THE ENCEPHALOMYELITIC ACTIVITY OF MYELIN ISOLATED BY ULTRACENTRIFUGATION*

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Myelin has long been implicated as the source of the antigen which produces experimental allergic encephalomyelitis (EAE), a disease which can be readily produced in laboratory animals by injection of whole central nervous tissue in Freund's complete adjuvant.

It has been suggested that EAE is an autoimmune phenomenon involving myelin, but convincing evidence that myelin is the exclusive source of the antigen has not been presented. Different brain fractions which have been implicated in the induction of EAE are white matter (1, 2), gray matter (3), brain mitochondria (4), and myelinated axon fragments (5). Chemical studies concerning the antigen have attributed activity to proteolipid fractions (6) and to water-soluble proteins (7, 8).

It is obvious that much of the contradiction in the literature has arisen from three major sources. First, some investigators have failed to assess the activity of their respective fractions quantitatively. Second, there are species differences in susceptibility of the test animals to antigen and adjuvant, as well as differences in the potency of the antigen from different species. Third, the inherent complexity of brain tissue precludes clean-cut separations of the tissue into types of cells by gross dissection or into subcellular particles by fractionation of homogenates. Any one of these difficulties could lead to erroneous interpretation of experimental observations; in some cases, all three have contributed to the lack of agreement in published reports on the encephalitogenic activity of nerve tissue components.

This report describes a procedure for the preparation of myelin from whole guinea pig brain and presents evidence that the encephalitogenic activity of nervous tissue is associated with it. Electron microscopy has been used to confirm its identity and to yield qualitative information regarding its purity. Bio-assay was carried out in the homologous species under optimal conditions for the quantitative estimation of encephalitogenic activity (9). The specific activity of myelin was found to be approxi-

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mately five times that of the tissue from which it was derived. The low specific activity of other cell fractions could always be attributed to a small amount of myelin which was shown by electron microscopy to be present as a contaminant.

A highly purified basic protein, previously isolated from whole brain and possibly representing the total encephalitogenic activity of that organ, has been isolated from myelin, confirming the locus of this protein in the fraction. Proof of the *in vivo* localization of this small protein was complicated by the fact that similar proteins (histones) are known to exist in cell nuclei, and it has been suggested (10) that cerebral nuclear histones do not remain firmly attached to the nuclei during homogenization and centrifugation, but migrate to and combine with other cell particles. The possibility that the encephalitogen originated in the nucleus and subsequently combined with acidic constituents of myelin has been considered. The results to be presented, however, confirm the hypothesis that the encephalitogenic protein is located in myelin and not in nuclei and that it is distinctly different in its specific biological activity from the basic proteins derived from cerebral nuclei.

A partial summary of these findings has been reported previously (11).

EXPERIMENTAL

Ultracentrifugation.—Centrifugal separation of cerebral cellular fragments based on particle density, excluding particle shape and size factors which influence sedimentation in differential centrifugation, was suggested by Dr. E. L. Kuff, National Cancer Institute. From his suggestions a technique of isopycnic centrifugation (12) was devised using a 30 per cent sucrose medium. Separation of particles based on their densities has also been used in other laboratories for fractionation of brain (5, 13–15).

Adult guinea pigs were decapitated, and the brains were removed as quickly as possible and rinsed in cold 0.9 per cent NaCl to remove adhering blood. The brains were homogenized with 9 volumes of 30 per cent (0.88 M) sucrose (w/v) in a smooth glass homogenizer with teflon pestle rotating at approximately 600 RPM. Nine complete up-and-down cycles in a period of 2½ minutes were sufficient to disintegrate cells without destruction of nuclei as seen by examination with a phase microscope. On top of 9 ml aliquots of this homogenate were carefully layered 3 ml of 8.5 per cent (0.25 M) sucrose in 12 ml plastic centrifuge tubes. Care was exercised to avoid disruption of the interface. The tubes were centrifuged for 60 minutes at 105,000 × g_{av} (40,000 RPM in the No. 40 rotor of the Spinco Model L Ultracentrifuge). There resulted a dense white layer at the interface plus some of the same material adhering to the side of the tube. This adhering material had broken away from the interfacial layer when the tubes had stopped spinning and the interface had returned to the horizontal from the vertical position. A higher density fraction had sedimented and formed a pellet at the bottom of the tube (Fig. 1).

The interfacial layer was carefully removed from each centrifuge tube with a fine-tipped pipette, resuspended in 30 per cent sucrose, carefully covered with 8.5 per cent sucrose, and recentrifuged at 105,000 × g_{av}. Likewise, the high density residue from the first centrifugation was resuspended in 30 per cent sucrose, covered with 8.5 per cent sucrose and recentrifuged. This procedure served to reduce the percentage of contaminating particles in each fraction. Further manipulations were carried out on the interfacial layer. It was suspended in approximately 8.5 per cent sucrose solution and centrifuged to obtain a tightly packed pellet which was homogenized in distilled water to release by osmotic shock axoplasm trapped in myelin figures. The material was again centrifuged into a pellet, then resuspended in 30 per cent sucrose, covered with 8.5 per cent sucrose and recentrifuged to free it from the more dense material.

Finally the preparations were resuspended in 8.5 per cent sucrose to yield pellets which could be used for enzyme assay, fixed for electron microscopy, or dialyzed and lyophilized for bio-assay and chemical analysis.

Guinea pig brain nuclei were isolated by a procedure developed by Sporn *et al.* (16) for the isolation of rat brain nuclei. The preparation involved isopycnic centrifugal separation of nuclei from other cellular constituents in 2 M sucrose in which the nuclei will sediment while other particles float. The extremely clean separation resulting from the use of 2 M sucrose was verified with phase microscopy. The nuclei were dialyzed and lyophilized before further procedures were carried out.

Bio-assay.—Disease-free adult male guinea pigs, weighing from 500 to 600 gms were used as the test animals. The adjuvant mixture consisted of 1 volume phenol-saline (0.5 per cent phenol—0.9 per cent NaCl), 1 volume melted Aquaphor (Duke Laboratories, South Norwalk, Connecticut), and 2 volumes of light paraffin oil (Fisher Scientific Co., Pittsburgh) which contained 2 mg/ml pulverized heat-killed *Mycobacterium butyricum* (Difco). The weighed antigen was placed in a motor-driven, ground glass homogenizer and the liquid constituents added and thoroughly mixed in the order stated. An intracutaneous injection of 0.1 ml of the adjuvant mixture containing varying amounts of antigen was made over the sternum. The animals were weighed and examined 3 times weekly for a period of 30 days after injection. Those animals which became ill prior to the end of the experiment were killed when their neurological signs became severe.

Histological studies were made on formalin-fixed brain and cord specimens which had been coded so that the histologist had no knowledge of the clinical state of the corresponding animals. Clinical and histological data were combined to yield a quantitative measure of the disease state in each animal as described by Alvord and Kies (9). The disease index for each dose of antigen was the average disease index of a group of animals (usually 5) which had received that dose.

Electron Microscopic Examination.—A small amount of each fraction was centrifuged into a pellet, fixed in 1 per cent OsO_4 in dichromate buffer (17), dehydrated in graded alcohols and embedded in 6:1, butyl:methyl methacrylate. With a Porter-Blum microtome, thick sections were made and viewed under the phase microscope; thin sections were examined with an electron microscope, RCA EMU-3.

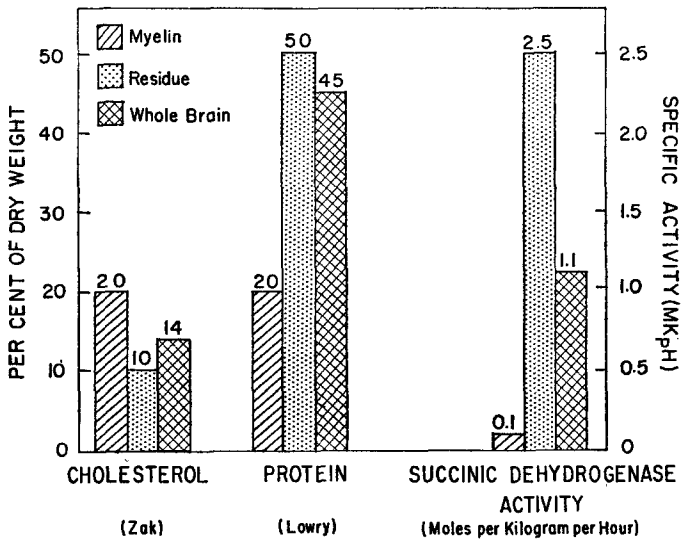
Succinic Dehydrogenase Activity.—The tetrazolium dye reduction method modified from Nachlas *et al.* (18) and Glick (19) was used for the estimation of succinic dehydrogenase activity of the fractions. The reaction mixture consisted of 100 mM sodium phosphate buffer, pH 7.5, 40 mM disodium succinate, 1 mM sodium cyanide and 4 mM INT (2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride, Dajac Laboratories, Philadelphia). A suspension of the fraction to be tested was thoroughly mixed and a 10 to 100 μl volume of suspension was added to 1.0 ml of reaction mixture in ice. For each sample a corresponding blank tube was prepared which contained all reactants except succinate. Standards consisted of similar concentrations of buffer, succinate, and cyanide to which an appropriate and exact amount of INT and excess fresh 0.1 M ascorbic acid were added. The tubes were then incubated 10 minutes at 37°C, returned to the ice bath, and 0.1 ml of 12 N HCl was quickly added to stop the reaction completely. The contents of the tubes were then extracted with 1.0 ml ethyl acetate and the optical density of the resulting red ethyl acetate layer was determined at 492 mμ in a Beckman DU spectrophotometer. The molar extinction coefficient of the INT formazan in ethyl acetate is approximately 2×10^4. Protein determinations were made on the same suspensions and the specific activities were expressed as moles of substrate converted per kilogram protein per hour of incubation.

Other Chemical Procedures.—Protein was determined on suspensions by the method of Lowry *et al.* (20), while concentration of protein in solution was obtained by an adaptation of Waddell's ultraviolet absorption procedure (21). Cholesterol was determined by the method

of Zak *et al.* (22). Protein solutions were compared electrophoretically on a Spinco Model R paper strip electrophoresis apparatus.

The procedure for extraction of basic protein from myelin was a small scale adaptation of the preparation of a highly encaphalitogenic protein from guinea pig whole brain (8).

The isoelectric point was determined by measuring the turbidity of buffered protein solutions in a Beckman spectrophotometer set at 610 mμ. The buffers were Sörensen's glycine-NaCl-NaOH solutions, 0.1 M concentration. One ml of buffer was mixed with 0.1 ml protein solution (10 mg/ml) and the mixture allowed to equilibrate 30 minutes at room temperature. The pH of each mixture was determined after the spectrophotometric measurements were made.



TEXT-FIG. 1. Chemical data on brain fractions. Cholesterol and protein expressed as per cent of dry weight; succinic dehydrogenase activity expressed as moles of dye reduced per kilogram protein per hour.

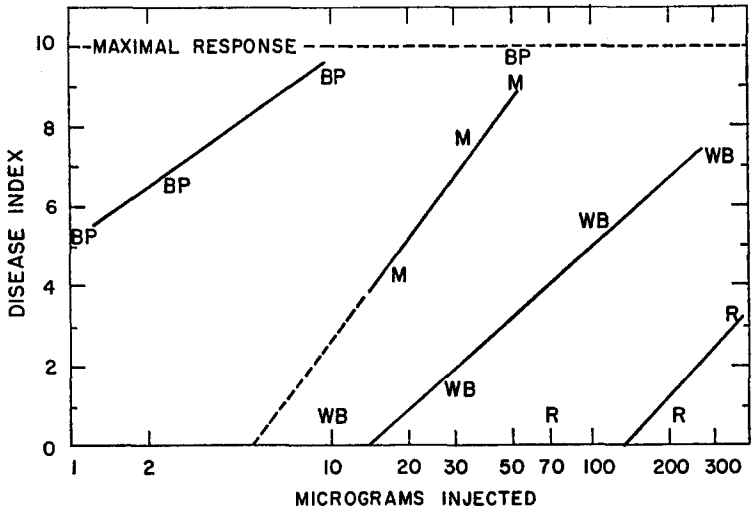
RESULTS

An electron micrograph of the less dense material which collected at the top of the 30 per cent sucrose is shown in Fig. 2. Micrographs of the osmotically shocked myelin fraction are seen in Figs. 3 and 4. A further increase in percentage of myelin fragments by this procedure was achieved. These micrographs show the high concentration of membranous structures, predominantly myelin. Even the best preparations of myelin contained occasional recognizable axoplasmic elements trapped in myelin lamellae, and rare intact mitochondria (Fig. 5).

The higher density material ("residue") which sedimented through the 30 per cent sucrose contained capillary fragments, erythrocytes, neuronal and

glial nuclei, mitochondria, occasional myelin forms, and some unrecognizable debris (Figs. 6 and 7).

The cholesterol and protein contents of the residue and osmotically shocked myelin preparations are compared in Text-fig. 1. The myelin fraction had 20 per cent cholesterol whereas there was only 10 per cent in the residue. The protein proportions were reversed; 20 per cent in the myelin fraction, 50 per cent in the residue. Succinic dehydrogenase, an enzyme thought to be located only in mitochondria, was used as an index of the concentration of mitochondria in the two fractions. As expected from the electron micrographs, the succinic



TEXT-FIG. 2. Semilogarithmic plot of encephalomyelitic activity of brain fractions. *R* = residue; *WB* = whole brain; *M* = myelin; *BP* = basic protein extracted from myelin. Positions of individual symbols indicate assay values derived from groups of 5 animals.

dehydrogenase activity of the myelin fraction was only 1 to 2 per cent of that of the high density residue, when compared on a dry weight basis. Whole brain homogenate had intermediate values between myelin and residue for all sets of determinations.

Quantitative bio-assay of the two fractions for encephalitogenic activity indicated that most of the activity was contained in the myelin layer (*M* in Text-fig. 2). This material showed half-maximal activity at 20 μg dose levels whereas the residue (R) showed less than half-maximal activity at 300 μg. Thus the myelin fraction was approximately 15 times more potent on a dry weight basis or 40 times as active as the residue when compared on the basis of their protein contents. The small amount of encephalitogenic activity in the residue could be correlated with small amounts of myelin contaminating this fraction, as seen by electron microscopic examination. The myelin fraction

was also about 5 times as active as whole brain (WB) in the bio-assay. Isolated brain nuclei were inactive even at 300 μg dose levels. The soluble supernatant fraction was also inactive at 100 μg.

A basic protein preparation (BP) similar to the one reported by Kies, Murphy, and Alvord (8) was isolated from the myelin fraction. This protein could be readily extracted from the 2:1, chloroform:methanol–insoluble portion of myelin with 0.01 N HCl but could not be extracted from whole myelin by 0.1 N HCl. The protein had a molecular weight of approximately 20,000, an isoelectric point of 10.5, and an arginine content of 11 to 12 per cent. The basic protein fraction extracted from myelin showed similar electrophoretic mobility (Fig. 8) to the one from whole brain and had the same isoelectric point and arginine content. The encephalitogenic activities of the two protein preparations (one from whole brain and one from myelin fraction) were approximately the same: 1 to 2 μg of each fraction showed at least half-maximal activity (Text-fig. 2).

The possibility that the encephalitogenic protein existed in cell nuclei *in vivo* and had been liberated into the medium during homogenization was suggested by the results of Wolfe and McIlwain (10). The speed and time of homogenization used in the present experiments were chosen to avoid damage to fragile nuclei, but there was no real evidence that all nuclei remained intact throughout the procedure. Therefore, nuclei were prepared by the procedure of Sporn *et al.* (16) and compared with myelin isolated as described above. The major difference between the two fractions involved the density of the particles, which allowed for very little contamination of either fraction with the other. Nuclei contained less than 1 per cent cholesterol and were sufficiently dense to sediment in 2 M sucrose. Myelin, on the other hand, floated on 0.88 M sucrose and had a cholesterol content of 20 per cent (total lipid of 70 to 80 per cent). The two preparations also differed markedly in their physical appearance. The nuclear preparation consisted of spheres with clearly visible nucleoli as seen in phase micrographs (Figs. 9 and 10). Myelin prepared as above was membranous, and the particles (1 to 2 μ) were not large enough to be visualized clearly in the phase microscope. Nuclei are 33 per cent protein in contrast to myelin which is 20 per cent protein. No significant encephalitogenic activity was detected in 300 μg of the nuclear preparation, a level at which whole brain showed maximal activity. Encephalitogenic activity of the myelin preparation could be detected at 20 μg.

Differences in the basic proteins extracted from the two fractions are indicated in Fig. 11. Whereas 0.1 N HCl readily extracted a mixture of histones from nuclei, this same extraction procedure extracted little protein from whole myelin. After the myelin was defatted with cold 2:1, chloroform: methanol, basic proteins could be extracted from it. As a group, the basic proteins extracted from myelin had electrophoretic mobilities similar to the nuclear histones but with certain differences in their pattern.

DISCUSSION

Ultracentrifugal Fractionation of Brain.—The classical procedures for fractionation of tissue homogenates by differential centrifugation which were first developed for liver have subsequently been applied to all types of biological tissues. Unfortunately, these techniques are not satisfactory for brain because myelin fragments are distributed more or less uniformly in all centrifugal fractions. In enzymatic studies, the presence of myelin probably has little influence on the results. On the other hand, data on the lipid constituents of subcellular particles of brain (23) must certainly be weighted heavily by the chance distribution of myelin in the particulate fractions. Failure to identify myelin in cellular fractionation studies on rat brain led Dahl *et al.* (24) to postulate a second mitochondrial particulate fraction characterized by its high lipid content, relative enzymatic inertness, and rapid increase during the neonatal period, all characteristics of myelin.

These misinterpretations could have been avoided if the investigators had used adequate criteria for identification of the fractions, as Petrushka and Giuditta (27) have pointed out. Many papers have appeared recently in which some of the difficulties were overcome by use of centrifugation in media which utilized density differences to prepare a myelin-rich fraction (14, 15, 25) or to remove it as a source of contaminating material from other subcellular fractions (26). The technical difficulties in preparing brain subcellular fractions of high purity still exist, even with the procedures currently being employed, but they may eventually be overcome since techniques other than centrifugation have been suggested for separation of subcellular particles (28, 29).

Criteria for Identification of Subcellular Particles.—Morphological identification of subcellular particles depends largely on criteria established by electron microscopic studies of intact tissue. As biochemical information regarding the various particulate fractions has accumulated, criteria other than structure can be used to monitor the fractionation procedure. For instance, succinic dehydrogenase activity was used as an index of mitochondrial content. Morphological identification, however, must still remain the major criterion of satisfactory isolation of a given fraction of subcellular particles.

EAE Activity in Subcellular Fractions.—Roizin and Wechsler (4) had reported that their brain mitochondrial preparation was active in EAE induction and Scheinberg and Korey (5) had observed that chopped myelinated axon cylinders were encephalitogenic. The two observations were not necessarily contradictory since both preparations contained mitochondria and myelin as well as other cellular constituents. Neither report included quantitative data on the encephalitogenic activity of the fraction and therefore did not present positive proof for the cellular localization of the encephalitogen. Since encephalitogenic activity had been reported in both fractions (myelin-enriched and "mitochondria"), the original goals of the present investigation were to prepare mitochondria free of myelin, and conversely, myelin free of mitochon-

dria and to subject both fractions to quantitative assay. For reasons to be discussed later, nuclei were also considered as a possible source of the active fraction.

The homogenization and centrifugation techniques were designed to separate the brain homogenate as simply as possible into two particulate fractions—one consisting mainly of myelin, the other of the remaining particulate matter. These two fractions were then compared morphologically, enzymatically, and chemically and these characteristics correlated with their respective encephalitogenic activities.

As expected, the myelin fraction was high in cholesterol and low in protein and had a low succinic dehydrogenase activity, confirming the electron microscopic evidence of very little mitochondrial contamination. On the other hand, its high specific activity in the bio-assay for experimental allergic encephalomyelitic activity indicated that this activity had been increased by removal of other cellular constituents. Conversely, the high succinic dehydrogenase activity of the residue correlated well with the electron microscopic evidence of many mitochondria and contrasted with the low encephalitogenic activity of this fraction. Since nuclei were present in the high density fraction and not in the myelin layer, the low encephalitogenic activity of the dense material could also be interpreted as evidence against the participation of nuclei in the encephalitogenic activity of nerve tissue.

Localization of Basic Proteins in Nerve Tissue.—The isolation of the specific encephalitogenic protein from myelin further supported the theory that the protein occurred in myelin *in vivo*. However, the physicochemical characteristics of the protein were so similar to those of nuclear histones that further evidence was sought for the specificity of the attachment of the basic protein to myelin.

The ability of basic proteins to alter the *in vitro* excitability of brain slices had been demonstrated by McIlwain (30) and Wolfe and McIlwain (10). In attempting to explain the similar effect of cold incubation on brain slice excitability, they suggested that basic proteins had migrated from nuclei to other subcellular fractions.

If their hypothesis were correct, it was possible that the basic (encephalitogenic) protein described in the present report had actually originated in nuclei and had shifted during homogenization and centrifugation to the myelin layer. However, the "nuclear fraction" prepared by Wolfe and McIlwain from cold-treated brain slices was the fraction which floated on 1 M sucrose. This density characteristic is similar to the density which we have observed for myelin. From our experience with guinea pig brain nuclei, and from most published reports on the preparation of nuclei from any source, they would not be expected to float on 1 M sucrose but should sediment readily unless they were trapped in an unusually cohesive mass of much lighter material (*e.g.*, myelin). The disparity between the density of brain nuclei prepared by Wolfe and

McIlwain (10) and those prepared in other laboratories (16, 31) casts considerable doubt on the identity of their nuclei and consequently on their conclusion that nuclear histones were involved in the differences in protein extractability observed. It is also possible that cold incubation of slices had affected the distribution of myelin in the subsequent fractionation and that this could account for observed differences in basic protein extractability.

With microscopically identified nuclei and myelin (see Figs. 3, 4, 9, 10), we have found that basic protein could be extracted from both fractions but *not with equal facility* (Fig. 11). Only after chloroform-methanol treatment did the basic protein of myelin become susceptible to extraction by dilute acid. In other words, the small amount of extractable basic protein from the so called nuclear fraction of Wolfe and McIlwain suggests that their fraction had consisted largely of myelin, and not nuclei which had lost their basic protein through prior experimental treatment. Furthermore, in our experiments there was no detectable encephalitogenic activity in freshly prepared nuclei even though a basic protein fraction could be extracted from them with 0.1 N HCl. Reconciliation of these facts with the theory that the "histone-like" material in myelin originated in nuclei requires the further postulate that this was a highly specific labile component of nuclear proteins—specific with regard to its encephalitogenicity as well as its lability, yet resembling its more firmly attached congeners in electrophoretic mobility, arginine content, and molecular weight.

It should be emphasized that the present experimental results do not contradict the hypothesis that basic proteins may be of importance in the regulation of nerve tissue excitability either *in vivo* or *in vitro*. They merely suggest that the concept of localization or migration of basic proteins in nerve tissue should be reexamined.

Although much electron microscopic and x-ray diffraction evidence has accumulated which indicates that myelin has a lamellar structure consisting of concentric bimolecular layers of protein and lipid, little information is available regarding the nature of the protein or proteins involved. The old concept of an insoluble trypsin-resistant protein residue as the structural framework for myelin has yielded in recent years to a more dynamic concept of protein-lipid interrelationships. The proteolipids described by Folch and Lees (32) are presumed to occur in myelin, but their variable concentration from one part of the CNS to another (33) casts doubt on their role as a structural component. The neuroanatomical distribution of the basic protein has not been investigated, so it cannot be concluded *a priori* that it is an important structural element of myelin. However, its basicity coupled with its ability to combine with acidic lipids (34) suggests that this type of protein might play an important role in the structure and function of myelin.

If the *in vitro* data regarding ganglioside and protamine effects on ion move-

ments in nerve tissue (McIlwain *et al.* (35)) can be translated to the *in vivo* situation, the basic protein may possess the important biological function of regulation of ion movements *in vivo* in addition to its ability to function as an "autoantigen."

SUMMARY

A relatively simple preparation of guinea pig brain myelin, free of gross contamination by other cellular elements has been described. Electron microscopic evidence of the predominance of membranous (lamellar) forms was used as the criterion of purity of this fraction. The slight mitochondrial contamination of the myelin fraction was confirmed by its low succinic dehydrogenase activity. Quantitative bio-assay of the encephalitogenic activity of myelin showed it to have a higher specific activity than whole guinea pig brain. The low encephalomyelitic activity of the other subcellular constituents (nuclei and mitochondria) which were removed from myelin by ultracentrifugation in 30 per cent sucrose could be explained by a small amount of myelin contamination.

A basic protein of high specific encephalitogenic activity has been isolated from myelin by methods previously applied to whole brain. Although the protein is similar to nuclear histones, the following facts point to certain significant differences. Nuclei prepared by a different procedure from the one developed for the isolation of myelin were found to be non-encephalitogenic. Although basic protein could be extracted readily from these nuclei by dilute HCl, the same extraction procedure yielded little extractable protein from whole myelin. Myelin which had been defatted by cold chloroform-methanol yielded a basic protein which was highly encephalitogenic. The evidence presented thus supports the view that there exists in myelin a new basic protein responsible for the induction of experimental allergic encephalomyelitis, which is distinctly different from nuclear histones. The possible relationship of this protein to myelin structure and function has been discussed.

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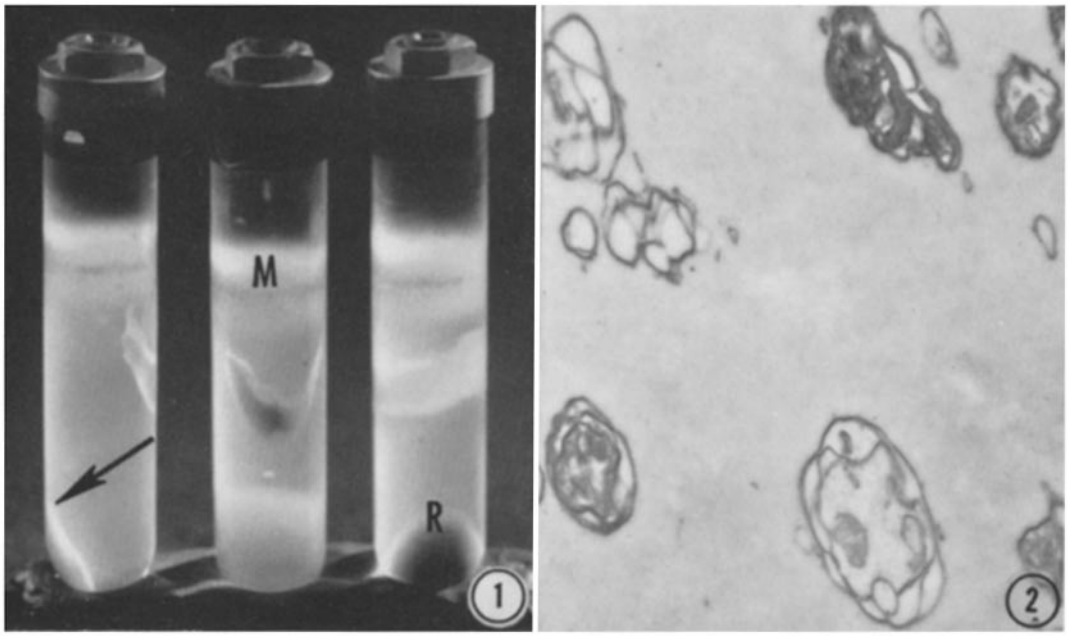
EXPLANATION OF PLATES

PLATE 61

FIG. 1. Centrifugation of brain homogenate in sucrose layers. Tubes are shown in lateral, rear, and front views, respectively. Arrow indicates direction of centrifugal force. The myelin layer (M) floats on 30 per cent sucrose and residue (R) is material which has sedimented in 30 per cent sucrose. Piece of myelin layer adhering to the side of tube is clearly visible.

FIG. 2. Myelin "spheres" with trapped axoplasmic elements. × 11,000.



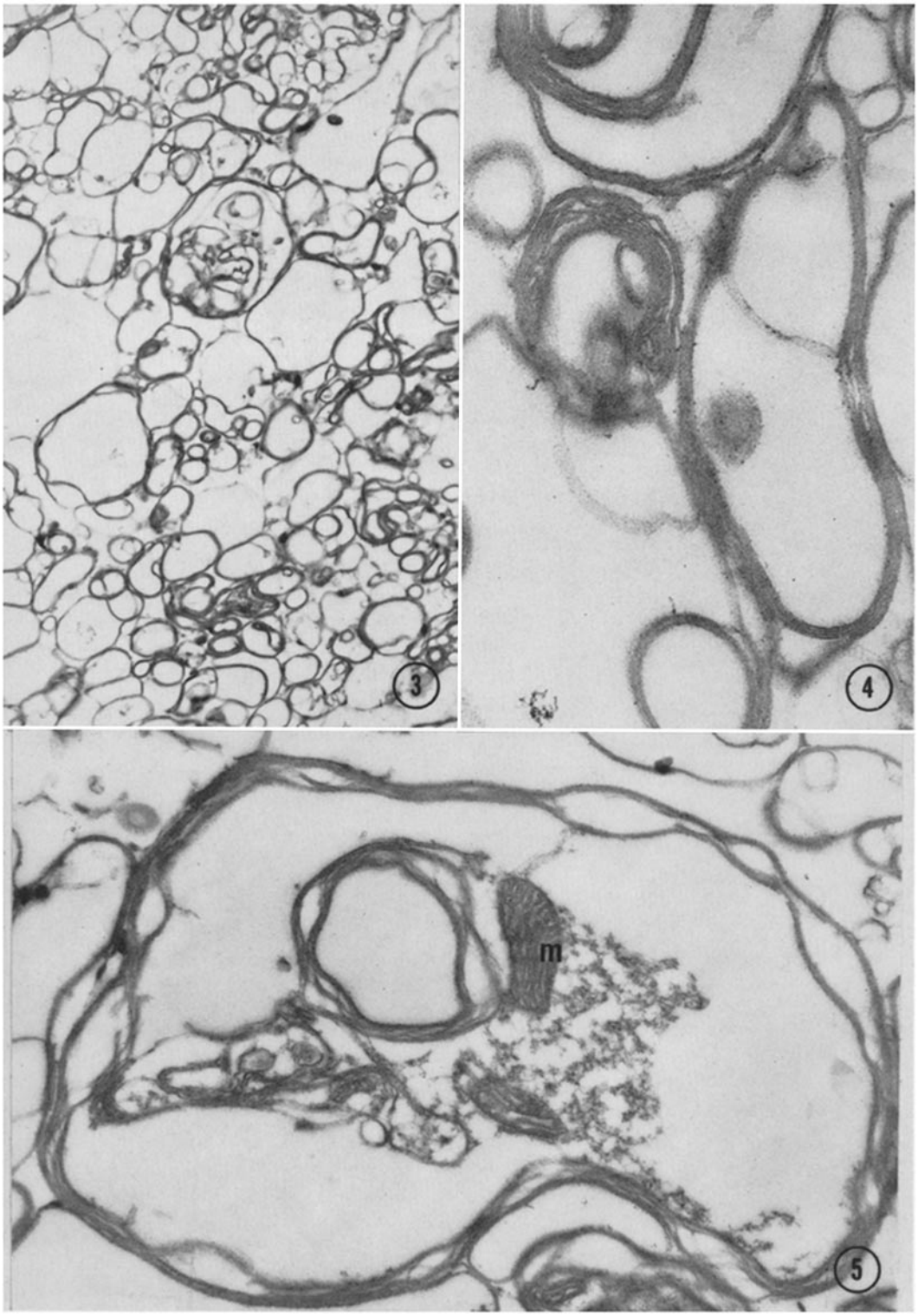
(Laatsch *et al.*: Encephalomyelitic activity)

PLATE 62

FIG. 3. Low power view of osmotically shocked myelin fraction after centrifugation in 30 per cent sucrose. × 10,000.

FIG. 4. Higher magnification of a portion of osmotically shocked myelin fraction showing lamellar structure. × 55,000.

FIG. 5. Osmotically shocked myelin fraction showing infrequently occurring axoplasmic elements with mitochondrion (m). × 34,000.

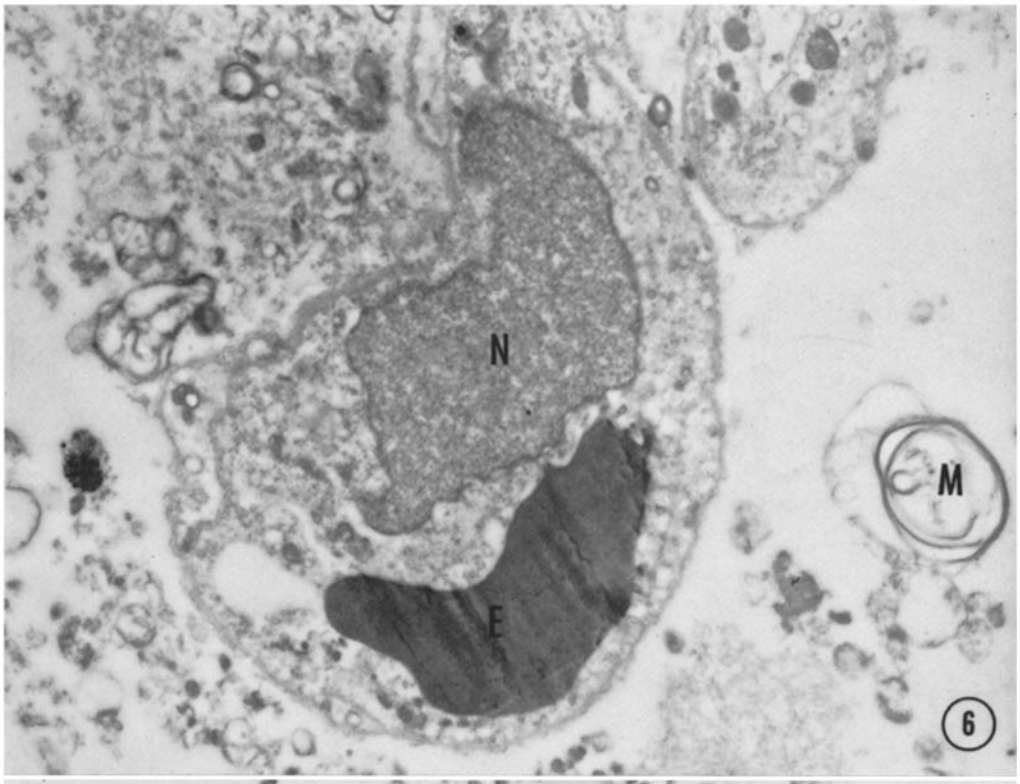


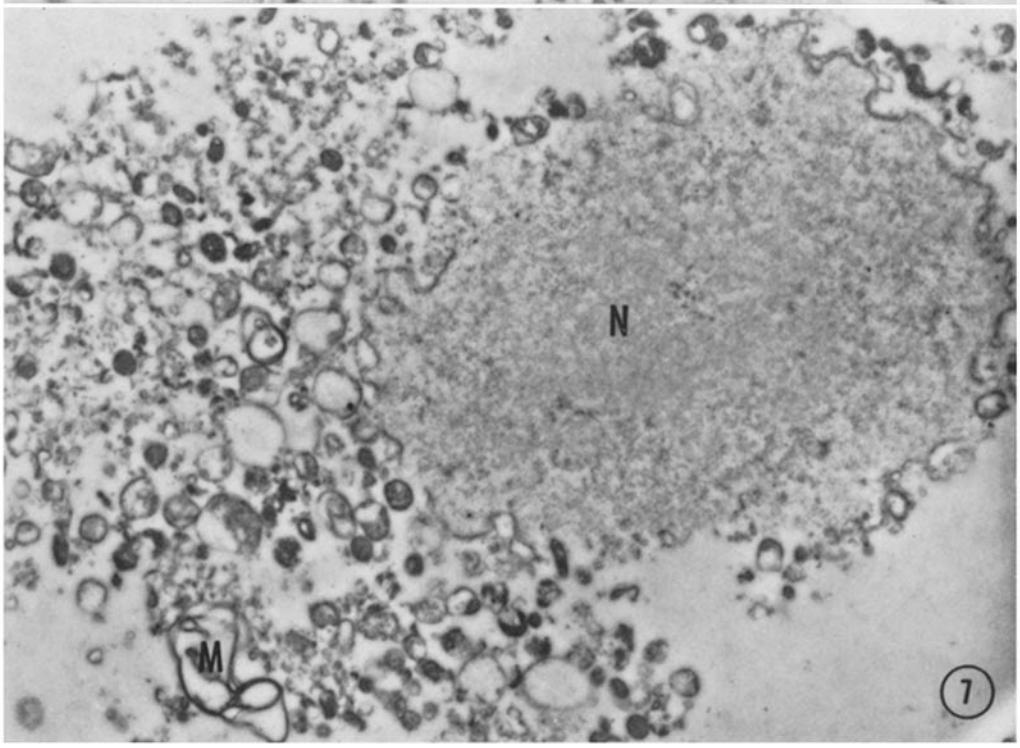
(Laatsch *et al.*: Encephalomyelitic activity)

PLATE 63

FIG. 6. Portion of residue showing capillary with endothelial nucleus (N) and erythrocyte (E) in capillary lumen. Myelin figure (M) at right of figure. × 11,000.

FIG. 7. Section through residue showing numerous particulate elements, myelin (M) and large nucleus (N). × 9,500.





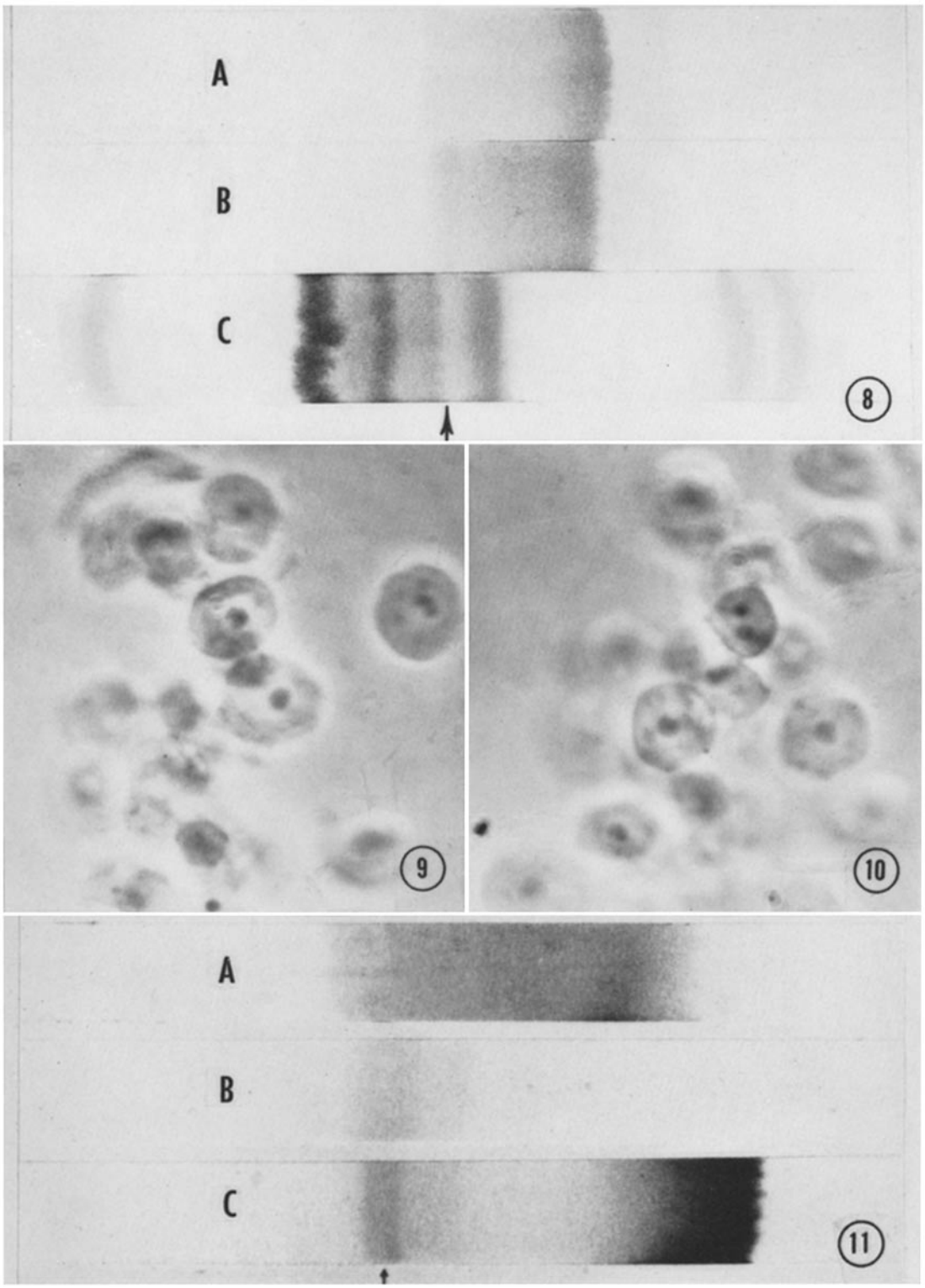
(Laatsch *et al.*: Encephalomyelitic activity)

PLATE 64

FIG. 8. Paper electrophoresis of basic protein from myelin (*A*), similar protein from whole brain (*B*) and normal guinea pig serum (*C*). Arrow indicates origin. Electrophoresis in 0.075 M veronal buffer, pH 8.6, 4 hours, 30 ma, Whatman 3MM paper, ninhydrin stain.

FIGS. 9 and 10. Phase micrographs of intact guinea pig brain nuclei, showing nucleoli. × 1300.

FIG. 11. Paper electrophoresis of acid-soluble proteins from guinea pig brain nuclei (*A*), untreated myelin (*B*) and defatted myelin (*C*). Electrophoresis in 0.1 M acetate buffer, pH 5.1, 3 hours, 30 ma, S & S 2043B paper, bromphenol blue stain.



(Laatsch *et al.*: Encephalomyelitic activity)