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MYELOTOXICITY OF SERUM AND SPINAL FLUID IN MULTIPLE SCLEROSIS: A CRITICAL ASSESSMENT

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SUMMARY

Explants of neonatal rat cerebellum have been cultured on rat-tail collagen coated cover-slips for 2–4 weeks under conditions which yielded well myelinated nerve fibres suitable for testing myelotoxicity of serum. Eighty-four per cent of twenty-five acute multiple sclerosis serum samples were myelotoxic, as opposed to 62% of twenty-six sera from cases of motor neurone disease. Twenty-four per cent of thirty-four normal sera also showed distinct myelotoxicity. The difficulties in setting up and interpreting tests for *in vitro* demyelinating activity of serum are discussed. Six spinal fluids from acute cases of multiple sclerosis were without activity. It is concluded that although myelotoxicity exists in most samples of serum from acute multiple sclerosis, it is not limited to such subjects and that its high incidence in motor neurone disease sera indicates that it may well be a consequence of myelin destruction rather than a primary factor in its causation.

INTRODUCTION

The basic researches of Hild (1957) and Bornstein & Murray (1958) laid down the experimental conditions under which myelination occurs in explants of neonatal mammalian cerebellum maintained *in vitro*. The technique is arduous and its application to the problem of demyelination has been attempted in only two or three laboratories. The present work summarizes 3 years experience with myelinated tissue cultures, discusses the significance of the findings and reviews some of the difficulties in interpretation.

MATERIALS AND METHODS

Essentially the methods employed have been those of Bornstein & Appel (1961) and have been described in detail elsewhere (Field & Hughes, 1965). Explants of neonatal rat cerebellum, about 0·5–0·75 mm thick, obtained by slicing sagitally the whole cerebellum into twelve

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fragments, were cultured on rat-tail collagen coated coverslips (Bornstein, 1958) in roller tubes at 35°C for 2–4 weeks, during which time well differentiated myelin sheaths were formed. The nutrient medium comprised Simms' balanced salt solution 45%; tissue culture medium '199' (Glaxo) 5%; bovine serum ultrafiltrate (Microbiological Associates, Bethesda, Maryland) 25%; human ascitic fluid 25% with glucose added to give a final concentration of 600 mg/100 ml. The culture medium was maintained within the pH range 6·9–7·1 by adjustment with CO_2 as required, and was totally replaced once a week. Chick embryo extract (9 days *in ovo*), previously incorporated at a 5% level (Field & Hughes, 1965), was eliminated entirely for a time as it was responsible for contamination by pleuro-pneumonia-like organisms (PPLO). Since, however, the myelin, which formed satisfactorily in its absence, was very prone to spontaneous degeneration, it became necessary later to add it as a supplement (5%) to the nutrient medium after the 1st week of culture, using batches of embryo extract known to be free from PPLO. The majority of cultures, when screened to ascertain their suitability as test objects, showed a very occasional myelin ball or minor bleb on otherwise healthy sheaths, and these were considered suitable for myelotoxicity tests. A small proportion of cultures, however, exhibited more marked spontaneous degeneration of the myelin sheaths (Bornstein & Murray, 1958) with well formed swellings at times indistinguishable from the changes caused by a toxic serum (described below). Such degenerate cultures were discarded.

In addition to photomicrographic records, time-lapse cinephotomicrographic analysis of tests has enabled detailed study of the changes that occur when a serum is applied to a myelinated culture. For observing cultures bright field was more successful than phase contrast illumination. With bright field (Bornstein & Murray, 1958; Bornstein & Appel, 1961; Bornstein, 1963; Appel & Bornstein, 1964; Bornstein & Appel, 1965; Lumsden, 1965), myelinated fibres, because of their high refractility, appear as parallel bright seams bordering axis cylinders and stand out clearly from the background of glia. In our experience, phase contrast illumination, used by Lamoureux, Boulay & Borduas (1966), requires a very thin culture and even then does not give a convincing picture of myelinated nerve fibres, so that it is difficult to distinguish them from drawn out glial fibres.

In each test three groups of preparations were set up: (a) culture plus serum under test (25% in nutrient fluid), (b) culture plus a normal human serum (25% in nutrient fluid), and (c) culture plus nutrient fluid alone. The test serum was incorporated in the nutrient fluid by reducing the balanced salt solution only. At least two, and usually three or four, cultures were set up within each group. Since the sera used were stored at −60°C it was found unnecessary to add complement (Field & Hughes, 1965). Occasional additional tests with 20% and 33% serum were made and in all cases the final glucose concentration was adjusted to the usual 600 mg/100 ml to prevent degeneration due to glucose withdrawal (see 'Discussion'). Spinal fluids were added to the cultures at 50% concentration in the usual nutrient medium.

During testing, cultures of the same batch and age were mounted in Maximow slides or an optical glass viewing chamber (Rosenthal, New York) with two drops of the test medium applied to each culture, maintained at 35°C in the lying-drop position in an incubator and removed at intervals for examination. Sometimes the chambers were kept on a hot stage at 35°C in order to follow a selected field more closely. In all cases an overall study of each culture was made and changes in specific fields recorded photographically.

A total of eighty-seven sera have been tested: twenty-five from acute cases of multiple sclerosis; twenty-six from cases of motor neurone disease (reported by Field & Hughes, 1965); two from general paralysis of the insane, and thirty-four from normal subjects—healthy blood transfusion donors matched with the patients for age and sex. In addition five spinal fluids from acute cases of multiple sclerosis have been tested and one sample of ventricular fluid from a chronic case of multiple sclerosis after concentration about fifty times by freeze drying and reconstitution. A total of 400 cultures have been used. At the end of a test, cultures were fixed in formol–saline and either stained for myelin by Weigert–Pal's method or axis cylinders impregnated by the method of Bodian.

The criterion of characterising a serum as demyelinating or positive was that adopted by Bornstein (1963), who writes 'the undoubted presence of the characteristic pattern of demyelination . . . in the absence of a gross, general cytotoxic effect was the essential criterion for designation of a particular sample of human serum as positive or demyelinating'. Whilst the assessment of the degree of demyelinating activity is necessarily subjective, some attempt at quantitation has been made by estimating the proportion of myelin sheaths which showed degeneration at specified intervals after the application of a test serum. Since it was not practicable to count individual sheaths the degree of demyelination was arrived at by scanning the whole culture and estimating what proportion of myelinated sheaths had been affected. A score was then assigned as follows. For the degeneration present at 6 hr after the application of the test medium, + + + + if 75% or more of myelin showed degeneration; + + + if 50% did so; and + + if 25% was degenerate. Where degeneration was present in only a few sheaths, approximately 5%, + was scored. At 24 hr, 75% degeneration was scored as + + +; 50% as + +; 25% as +; and 5% as ±. At 48 hr, 75% was rated + +; 50% as +; 25% as ± and 5% as negative. A serum which produced 75% degeneration after 72 hr was classed as +; if it produced 50%, as +; and 25% was rated as ±. These ratings are summarized in Table 1. Each culture was given the nearest applicable score and the final assessment for a particular serum arrived at by averaging the individual scores for the duplicate cultures.

TABLE 1. Serum myelotoxicity or demyelinative activity grading

Time elapsed since application of serum (hr)	Demyelinative activity (percentage estimated of proportion of myelinated fibres damaged)			
	5%	25%	50%	75%
6	+	+ +	+ + +	+ + + +
24	±	+	+ +	+ + +
48	−	±	+	+ +
72	−	±	+	+

Cultures were examined at the stated intervals following the application of the test serum and a score given −, ±, +, + +, + + + or + + + + according to the amount of myelin destruction present at the time, e.g. 50% of the sheaths damaged after 24 hr was scored + +.

RESULTS

In positive tests, changes closely resembled those described by Bornstein & Appel (1961) working with serum from rabbits with experimental allergic encephalomyelitis. An unequivocably active serum (scored +++) would produce well marked changes within 30 min especially if applied in 50% concentration. Since in our hands 50% serum even from normal subjects commonly showed toxicity, 33% was considered to be the highest concentration safe to use without giving a false positive result, and the majority of tests were in fact conducted using 25% serum.

Very real difficulties in interpretation soon became apparent. In general, myelinated fibres were deeply situated within a culture and grew close to the layer of collagen as revealed by electron microscope studies of cultures (Field, Hughes & Raine, unpublished observations) and were frequently covered by a varying thickness of glial cells. Only in a 'monolayer' culture does an applied serum have ready access to the myelinated fibres, so that a thick culture may show no demyelination whilst a thin one with well spaced and easily accessible fibres may show a positive result with the same serum. Moreover, the same factor might operate within a single culture so that different conclusions might be drawn from examination of thick and thin areas. Even at 25% strength nearly all sera (both normal and pathological) were found to be toxic in greater or lesser degree for the isolated and highly exposed glial cells at the periphery. All readings were, therefore, made from thin cultures in which myelin sheaths were evenly distributed and virtually free from any blebs or swellings due to spontaneous degeneration. In every case, a total examination was made and selected areas identified for serial photographic recording.

The demyelinative response was characteristic, with a time sequence dependent upon the activity of the serum, its concentration and ease of access to the myelin sheaths. The first changes affected the exposed and vulnerable glial cells at the margin of the culture. Their delicate processes became thickened and retracted and the whole cell rounded. These changes commonly occurred within $\frac{1}{2}$ hr. Cytoplasmic granules showed gradually intensifying Brownian movement and the cell bodies became swollen and some ultimately burst. Not all glial cells, however, even at the periphery of the culture showed such changes. Nearer the centre degenerative changes were less rapid and less severe. Enlarged glial bodies displaced nerve fibres which consequently took on a devious course. Signs of degeneration soon began in the myelin sheaths themselves (Fig. 1). The parallel bright lines they presented became irregular with small lateral buddings which gradually increased to become pinched off and form free myelin balls. When changes were severe the sheaths lost continuity and were converted into rows of ellipsoids. Glial cell swelling usually became marked after myelin destruction was well under way. With a strongly myelotoxic serum, devastation of myelin might be so wide-spread as to reduce it to scattered globules (Fig. 2). With a 25% serum such destruction usually came about after 18 hr.

In addition to the sequence of changes set out above, in some instances the serum caused myelin sheaths to degenerate without prominent refractile swellings and blebs. In these cases they gradually lost their refractility until only non-refringent fibres remained. Bornstein & Appel (1961) observed this with sera from rabbits with experimental allergic encephalomyelitis and reported that 'the myelin seems simply to melt away'.

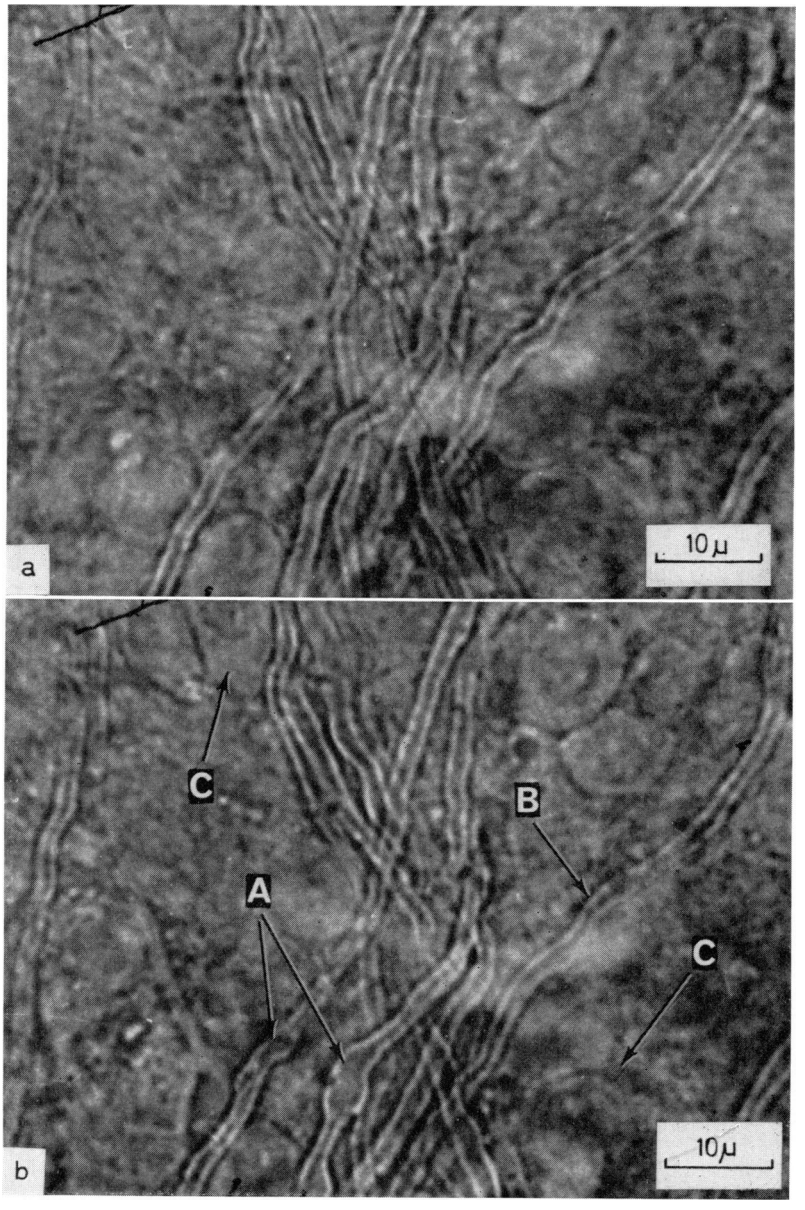

FIG. 1. Series taken from time-lapse 16-mm cine film of a culture of newborn rat cerebellum 30 days *in vitro* undergoing demyelination in a Rosenthal optical chamber.

(a) Selected field before application of 33% dilution in normal nutrient of serum from active multiple sclerosis case (this serum was one already known to produce demyelination at 20% concentration, but in order to accelerate the effect for filming purposes was here used at 33% concentration). Note absence of swellings on myelin sheaths. Bright field illumination ×40 Zeiss water immersion objective. ×1400.

(b) Same field 12 min after application of test feed. Note swellings appearing in two of the sheaths (A). Another sheath (B) and two glial cells (C) not yet affected.

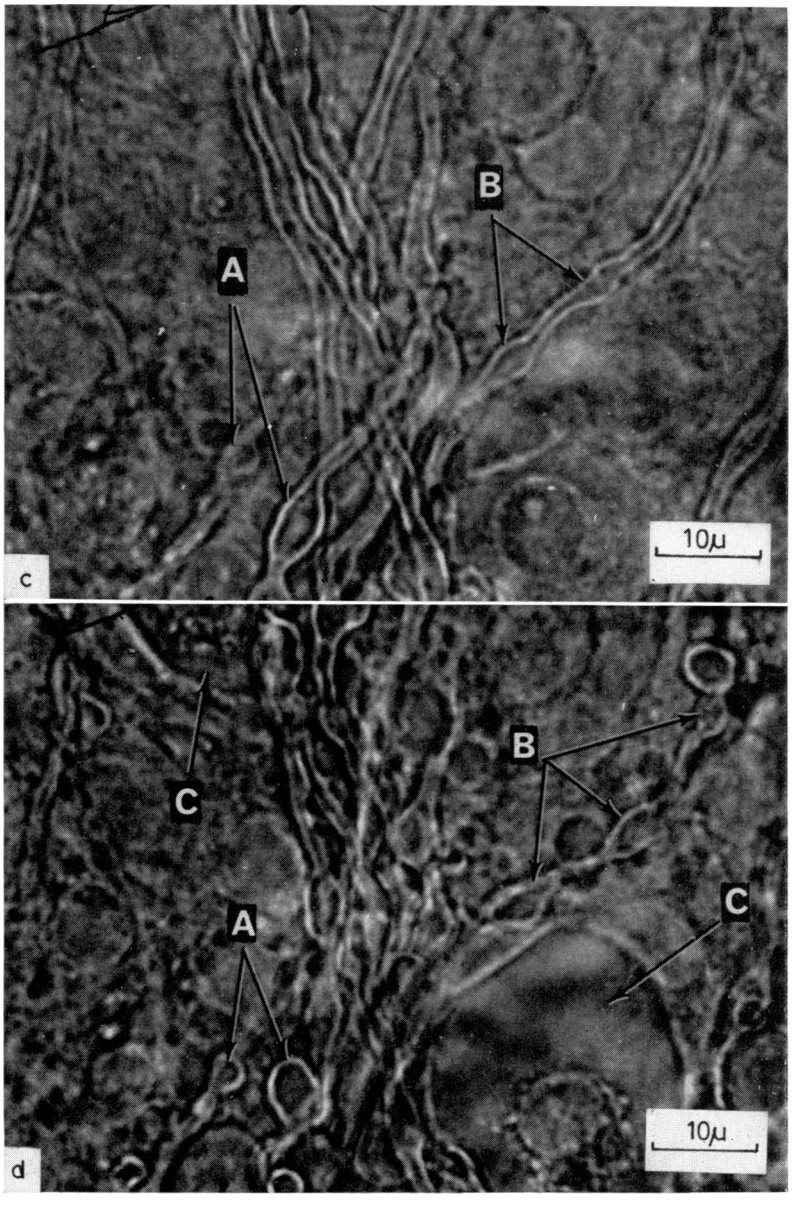

FIG. 1 (*continued*). (c) Twenty-one minutes after application of test feed. Previously formed swellings (A) increased in size and sheath (B) has begun to show swellings.

(d) Eighty-seven minutes after application of test feed. All sheaths show some degeneration. The more advanced degeneration has progressed so that lines of discrete myelin ellipsoids (A) follow the course of the nerve fibre. Other sheaths show irregularly shaped blebbings and swellings (B) still apparently in association with the parent fibre. Two glial cells (C) have swollen up with granules in Brownian movement in their cytoplasm.

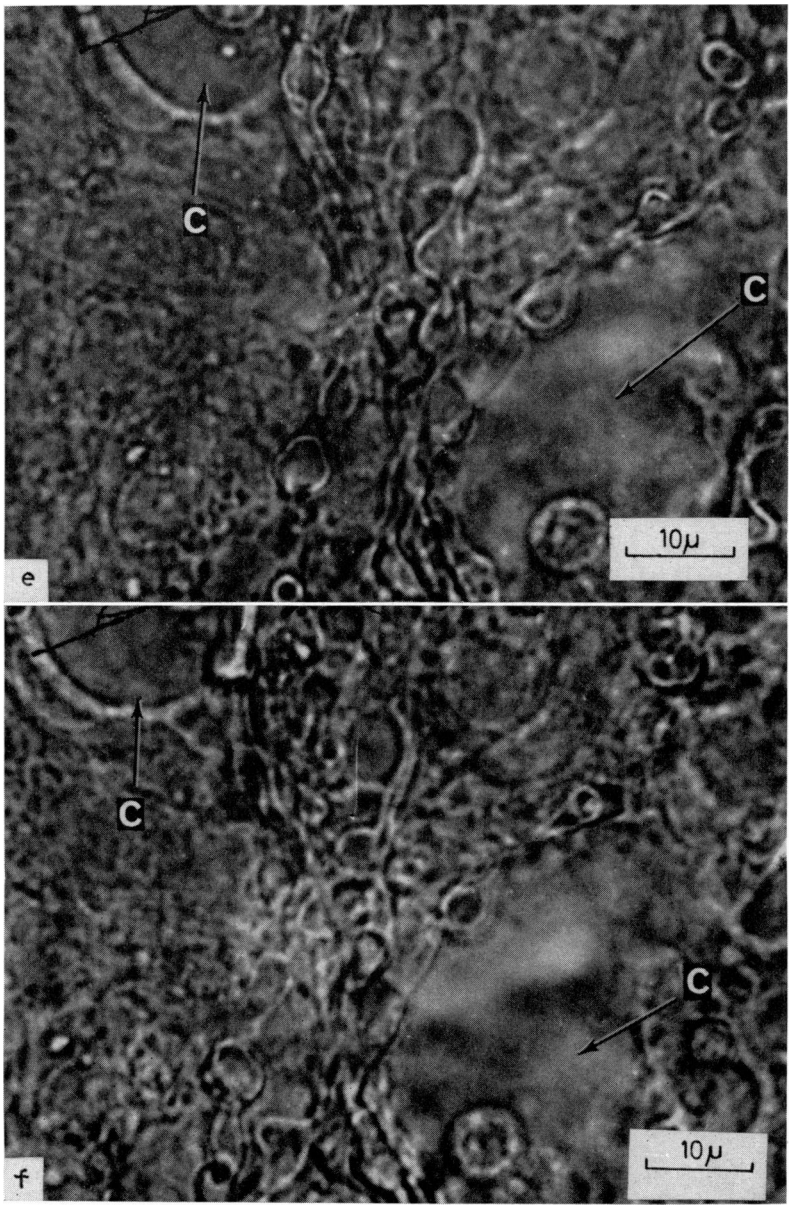

FIG. 1 (*continued*). (e) One hundred and fifty-three minutes after application of serum. The swollen glia (C) have increased in size while their nuclei have contracted.

(f) Two hundred and eight minutes after application of serum. The most rapid stage of the degenerative process is now over. The movement of the cytoplasmic granules in the glia (C) has ceased and the swollen cell eventually bursts. All sheaths are affected and the degeneration progresses to complete myelin devastation.

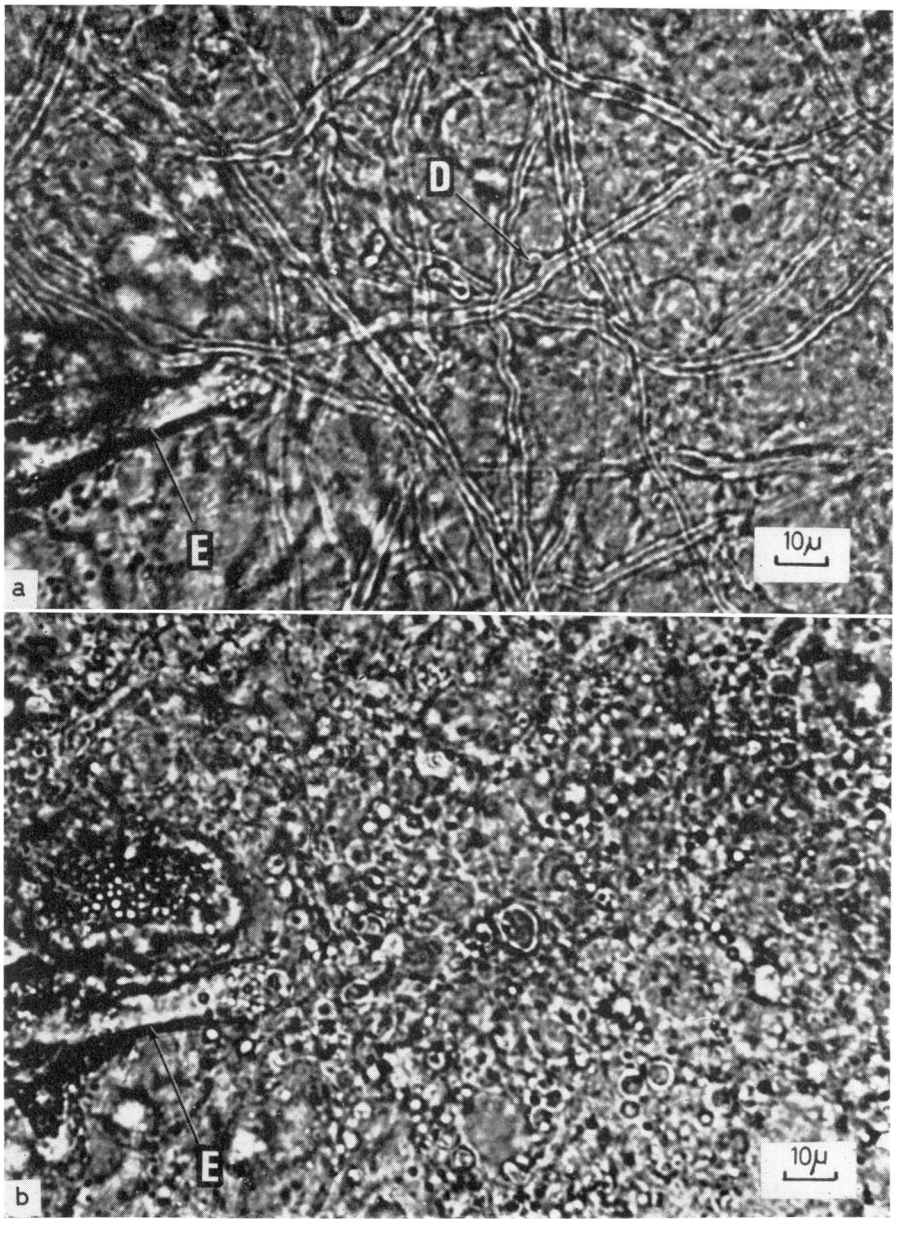

FIG. 2. (a) Field of myelinated fibres in 30-day-old culture of newborn rat cerebellum. Note the occasional very minor blebbings on sheaths (D) occasionally present even in healthy cultures. Foreign material (E) provides a marker for relocation of field. × 690.

(b) Same field 24 hr after the addition of serum from an active multiple sclerosis case diluted to 33% in nutrient medium. All myelin is devastated and reduced to myelin balls and granular debris. Only the presence of the inert marker (E) enables the field to be located again. × 690.

Results, with myelotoxicity scored according to the arbitrary scale set out in Table 1, are summarized in Table 2. Because it was impossible to rule out minor non-specific degenerative changes, ± results have been regarded as negative and only definite changes, well

TABLE 2. Serum myelotoxicity or demyelinative activity of eighty-seven human sera on cultured rat cerebellum

Clinical diagnosis	Total sera tested	Demyelinative activity							Proportion positive	
		Positive				Negative				
		+++	++	+	Total	±	−	Total		
Multiple sclerosis	25	5	5	11	21	2	2	4	21/25	(84%)
Motor neurone disease	26	2	8	6	16	4	6	10	16/26	(62%)
General paralysis of the insane	2	0	1	0	1	0	1	1	1/2	(50%)
Normal	34	1	4	3	8	9	17	26	8/34	(24%)

Sera have been graded according to the scheme set out in Table 1. For reasons explained in the text (±) have been regarded as negative. No multiple sclerotic serum tested was sufficiently toxic to be scored (++++).

outside the limit of what may be seen in well-maintained cultures, regarded as positive. Minor changes, especially from the application of normal serum tended to regress after some hours and did not seem to interfere with the vitality of the sheaths.

DISCUSSION

Although 5 years have elapsed since Bornstein & Appel (1961) introduced the use of tissue cultures for the study of myelotoxicity, apart from their publications (Bornstein, 1963; Bornstein & Appel, 1965) no detailed study with respect to multiple sclerosis has come from any other laboratory, though preliminary reports have been presented by Lumsden (1965, 1966).

The criterion of a demyelinative serum has already been established as demyelination in the absence of any general cytotoxic effect. No serum, however toxic to myelin, appeared to cause appreciable damage to nerve cell bodies or axons, ependymal cells (whose ciliary activity was often maintained even in the presence of widespread degeneration) or connective tissue cells. Non-specific toxicity was usually manifested in the extremely sensitive neuroglia. Toxic activity was, therefore, assessed entirely on the basis of demyelinative action, as was done by Lumsden (1966) who also attempted to grade the activity of sera. Lamoureux *et al.* (1966) on the other hand carrying out somewhat similar testing with experimental allergic encephalomyelitis sera from rhesus monkeys have taken account of damage to neurones and glia, as well as myelin in computing their cytotoxic index.

From what has already been said of the difficulties in interpreting myelotoxicity tests, it will be apparent that the method calls for healthy cultures free from all spontaneous

degenerative changes at the start of a test. Myelin degeneration under the conditions of testing may occur from many causes other than genuine serum myelotoxicity and these must be fully appreciated in interpreting the tests or false results will be recorded.

Apart from the occurrence of spontaneous degenerative changes which may already be present in a culture, non-specific degeneration may also be induced in some degree by the change of medium when a test is set up. A myelinated culture is a very sensitive test system and it is not surprising that a sudden change to a foreign serum (i.e. different to that used as nutrient) causes immediate glial damage and even degenerative changes in myelin sheaths. The effects of this 'shock' due to serum change are rarely severe and permanent, but generally mild and non-progressive. Frequently they are reversible. However, they differ only quantitatively from the early phases of severe myelin degeneration. Blebbing appears along the sheaths but gradually regresses and the sheaths revert to their normal smooth appearance.

Gliotoxicity is not considered in the present work as it is not considered to be a specific characteristic of sera that are truly demyelinative. In our experience nearly all sera are toxic to the highly sensitive delicately branched neuroglial cells, especially when they are so openly spaced as are the oligodendrocytes and 'Schwann cells' (*sic*) in the cultures of neonatal-rat central nervous tissue figured by Lamoureux *et al.* (1966), and this gliotoxic effect confused the interpretation of weakly positive results. The severe and rapid myelin degeneration produced by strongly demyelinative sera is, however, rare with normal sera. Non-specific degeneration caused by a change of serum is reduced by dilution of the serum to the 20% level as used in the present experiments.

Berg & Källen (1962) on the other hand, used undiluted serum from animals with experimental allergic encephalomyelitis in tests on neonatal glial cells in plasma clot tissue culture. Bornstein & Appel (1961) tested sera from rabbits with experimental allergic encephalomyelitis at concentrations varying between 3 and 25%, but increased the concentration to a maximum of 50% for the weaker activity found in human sera (Bornstein, 1963). Lumsden (1965, 1966) gives no indication of the concentration of serum used.

Cultures may also be affected by adverse conditions developed in the small testing chambers. Degeneration through excessive light exposure may occur (and control cultures may not always be examined so exhaustively as experimental cultures), or from partial drying up of the small volume of test medium on the culture, with resulting damaging osmotic changes. Another consequence of the small volume of test medium is that rapid pH changes may occur which can be deleterious. Exhaustion of the medium, particularly with regard to glucose, is an important source of error, especially in tests exceeding 24 hr.

Non-specific changes due to glucose deprivation may lead to errors since they may be mistaken for those produced by a myelotoxic serum. For adequate myelination, and maintenance of a sheath once formed, there must be present in the medium a concentration of glucose of at least 200 mg/100 ml and optimally 500–600 mg/100 ml (Murray, Peterson & Bunge, 1962). A lowering of glucose concentration will induce a characteristic myelin degeneration pattern (Bornstein & Appel, 1961), and this could occur through natural depletion over a period of 1 or 2 days in a small volume of feed, or through dilution errors in making up the test media, as pointed out by Lumsden (1965, p. 374). Myelin degeneration due to glucose withdrawal (Fig. 3) can be distinguished from that caused by serum toxicity, as in the former the myelin sheath breaks up into a series of regular balls strung more or less as uniform pearls along a thin axis cylinder. Glial cells do not as a rule undergo marked

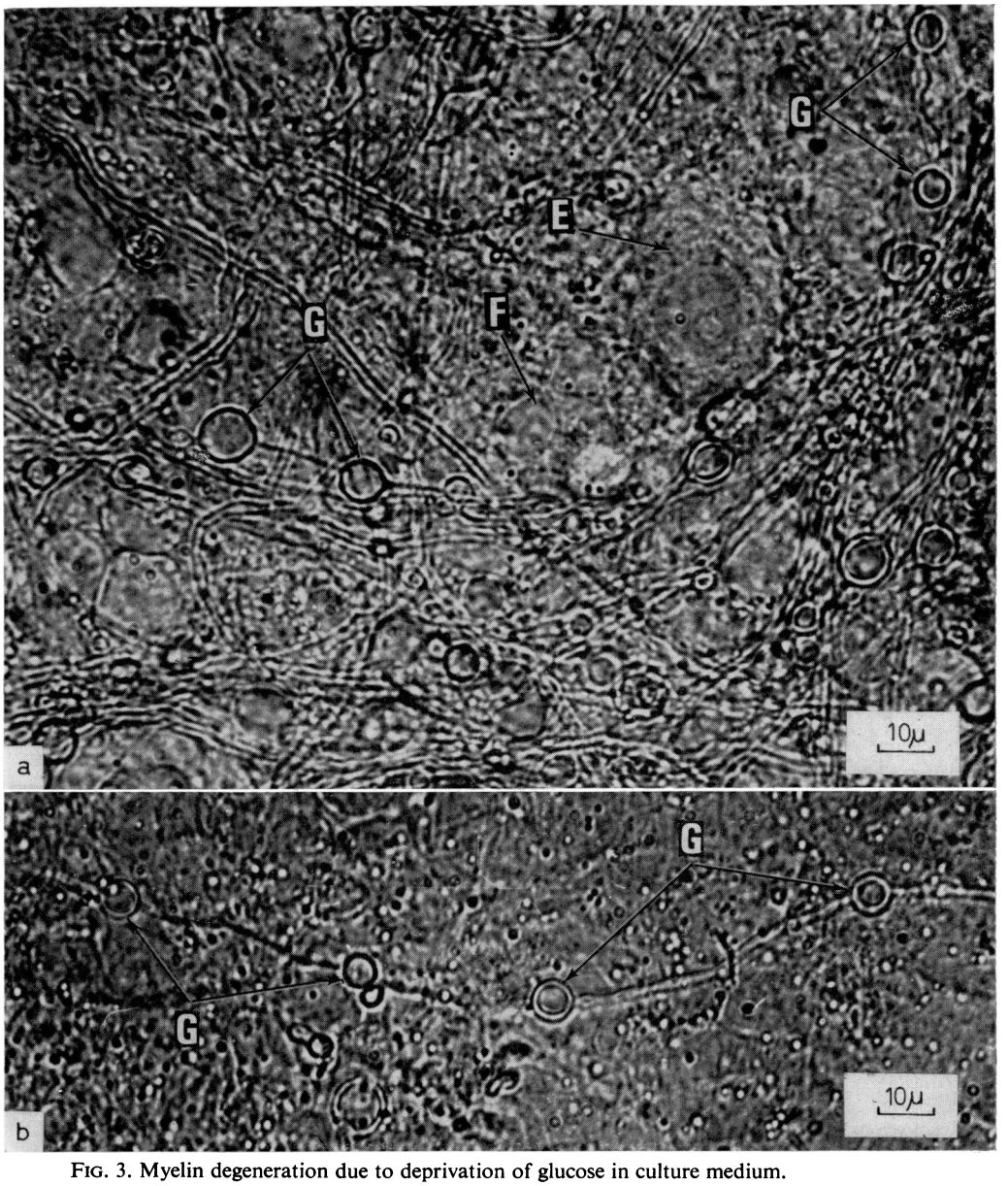

FIG. 3. Myelin degeneration due to deprivation of glucose in culture medium.

(a) Culture of newborn rat cerebellum 23 days *in vitro* taken straight from roller tube showing spontaneous myelin degeneration. This is typical of the degeneration caused by a decrease in glucose concentration. Note that only the sheaths are affected—nerve-cell bodies (E) and glia (F) show no damage. Perfectly regular spherical swellings (G) (contrasted with the irregular blebbings due to a toxic serum) form at regular intervals along the sheaths which become very thin and lose some of their refringence. × 770.

(b) Another field showing the regular spherical swelling (G) along the sheath like a widely spaced string of pearls. × 770.

swelling so that nerve-fibres are not displaced. However, if true toxicity and glucose deprivation were operating together it would be difficult to estimate the contribution of the former.

The degree and extent of demyelination will be influenced by variable factors within each culture as it is impossible to produce identical cultures. Different results may sometimes be observed in different regions of a given culture through variations in thickness of the preparation, or fortuitous variations in other factors not recognized.

Difficulties in interpretation have been met by other workers, and Patterson (1966) reporting some tests carried out by Bornstein's group, records 'equivocal results with respect to cytotoxic antibody because of technical problems, viz. 'background toxicity' noted in all brain cultures including control cultures' (p. 183). Recently, Bornstein & Appel (1965) have pointed out that 'the interpretation of tissue culture techniques in the study of specific immunological phenomena and their related or potentially related disease processes must be viewed with considerable skepticism in view of the general cytotoxic properties displayed by almost all sera, particularly when applied to short-term or young cultures'. Taking into account all the pitfalls and variations relating to the technique our quantitation is necessarily to some extent subjective, and the same may be true of the cytotoxic index (stated to be semi-quantitative) adopted by Lamoureux *et al.* (1966). To assign a figure which acts as a parameter of destruction under these circumstances may sometimes engender an unjustified confidence in interpretation.

In our experience, the great majority of normal human sera show some minor degree of toxicity, and this is true also of animal sera. In our own unpublished work on experimental allergic encephalomyelitis serum myelotoxicity in the guinea-pig, it quickly became apparent that so many normal animals displayed appreciable toxicity that only those which were free from it could reasonably be used as the subjects for experiment. Bornstein & Appel (1965) state that the specific pattern of demyelination was not produced by serum from any normal rabbit, guinea-pig, rat or mouse they tested (numbers unspecified) but occurred with two of twenty-six (8%) normal human sera. Lumsden (1966) reported that 15–20% of normal guinea-pig sera showed natural toxicity for tissue cultures, whilst no control human serum (of an unspecified number tested) did so.

Although in the present work 24% of normal sera were found to be myelotoxic, most possessed only mild demyelinative activity and only one was scored as $+++$. The proportion of positive sera from cases of multiple sclerosis and motor neurone disease exceeds significantly that found with normal sera. Multiple sclerosis cases (84% positive) show a higher incidence of demyelinative activity than motor neurone disease cases (62% positive). Furthermore five of the twenty-five multiple sclerosis sera were scored ($+++$) compared with only two of the twenty-six motor neurone disease sera. Thus although motor neurone disease serum was sometimes markedly toxic, this occurred less frequently than in the case of multiple sclerosis.

If our own results are compared with those published by Bornstein & Appel (1965) and Lumsden (1966) substantial similarities emerge except in the case of normal sera. Thus our own figure of 84% positive sera from cases of acute multiple sclerosis compares with Bornstein's 68% and Lumsden's 92% (*severe* active cases). In motor neurone disease our own 64% compares with Bornstein's 60%. Lumsden (1966) has 'deliberately . . . exclude(d) neurological diseases other than multiple sclerosis' and has selected his controls from normal laboratory staff and haematological cases without neurological symptoms. A difference,

however, emerges in the figures for normal sera since our 24% differs substantially from Bornstein's 8% and Lumsden's nil. From these results it may be concluded that myelotoxicity of serum, whilst undoubtedly increased in acute multiple sclerosis is not essentially specific to this condition.

Our own experience with spinal fluid has been limited. Bornstein & Appel (1965) tested one spinal fluid (presumably unconcentrated) of a rabbit with experimental allergic encephalomyelitis which produced the specific pattern of demyelination in the presence of complement. Lumsden (1966) has obtained positive results with three of five cerebrospinal fluids from multiple sclerosis cases concentrated by a factor of 100–150 using concentration dialysis against carbowax and therefore containing a globulin level comparable with that of serum. None of the six specimens of unconcentrated spinal fluid we have tested has been positive. The rationale of concentrating the fluid 100–150 times is dubious. If some toxic material in spinal fluid were to be of pathogenetic significance, it should be active at concentrations obtaining normally. This is especially the case since it has been claimed that γ-globulin is made within the subarachnoid space and that it may be the antibody responsible for demyelination (Lumsden, 1965, 1966). Toxicity might indeed be expected if the globulin constituents of cerebrospinal fluid are concentrated to a level 100–150 times that at which they are normally in contact with nervous tissue. However, only three of Lumsden's five concentrated spinal fluids were toxic. The six unconcentrated spinal fluids tested by us and found to be negative all had γ-globulin levels exceeding the normal upper limit (28·5% of total protein).

The biological significance of the myelotoxic activity found in multiple sclerosis serum with greater strength and frequency than in normal serum, is still far from clear. That myelotoxic antibodies may be formed secondarily to myelin destruction, is a possibility in view of the occurrence of antibody to encephalitogenic factor in a variety of neurological conditions reported by Field, Caspary & Ball (1963). Antibody to encephalitogenic factor was found with significantly greater frequency in the serum of multiple sclerosis patients, those suffering from general paralysis of the insane, or presenile dementia, and in those with other neurological disorders in which there is reason to suppose that considerable cerebral atrophy had taken place, than in normal people, i.e. whenever there had been brain disintegration from any cause. The toxicity shown by motor neurone disease sera (Bornstein & Appel, 1965; Field & Hughes, 1965) supports this view. No doubt examination of further cases of general paralysis of the insane would reveal similar myelotoxicity. In this respect, it is interesting to note that Lamoureux *et al.* (1966) found pronounced myelo- and gliotoxicity in tissue culture tests in the serum and (unconcentrated) spinal fluid of rhesus monkeys with experimental allergic encephalomyelitis. This species certainly reacts very violently to inoculation of human brain antigen (Field, 1966) so that large necrotic lesions are commonly produced in the central nervous system. Lamoureux *et al.* used rabbit antigen but, if anything like the severe reaction to human antigen occurred, there would be ample opportunity for breakdown products to reach the general circulation and also the immunologically competent cells of the subarachnoid space. Apart from a possible local immunological response, gross lesions in the nervous system resulting in lowered blood-brain barrier offer ample opportunity for access to the spinal fluid of any toxic agency developed in the circulating blood. The undoubted occurrence of toxic factors circulating in the blood in multiple sclerosis and experimental allergic encephalomyelitis in the presence of a lowered

blood-brain barrier at lesions (Broman, 1947, 1964; Barlow, 1956; Vulpe, Hawkins & Rozdilsky, 1960; Field, 1961) might well be of significance in the development or extension of lesions.

In connection with 24% of normal sera found to be myelotoxic it may be recalled that Field *et al.* (1963) were able to demonstrate antibody to encephalitogenic factor in 20·4% of forty-four normal subjects. From the work of Hashem & Barr (1963) it is possible that normal subjects may become sensitized to brain or its degradation products if these are ingested.

Although Lumsden (1966) in a preliminary report refers to 'myelotoxic antibody' in cases of multiple sclerosis, Bornstein & Craine (1966) are careful to point out that whilst the toxic factor of serum is complement dependent, its antibody nature has not yet been demonstrated.

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