is good, then for any given increase in permeability the C.S.F. in disseminated sclerosis contains more than the expected amount of protein.

Discussion and Conclusions

The idea that hypersensitivity plays a part in the natural history of disseminated sclerosis is, of course, not new. The discovery that, under suitable circumstances, brain tissue could act as an antigen and provoke a reaction resulting in demyelination lent strong support to this idea, and numerous attempts have been made to incriminate allergy as a factor in pathogenesis (McAlpine et al., 1955). But, so far as we know, these attempts have been exclusively concerned with the immediate or anaphylactic type of immune response, whereas the present study is solely concerned with delayed or tuberculin type sensitivity.

The observations recorded here on the intrathecal tuberculin reaction fully support our preliminary report that, in the great majority of cases of disseminated sclerosis, the pattern of the reaction is
consistently distorted. Moreover, attempts to produce Mantoux conversion in those patients who were tuberculin negative not only confirm the presence of an immunological abnormality in this disease, but show that it is not confined to the central nervous system but affects the body as a whole. The nature of the mechanism whereby the acquisition of skin sensitivity is interfered with, and the cellular component of the intrathecal reaction depressed, while the production of protein is enhanced, remains obscure though, from the sex differences described, it is presumably susceptible to hormonal influence.

The part, if any, which the immunological abnormality plays in the pathogenesis of the disease is almost equally obscure. The presence of a demonstrable immunological abnormality with systemic involvement, and the possible hormonal factor, even the classical relapsing-remitting course, at once suggest an auto-immune disease. On the other hand, it is probable that the abnormality in Mantoux conversion develops together with the disease, and there is also the curious finding that it may be associated with facilitation of remission. Somewhat in line with this is the, to us, unexpected finding that, after treatment, the immediate relapse rate has varied inversely with the abnormality of the reaction. In view of this, it is possible that the immunological anomalies, so far from reflecting the disease process as such, are the expression of some defence mechanism.

So far the question has been begged of whether the freedom from relapse in the "low-cell, high-protein" cases (Table XIII) is the result of treatment, or whether the pattern of the reaction has merely served to distinguish those cases in which the prognosis is naturally good. We incline to the former view. Certainly there was nothing in the course of the disease before treatment in these cases to suggest that so many patients were about to enter on a period of arrest; on the contrary, in many the disease was taking a progressive course. It is also worth noting that of those in whom the protein content failed to reach a high level, 33% relapsed during the subsequent year, a figure which agrees very well with the average annual relapse rate in many published series (e.g., McAlpine et al., 1955). We, therefore, postulate that in those cases in which a high protein content is achieved the chance of a relapse during the next year is lessened while for the remainder it is unaffected.

But whichever is the right interpretation, one thing is very clear: the demonstration that the relapse rate is related both to the cell count and to the protein content of the reaction has enormously increased the difficulty of planning a valid controlled therapeutic trial. On the one hand, only those among the treated group in whom a high protein level is reached can be reckoned as adequately treated; and on the other, factors of demonstrable prognostic significance are now so numerous that inconveniently large numbers will be required before it can be assumed that the controls and treated cases are properly matched.

Summary

Former studies on the place of allergy in the natural history of disseminated sclerosis have usually been limited to the immediate or anaphylactic type of hypersensitivity. The present study is confined to the delayed or tuberculin type of immune reaction.

Study of the intradermal and intrathecal response to tuberculin in 248 cases of disseminated sclerosis has confirmed our earlier finding of an immunological disorder in this disease.

Serial Mantoux tests have shown that, by contrast with the findings in tuberculosis and in the general population, men are significantly more sensitive to tuberculin than women.

B.C.G. or vole bacillus vaccination of Mantoux-negative patients has shown that the conversion rate is low and the reversion rate high, findings reminiscent of sarcoidosis. By contrast with that disease, the addition of small amounts of cortisone to the tuberculin has not affected the results of the test.

The intrathecal reaction was examined with special reference to (a) the first phase of the reaction, i.e., to the C.S.F. changes 24 hours after the lumbar injection of P.P.D.; and (b) to the highest protein content in the C.S.F. at any time during the reaction.

The cellular component of the first phase of the reaction was found to be partially suppressed in approximately 90% of cases. Moreover, those in which it developed normally differed from the majority in regard to the protein content of the same specimen of C.S.F., in the distribution of the sexes, and in the immediate prognosis. It is therefore concluded that this series is not homogeneous but consists of a large majority group—called the "low-cell" group, and a small minority group, the "high-cell" group—that differ qualitatively from one another.

The vaccinated tend to have rather higher cell counts and considerably lower protein contents than do the natural conversions. The reaction thus approximates more closely to the normal. This does not seem to vary with the ease or difficulty of Mantoux conversion.

Within the low-cell group the distribution of the sexes is not uniform in that, as judged both by the cell counts and the protein contents, the greater the
departure from the normal, the higher the proportion of males.

Throughout the high-cell group the immediate prognosis is bad, as judged by the relapse rate during the first year after treatment. Among the low-cell cases the prognosis for those in which the protein content of the first phase reached 550 mg. per 100 ml. is significantly better than in the rest. Reasons are advanced for attributing this improved prognosis to the treatment.

It is pointed out that the demonstration that the immediate relapse rate varies both with the cell counts and the protein contents makes the planning of a valid controlled trial very difficult.

Comparison of the relationships between the maximal protein content and bromide ratio in disseminated sclerosis, tuberculous meningitis, and in different kinds of psychosis, shows that, for any given increase in the permeability of the blood-C.S.F. barrier as measured by the bromide test, the C.S.F. in disseminated sclerosis contains an excess of protein.

It is concluded that in disseminated sclerosis there is a disorder of the delayed type of hypersensitivity and that this is not limited to the central nervous system but affects the body as a whole. The possible bearing of these findings on pathogenesis is discussed, and it is pointed out that they are compatible with the hypothesis that disseminated sclerosis is an autoimmune disease. It is, however, possible that they reflect a defence mechanism rather than the disease process itself.

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