27 Nielsen, V.K., The peripheral nerve function in chronic renal failure. VI. Acta med. scand. *194* (1973) 455–462.
29 Rasminsky, M., and Sears, T.A., Internodal conduction in undissected demyelinated nerve fibers. J. Physiol., Lond. *227* (1972) 323–350.
30 Ritchie, J.M., Rang, H.P., and Pellegrino, R., Sodium and potassium channels in demyelinated and remyelinated mammalian nerve. Nature *294* (1981) 257–259.
31 Rosenbluth, J., Intramembranous particle distribution at the node of Ranvier and adjacent axolemma in myelinated axons of the frog brain. J. Neurocytol. *5* (1976) 731–745.
32 Rosenbluth, J., Freeze-fracture approaches to ionophore localization in normal and myelin-deficient nerves; in: Demyelinating Disease: Basic and Clinical Electrophysiology, pp. 391–418. Eds S.G. Waxman and J.M. Ritchie. Raven Press, New York 1981.
33 Saida, K., Saida, T., and Nishitani, H., Rapid alterations of the axon membrane of peripheral nerve fibers: morphological basis for acute conduction block during antibody-mediated demyelination. Fifth Int. Congr. Neuromuscular Diseases, Marseilles, France, September 12–18, 1982.
34 Schoepfle, G.M., and Bloom, F.E., Effects of cyanide and dinitrophenol on membrane properties of single nerve fibers. Am. J. Physiol. *197* (1959) 1131–1135.
35 Sharma, A.K., and Thomas, P.K., Peripheral nerve structure and function in experimental diabetes. J. neurol. Sci. *23* (1974) 1–15.
36 Sherratt, R.M., Bostock, H., and Sears, T.A., Effects of 4-aminopyridine on normal and demyelinated mammalian nerve fibers. Nature *283* (1980) 570–572.
37 Sima, A.A.F., Peripheral neuropathy in the spontaneously diabetic BB-Wistar rat. Acta neuropath. *51* (1980) 223–227.
38 Sima, A.A.F., and Hay, K., Functional aspects and pathogenetic considerations of the neuropathy in the spontaneously diabetic BB-Wistar rat. Neuropath. Appl. Neurobiol. *7* (1981) 341–350.
39 Smith, K.J., Bostock, H., and Hall, S.M., Saltatory conduction precedes remyelination in axons demyelinated with lysophosphatidyl choline. J. neurol. Sci. *54* (1982) 13–31.
40 Tegnér, R., and Brismar, T., Peripheral nerve function in acute and chronic uremia in rats. Acta physiol. scand. *115* (1982) 287–288.
41 Thomas, P.K., and Lascelles, R.G., The pathology of diabetic neuropathy. Q. J. Med. *35* (1966) 489–509.
42 Thomas, P.K., Jefferys, J.G.R., Sharma, A.K., and Bajada, Nerve conduction velocity in experimental diabetes in the rat and rabbit. J. Neurol. Neurosurg. Psychiat. *44* (1981) 233–238.
43 Ward, J.D., Barnes, G.G., Fisher, D.J., Jessop, J.D., and Baker, R.W.R., Improvement of nerve conduction following treatment in newly diagnosed diabetics. Lancet *1* (1971) 428.
44 Waxman, S., Clinicopathological correlations in multiple sclerosis and related diseases; in: Demyelinating Disease: Basic and Clinical Electrophysiology, pp. 169–182. Eds S.G. Waxman and J.M. Ritchie. Raven Press, New York 1981.

0014-4754/83/090946-08$1.50 + 0.20/0

V. Intramembranous particle distribution in nerve fiber membranes

J. Rosenbluth

Departments of Physiology and Rehabilitation Medicine, New York University School of Medicine, New York (N.Y. 10016, USA)

What are intramembranous particles?

When tissue specimens, either fixed or fresh, are frozen into a vitreous state and then mechanically cracked open, the fracture tends to cleave the lipid bilayer of membranes into outer and inner leaflets[16]. The interior of the membranes thus exposed can be replicated with evaporated platinum, and the replicas of the two 'fracture faces', designated 'E' (external) and 'P' (protoplasmic) respectively, can then be studied by electron microscopy (fig. 1).

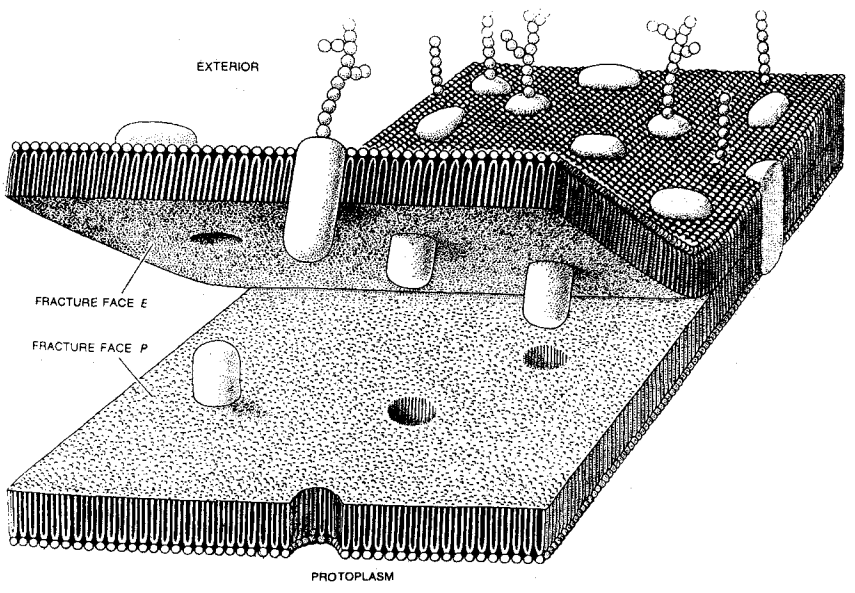

Figure 1. Diagram showing cleavage of a membrane bilayer into inner (protoplasmic) and outer (exterior) leaflets. Integral membrane proteins remain intact attached to one or the other leaflet and appear as 'particles' projecting from that fracture face with 'pits' in the complementary face. (From: 'The first steps in secretion' by B. Satir. Copyright 1975 by Scientific American, Inc. All rights reserved.)

Nearly all such membrane fracture faces display projections or 'intramembranous particles'. The latter are generally regarded as sites of integral membrane proteins that traverse the lipid bilayer entirely or are deeply embedded within it. Presumably these elements are not fractured with the bilayer, but are 'pulled out' of one leaflet of the membrane, leaving a pit, and remain attached to the other leaflet. Variable amounts of membrane lipid may remain associated with the protein, and it has also been proposed that lipids alone, e.g., when in a hexagonal phase, may appear as particles. Nevertheless, studies of protein-free and protein-containing artificial membranes show a clear correlation between protein content and particle presence[8], and it is likely that the great majority of intramembranous particles seen in freeze fracture replicas do represent sites of integral membrane proteins.

In a few instances, it has been possible to detect subunit structure in such particles, e.g., the putative acetylcholine receptor[5]. The more recently developed rotary shadowing technique may produce more accurate pictures of particle size and shape and may also bring out subunit structure not apparent in unidirectionally shadowed specimens[13]. In general, however, particles are not sufficiently distinctive to be identified specifically on the basis of morphological criteria alone. Moreover, a single species of protein within a membrane may vary considerably in size and shape because of variations in the level of the fracture within membranes, in the extent of mechanical deformation occurring during the fracturing process, and in the amount of associated lipid. Condensation of water or other contaminants onto particles before replication may also increase their apparent size and obscure subtle details of their shape. In addition, extramembranous structures may remain attached to integral membrane proteins and be 'pulled through' a membrane leaflet with the latter, again resulting in apparent variation among particles from a homogeneous group.

Measurements of particle concentration are also subject to error. For example, some intramembranous particles may be pulled out of *both* halves of the membrane during the fracturing process and thus lost. Or some particles may themselves fracture at the level of the bilayer, in which case neither half would extend far enough above the level of the background to be seen. The distribution of particles between the 2 leaflets of the fractured membrane also varies. In the case of some gap junctions, virtually all particles remain with the P face, leaving pits in the E face[15], but in the case of the diagonal strings of particles in the glial membrane at axoglial junctions, about $^1/_3$ of the particles remain with the E face and $^2/_3$ with the P face[25]. In addition, there is evidence that irregularities in membrane surfaces, e.g. pits, may act as sites for condensation of water vapor, leading to artifactually-created ice crystal particles ('decoration')[7]. Ice crystals are usually distinguishable, however, since they tend to be larger and more angular than intramembranous particles and also tend to sublime away when specimens are 'etched' before replication.

Despite these possible sources of error, measurements of particle concentration have proven reasonably consistent from specimen to specimen, and among different laboratories as well, and have produced significant structural information about a number of specialized membranes including the axolemma. Methods for specific labeling of intramembranous particles are currently being developed and will undoubtedly further increase the usefulness of the freeze fracture method in analyzing cell membranes when they become available.

Morphology of myelinated axons in freeze fracture replicas

Myelinated nerve fibers are customarily divided into nodal and internodal regions. Based on recent morphological studies of thin sections and freeze fracture replicas, it is now more useful to distinguish 4 regions: the node, a paranodal region immediately adjacent to the node, representing the site at which the myelin lamellae terminate and form junctions with the axolemma, the juxtaparanodal portion of the internode adjacent to the paranodal axolemma, and finally the remainder of the internode. The last 2 regions are not separated from each other by a clear boundary. However, the node is sharply demarcated from the paranode, and the paranode is sharply demarcated from the juxtaparanodal region.

In sectioned nerve fibers, the plasma membrane at the node of Ranvier tends to be convex outwards, and it is characterized further by the presence of a dense coating on its cytoplasmic surface, which has been shown to have an affinity for iron[27]. The axolemma of the paranodal region, in contrast, displays a characteristic scalloping resulting from the fact that the terminal loop of each lamella of myelin indents the axon forming a junction characterized by a narrow ($\sim$ 20–40 Å) intercellular gap containing regularly spaced, diagonally oriented dense ridges referred to as 'transverse bands'[21]. Thin sections of the juxtaparanodal and internodal axolemma show no distinguishing features. In freeze fracture replicas, where extensive surface views of the axolemma can be seen, further distinctions can be made.

Paranodal axolemma. Historically, the paranode was the first region to be examined in detail. It was found to have a paracrystalline structure (fig. 2) which was likened to that seen in a 'stretch weave fabric'[12]. A diagonal pattern corresponding to that of the transverse bands is visible in that portion of the paranodal axolemma that forms junctions with the terminal

myelin loops but is not present in the non-junctional region between loops where transverse bands are absent. Careful study of replicas whose orientation was followed throughout processing has shown that the orientation of the diagonal pattern is always the same[25].

In the E fracture face, representing the outer leaflet of this membrane, the pattern is especially conspicuous in preparations that have been 'etched' somewhat. Presumably during the etching process, some of the ice occupying the space between this membrane and the terminal loops sublimes away, resulting in collapse of the axolemma against the terminal loop membrane. Since the transverse bands, which are also situated within this space, presumably remain during the etching process, their outline becomes increasingly conspicuous as the surrounding ice is removed. Thus, the prominent pattern in the axolemma may represent an embossed pattern rather than an intrinsic axolemmal structure. Aside from this paracrystalline pattern, the E face of the junctional axolemma displays few intramembranous particles. Large particles do, however, occur in the thin non-junctional strip of axolemma that winds helically through the paranode between successive strips of the paranodal junction (fig. 3).

Examination of developing nerve fibers has demonstrated that the paranodal indentations can appear early, particularly in frog nerves, but with no sign of a diagonal pattern. This probably reflects the fact that the transverse bands are not fully formed at early stages of development and therefore do not emboss the axolemma until later stages. Both the indentations and the transverse bands appear first in the junctions formed by the outermost layers of the myelin sheath, i.e., those closest to the node of Ranvier, and even before the formation of transverse bands these indented regions of the axolemma are already virtually free of particles in comparison with the adjacent unindented membrane[26]. E face intramembranous particles thus appear to be excluded from the indented axolemma corresponding to sites of axoglial junctions. Prior

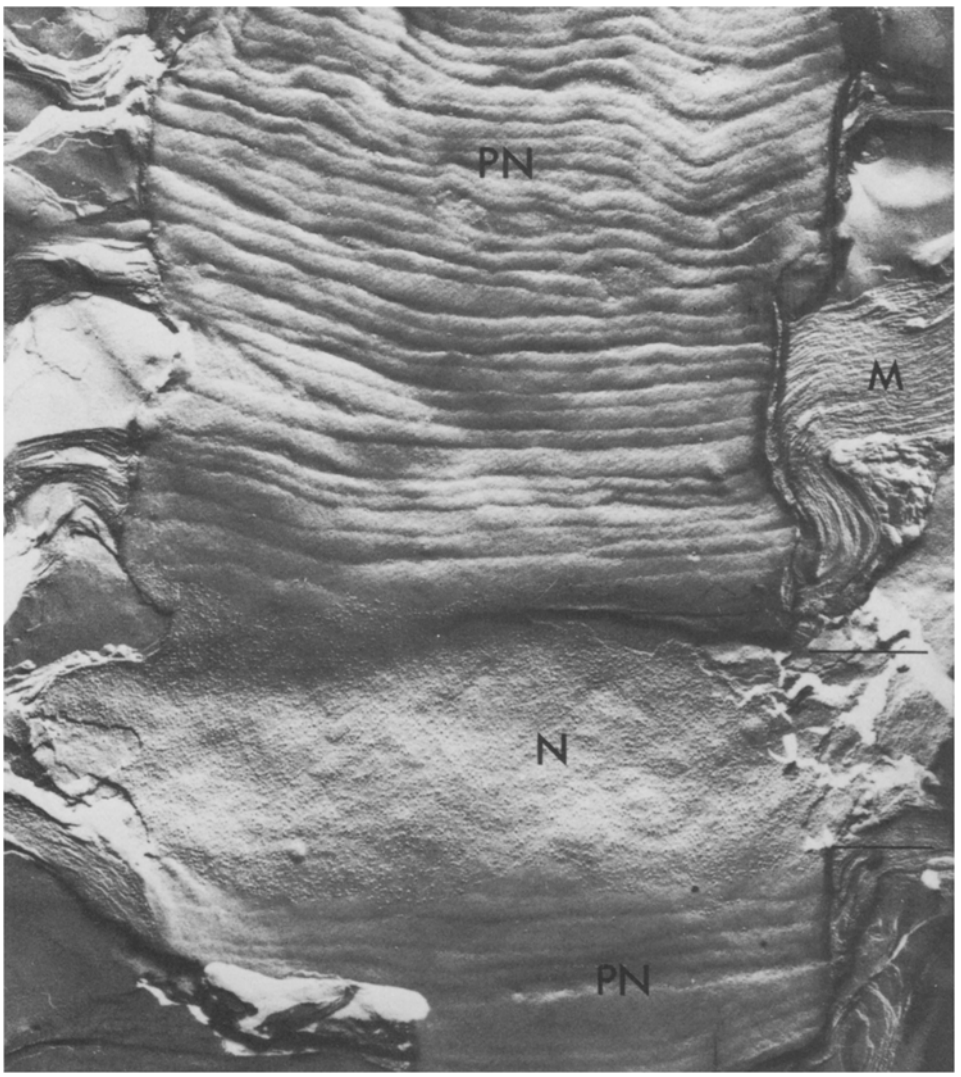

Figure 2. Axolemma showing portions of two paranodal regions (PN) separated by a node of Ranvier (N). Investing myelin lamellae (M) are visible alongside. The paranodal axolemma is marked by a series of strip-like indentations, each approximately 0.1–0.2 µm wide, which are free of particles but which display a faint diagonal pattern. Thirty-two such strips are visible in the upper paranode indicating that this internode is ensheathed by at least that number of myelin lamellae. The nodal axolemma is distinguished by large numbers of intramembranous particles distributed randomly within the 1 µm wide node (brackets). From frog sciatic nerve.

to clear indentation of the axolemma by myelin loops, however, particle distribution in the axolemma remains rather uniform with little sign of accumulation at nodal or juxtaparanodal regions (fig. 4a).

The P fracture face of the paranodal axolemma, representing the inner leaflet of the plasma membrane, is less distinctive in appearance and has given rise to more controversy because of variations from one preparation to the next[21]. In most cases, it displays a relatively high concentration of intramembranous particles of the type found in the internode. Sometimes the particle concentration is visibly lower than that elsewhere along the axolemma, but in no case does this membrane appear virtually particle-free

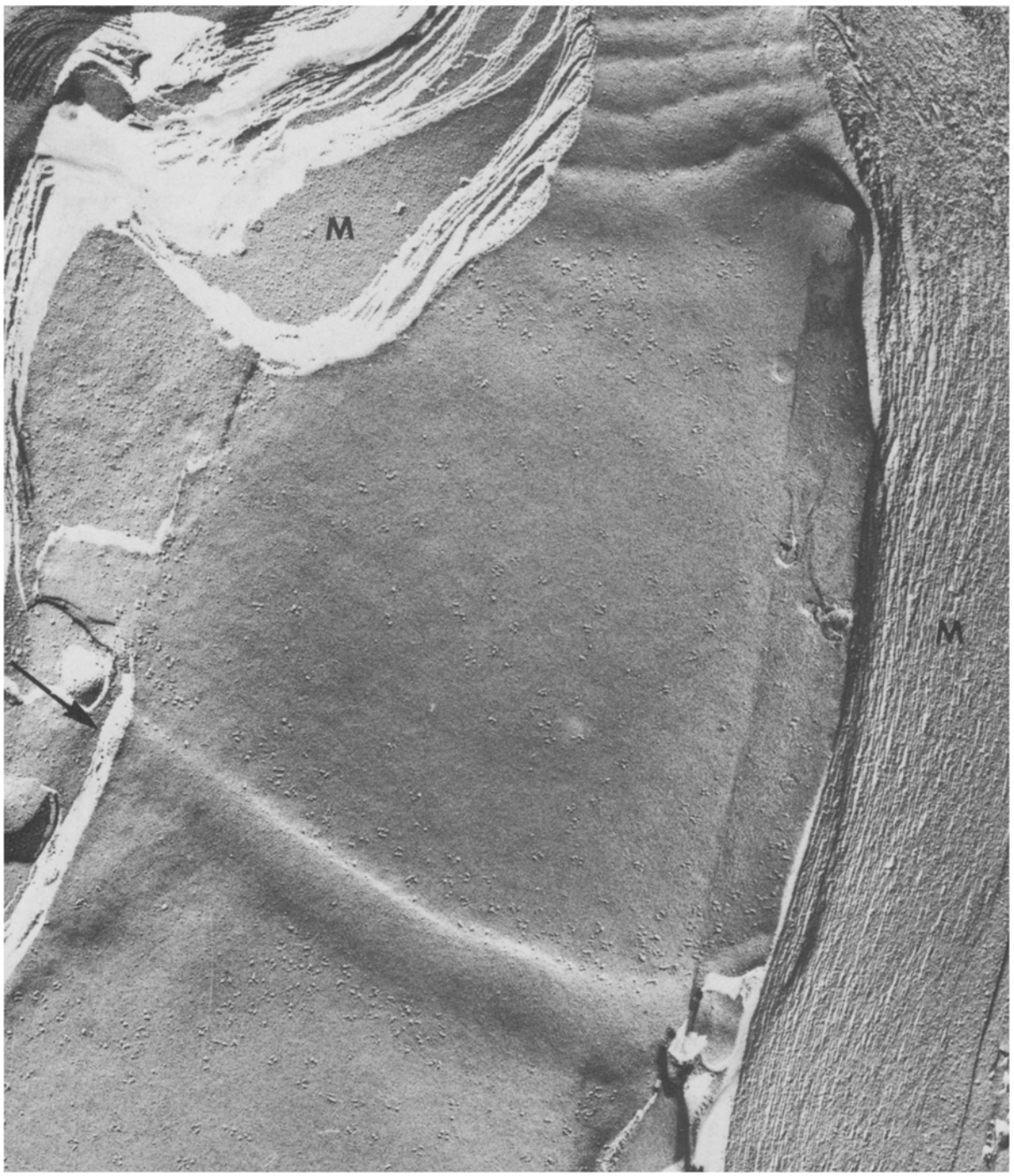

Figure 3. Juxtaparanodal axolemma (E face) from the peripheral nervous system of a twitcher mouse. Strip-like indentations identify the paranodal region of this fiber (top), and myelin lamellae (M) are visible on either side. The paranodal axolemma contains a few particles especially in the 'grooves' between strips. The juxtaparanodal region below the paranode contains moderate numbers of particles whose concentration diminishes in the direction away from the paranode. A single aberrant indentation near the bottom of the figure (arrow) is virtually particle-free, but moderate numbers of particles are visible in the internodal axolemma on either side of it.

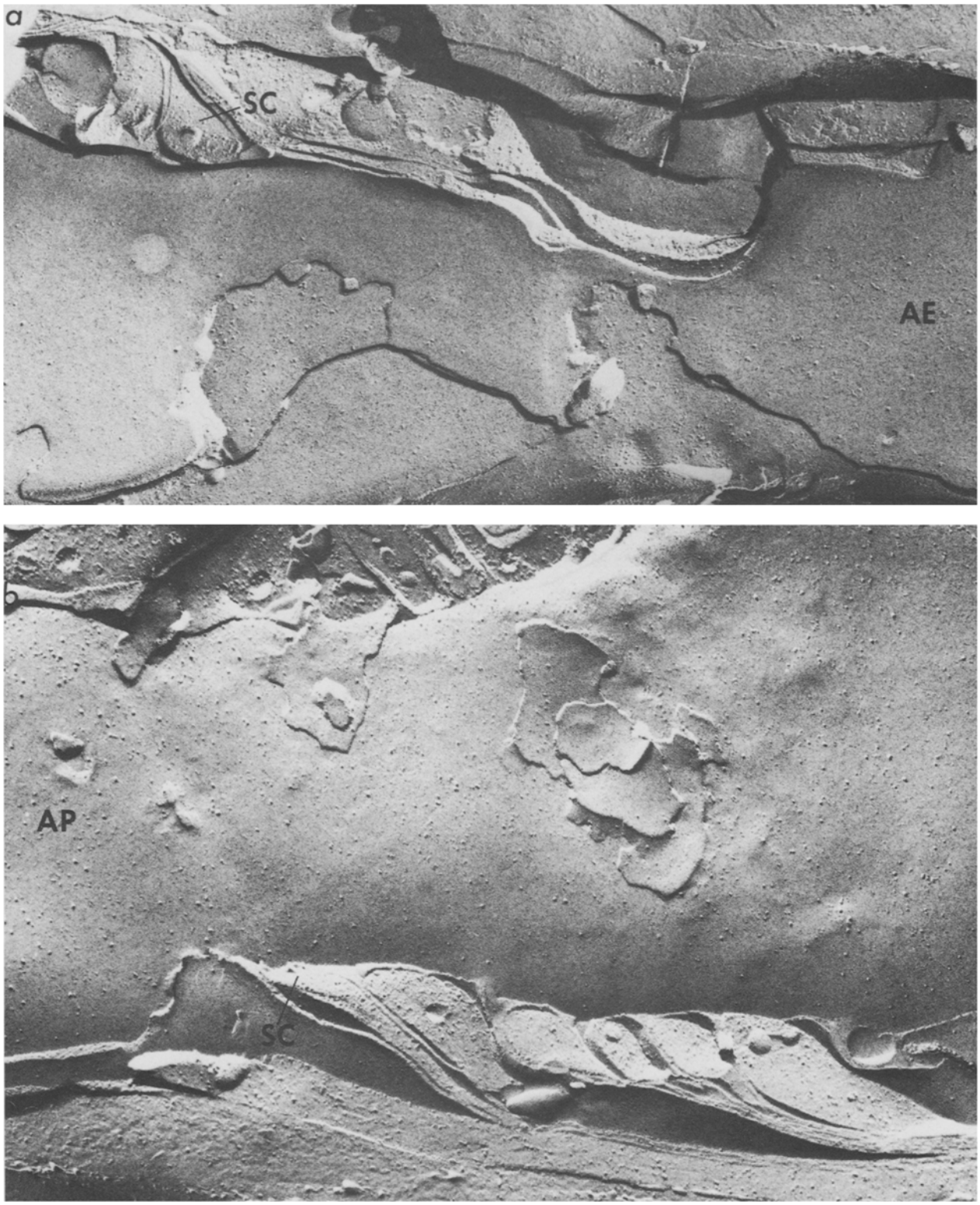

Figure 4. Developing fibers from rat peripheral nerve. *a* Several terminal Schwann cell loops (SC) are visible along the upper left edge of an axolemmal E face (AE), but do not yet indent the axolemma significantly. Sparse axolemmal particles are present in approximately the same concentration all along the axon. The striking annular particle aggregates characteristic of the adult nodal E face and particle-free paranodal indentations are both absent. *b* Approximately 6 Schwann cell loops (SC) are visible along the edge of an axonal P face (AP). Here too the terminal loops do not yet indent the axon significantly, and particles are distributed relatively uniformly along the axolemma. P face particle density is clearly lower in this immature axolemma than in the adult. (From Tao-Cheng and Rosenbluth, Devl. Brain Res. 1983, in press.)

as does the E face of the same membrane. Occasionally a diagonal pattern can be detected corresponding to that seen in the E fracture face. Presumably this too represents an embossed pattern. At early stages in myelinogenesis, before significant indentation of the axon by paranodal loops, particle density is relatively low throughout the axolemmal P face and is not visibly different in paranodal, nodal, or internodal regions (fig. 4b).

At present no clear evidence exists for any unusual intrinsic structure in the paranodal axolemma, and the simplest explanation for its distinctive appearance is that the transverse bands, which lie just outside this membrane, are closely enough applied to it to create a fine pattern of indentations most apparent in the E face and sometimes also visible in the P face.

Usually in the paranodal region the edge of the myelin sheath forms a very regular series of strips as it winds around the axon. However, particularly in regions further away from the node, the winding pattern may be irregular, and conspicuous spaces may appear between the strips of junctional axolemma. These non-junctional regions tend to contain large concentrations of large particles which appear in the E fracture face and are quite comparable to those in the nodal axolemma with respect to size and packing (see below). The existence of such 'lakes' of particles, not only in normal paranodes but also in abnormally myelinated axons (fig. 5a), has led to the suggestion that particles of this type may be mobile within the plane of the plasma membrane and become 'trapped' in regions where their movement is impeded. It has been postulated that the junctional axolemma, as a result of its close association with adjacent Schwann cell or glial processes, is 'structured' in such a way that large particles are not able to move freely through it and as a result 'pile up' at its margins or become caught in its interstices[19].

Nodal axolemma. The distinctive appearance of the paranodal junctional region in freeze fracture replicas so captured the attention of the investigators who originally studied the axolemma by this method that little attention was paid to the neighboring nodal and internodal regions of the membrane, and it was not until 1976 that the consistent presence of a relatively high concentration of particles was found in the E face of the nodal axolemma (fig. 2) and the possibility recognized that these might represent sodium channels[19]. The E face of most vertebrate plasma membranes has relatively few particles within it, and particle accumulations in certain specialized locations such as post synaptic membranes and the nodal axolemma are therefore very conspicuous. Nodal E face particles are distinctive not only because of their concentration but also because of their relatively large size. Recent attempts to demonstrate a sub-structure within nodal particles have not as yet succeeded, however, even in rotary shadowed specimens. Scrutiny of the latter has so far shown only that the particles at the node are not quite as large or irregular in shape as had been thought based on studies of unidirectionally shadowed membranes.

The concentration of nodal E face particles ranges from approximately 1000-1500/μm^2. The figure of 1200/μm^2 was originally derived from studies of axons in the amphibian central nervous system, but comparable figures have now been obtained from studies of the mammalian central nervous system[10] and of peripheral nerves as well[25]. An intriguing exception to this generalization occurs in the electric organ of the knifefish *Sternarchus,* which contains 2 types of nodes, one excitable and one inexcitable. Both have high concentrations of particles in the axolemmal P face, but only the nodes believed to be excitable have high concentrations of particles in the axolemmal E face[9]. Thus, excitability can be correlated with the presence of E face, but not P face, particle aggregates. More recently we have attempted to identify structural differences between active and quiescent excitable membranes by comparing frog sciatic nerves that were either anesthetized or stimulated repetitively prior to and during fixation. However, no apparent differences were found either in axolemmal particle concentration or in the size or shape of the individual particles.

Although nodal E face particle *concentration* seems to vary little in different locations and in fibers of different size, nodal *area* may vary considerably. Thus, the total number of particles at 2 different nodes may be very different even though the particle concentrations are comparable. It will therefore be important to obtain accurate morphometric assessments of nodal area from axons of different sizes, in different locations, and in different vertebrate classes in order to permit calculation of the total *number* of particles per node from freeze fracture data or the *concentration* of sodium channels at nodes from current fluctuation or gating current studies in order to correlate data obtained by these different approaches properly.

The P fracture face of the nodal axolemma is also distinctive. Although the concentration of particles here is not clearly higher than that in the internode, their mean size is larger[25]. The proportion of large particles in the nodal P face is, however, not as high as that in the E face. Pits can be found in both fracture faces of the nodal axolemma, presumably corresponding to the large particles in the complementary faces. One possible interpretation of these findings is that the nodal membrane contains a distinctive population of large particles which, during the fracturing process, becomes partitioned between the 2 fracture faces, with the majority remaining attached to the outer leaflet of the membrane and thus appearing in the E fracture face.

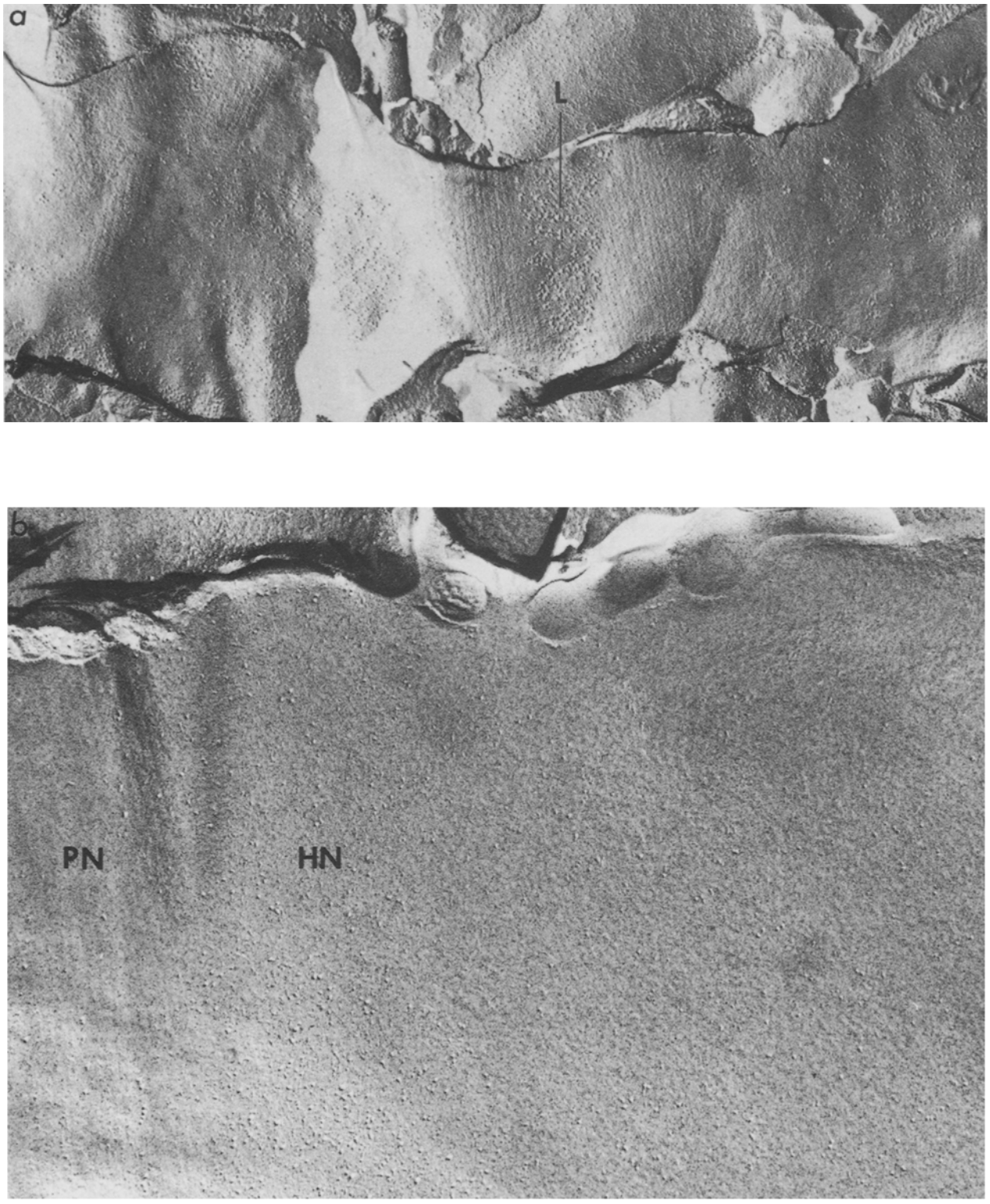

Figure 5. *a* Axolemma (E face) from the central nervous system of a shiverer mouse. Paranodal junctional regions characterized by indentation and a paracrystalline pattern are irregularly distributed along the length of the axon. These regions are relatively free of particles but the unspecialized regions around them do contain particles, and 1 'lake' (L) between 2 adjacent junctional regions contains large numbers of particles. *b* Heminode from a dystrophic mouse root. The paranodal region (PN) of the axolemma (E face) at the left exhibits a paracrystalline diagonal pattern with some particles visible in the grooves between the strip-like indentations. Immediately to the right of the paranode is a heminode (HN) which contains scattered particles at relatively low concentration in one region (above) and a more concentrated patch of particles in another region (below). The particle concentration diminishes gradually in the direction of the amyelinated region of the fiber to the right.

Alternatively, the large P face particles could represent a separate population having a completely different significance. For example, some of the intramembranous particles in the nodal axolemma could represent structural components involved in attachment of external perinodal elements or internal cytoskeletal elements to the axolemma or to each other. Such structural components could play an important role in maintaining the shape of the nodal axon and its relation to the perinodal structures and in preventing herniation of the axon into the perinodal space. As discussed above, E face particles of the type found at nodes also appear in 'lakes' in the paranodal axolemma where no attachments to equivalent external structures are possible, and it is therefore unlikely that the E face particles have such a structural role.

Juxtaparanodal and internodal axolemma. The juxtaparanodal region, which is adjacent to the paranode, also often displays accumulations of large particles comparable to those found at the node (fig. 3). These particles do not form a distinct circumferential band, as they do at the node, however, but rather have a sharp boundary against the paranodal axolemma and a gradually diminishing concentration in the direction of the internode. Based on their similarity to nodal particles, it has been postulated that these, along with the particles in paranodal 'lakes', represent particles of the same population en route to or from the nodal region. Other interpretations of the significance of juxtaparanodal particles have also been offered[24], but an essential role for juxtaparanodal particle accumulations in normal axonal function is unlikely inasmuch as they occur very commonly in central axons but not in peripheral fibers.

The remainder of the internodal axolemma has not been reported to have distinctive structural features in freeze fracture replicas. The P face has particles in a relatively high concentration and is not readily distinguishable from other plasma membranes. The E face has relatively few particles, numbering approximately $100/\mu m^2$, which are distributed randomly within the axolemma. Occasionally the innermost loop of the myelin sheath forms an indentation in the axolemma extending obliquely into the internode, but as a rule the internode does not display junctional specializations of the type found in the paranodal region.

Particle distribution in fibers lacking myelin

Mature axons that normally lack myelin sheaths have been examined in freeze fracture replicas of both central and peripheral nerve fibers. In addition, axons have been studied prior to the formation of myelin, as well as after demyelination. Animals with congenital myelin deficiencies in either the central or peripheral nervous system offer yet another opportunity for examination of axons without myelin, and these too have been studied in mutant mice.

Normal unmyelinated axons, both centrally and peripherally, exhibit particles in both P and E fracture faces with the great majority in the former. The particles of both fracture faces are randomly distributed in concentrations of approximately $100–200/\mu m^2$. The periodically-situated annular particle aggregates that characterize the nodal E face are missing.

At some 'transition zones' unmyelinated fibers may display a non-random distribution of particles[2]. The clearest case is at 'heminodes' that mark the transition between myelinated and unmyelinated regions of normal axons and also occur commonly in myelin-deficient mutants as a result of segmental demyelination or incomplete myelination. At these sites the particle concentration in the E face is usually markedly lower than that at nodes but often higher than that in internodes. The particles are sharply bounded on the myelinated side of the heminode with a gradually decreasing concentration over a considerable distance on the side lacking myelin (fig. 5b). In some instances no particle accumulation is visible at the heminode[21], and occasionally a circumscribed patch of particles occurs. Although the particle concentration may occasionally approach that at nodes, no examples of typical annular aggregates of particles in high concentration, sharply defined on both sides, have been found.

Examination of premyelinated peripheral axons during myelinogenesis has shown that ill-defined particle aggregates begin to appear in the axolemmal E face early in development, before compact myelin forms, but only after the axons are already individually ensheathed by Schwann cells[26]. Often these aggregates are seen just at the edge of the surrounding Schwann cell. Initially the particle aggregates have a diffuse form, of considerable longitudinal extent, with a low particle concentration and indistinct margins. Only gradually do they become more discrete and compact concurrent with the maturation of the myelin sheath.

Studies of demyelination have been carried out by electrophysiological as well as structural methods on a variety of experimental models. Exposure of myelinated fibers to antigalactocerebroside serum leads to disruption of the normal axon-Schwann cell relationship resulting in disintegration of paranodal axolemmal membrane specialization within hours, and loss of nodal particle aggregates by 6 days[21]. Thus, the axon-Schwann cell relationship appears to be necessary not only for the differentiation of the axon, but for maintenance of the differentiated state. In the case of demyelination produced by diphtheria toxin, axonal dedifferentiation is manifested by the demonstration of continuous conduction at 6 days[3]. In another model, produced by lysolecithin (LPC), in

contrast, discontinuous conduction can be found 6 days after demyelination even though compact myelin has not yet reformed at this stage[23]. Demyelinated axons in the LPC model are quickly re-ensheated by cytoplasm-containing Schwann cell lamellae, however, and the early discontinuous conduction seen in this model may simply reflect an early stage in remyelination. The apparent difference between these latter 2 models of demyelination at 6 days may therefore indicate only that recovery proceeds at a faster rate in the LPC model than in diphtheria demyelinated fibers. Thus, demyelination appears to result in axonal dedifferentiation, and the course of structural and functional changes that ensue seems to depend upon the degree and type of injury to the myelinating cells and on their ability to recover and

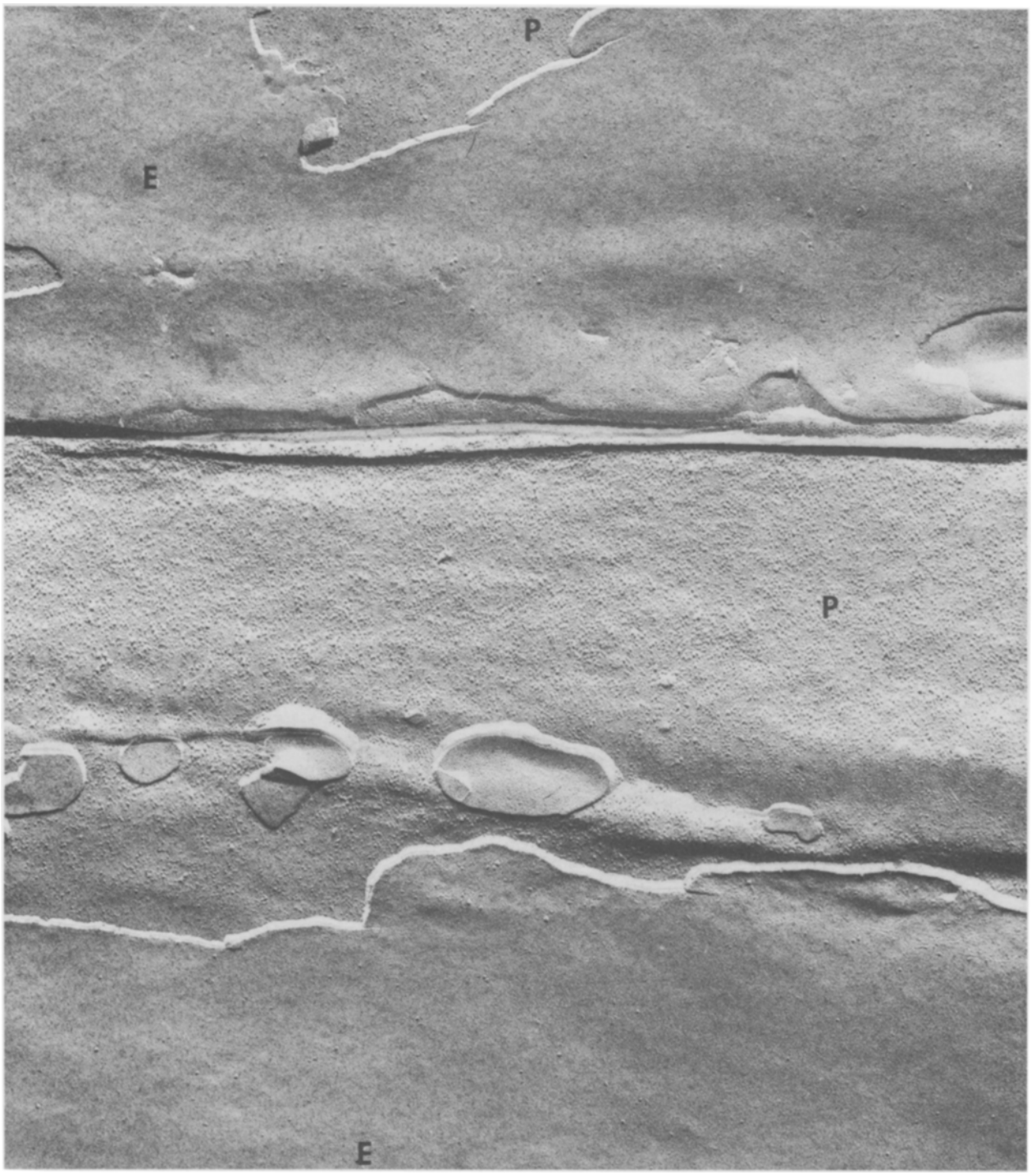

Figure 6. Amyelinated axons from dystrophic mouse roots. Portions of 4 different fibers are visible, 2 displaying P fracture faces (P), which contain large numbers of randomly distributed particles, and 2 displaying E faces, in which particles are very sparse.

re-ensheath the axons. Remyelination, once it begins, presumably recapitulates the events that occur during initial myelination.

Congenital lack of myelin has been studied in several mouse mutants[21] including dystrophic which displays 'amyelinated' segments of its spinal roots (fig. 6). Here too, electrical studies show continuous rather than saltatory conduction[17]. Axolemmal particles in these regions are randomly distributed, but rare circular E face particle patches have been seen[4] which could be the remnants of disintegrating nodes of Ranvier, in view of the presence of structural evidence of demyelination in these mutants. Alternatively, they could represent aberrant nodal specializations corresponding to sites of contact between the amyelinated axons and Schwann cells along the periphery of the amyelinated bundles. Although Schwann cells normally form myelin around a single axon and are isolated from neighbors by the endoneurium, a basement membrane defect in the dystrophic mutant permits unusually close contact between Schwann cells and adjacent axons, and at such sites aberrant, noncircumferential paranodal and nodal specializations can be seen in occasional thin sections[20]. Similarly in the central nervous system of another mouse mutant, shiverer, oligodendrocyte processes, or groups of processes, sometimes cross groups of axons transversely forming focal junctions, or groups of such junctions, with each. In these instances too the junctions are not circumferential. The distribution of E face particle aggregates is highly abnormal in this mutant corresponding to the gross irregularities in axonal ensheathment (fig. 5a).

Thus, in general, axons lacking myelin display randomly distributed intramembranous particles at low concentration in the E fracture face without evidence of particle aggregates of the type present at nodes of Ranvier. This is true of adult unmyelinated fibers, immature premyelinated fibers, congenitally myelin-deficient fibers, and demyelinated fibers. Occasional exceptions to this generalization may occur at sites of fortuitous contact with Schwann or glial cells, especially in cases of myelin abnormality, at 'transition zones' between myelinated and nonmyelinated regions, and during myelinogenesis. Presently available evidence indicates that well-defined, annular aggregates of nodal E face particles in high concentration are seen only where paranodal junctions girdle the immediately adjacent axolemma on both sides.

Identity of intramembranous particles in the axolemma

When unusual aggregates of E face particles were observed in the nodal axolemma, but not in internodes or unmyelinated axons, it was immediately suggested that these play some important functional role related to nodal physiology. Both pharmacological and physiological studies carried out at about the same time indicated that the nodal axolemma is unusual in having high concentrations of voltage-sensitive sodium channels, and thus the possibility that the E face particles might correspond to the sodium channels was explored. At first it appeared impossible that there could be a one-to-one relation between particles and channels because of saxitoxin-binding studies from which it was concluded that the concentration of sodium channels in the nodal axolemma is approximately 12,000/μm^2, or approximately 10 times the particle concentration in the axolemmal E face[18]. However, the assumption was made in that study that saxitoxin is capable of binding only to the exposed axolemma at the node of Ranvier. Studies of the paranodal junction using tracer methods have shown that molecules much larger than saxitoxin are able to penetrate this junction and gain access to the periaxonal space of the internode. Thus, over the time course of exposure to saxitoxin it is entirely possible that this material was able to label sodium channels not only at the nodes but also at the paranodal and internodal regions.

Other studies suggest that internodal sodium channel concentration is exceedingly low. However, the length of the internode is so great that even if the internodal sodium channel concentration were only one per cent of that at the node there would still be 10 times as many channels in the internode as at the node in a fiber with a nodal gap of 1 μm and an internodal length of 1000 μm. Penetration of saxitoxin into the internodal region would thus grossly distort the estimate of sodium channel concentration. Given this consideration, and the concurrence of the more recent estimates of nodal sodium channel concentration based on current fluctuation analyses and gating current measurements[6,14,22], it now seems likely there is very good correspondence between sodium channel and E face particle concentrations, and the original proposal that the latter represent the sodium channels remains realistic. Since the evidence for this correlation is still circumstantial, specific labeling methods will be required in order to establish this point.

In addition to comparing channel density with particle density, it is also possible to study the apparent molecular dimensions of sodium channels by entirely different methods. Estimates based on irradiation inactivation[11] indicate that sodium channels have a molecular weight of approximately 230,000, and indeed this figure corresponds well to a recent estimate derived from biochemical studies[1]. Although the size of intramembranous particles is not necessarily a reliable measure of the size of the underlying proteins, the dimensions and molecular weight of sodium channels, as determined by biochemical and biophysical methods, are at least compatible with the size of the particles seen in freeze fracture replicas.

Recent studies have demonstrated that it is not only sodium channels that have an uneven distribution in the axolemma of myelinated fibers, but also voltage-sensitive potassium channels, which appear to have a distribution converse to that of sodium channels in mammalian fibers. Attempts to identify a structural component corresponding to potassium channels have not been successful thus far, however. As discussed above, the distribution of E face axolemmal particles does not match what would be expected for potassium channels, and P face particles have roughly the same concentration in nodal and internodal regions. A subpopulation of P face particles could correspond to the potassium channels but may not be distinguishable with present techniques.

Other examples of channels that may have visible structural counterparts elsewhere are calcium channels, which may correspond to the rows of large P face particles in presynaptic nerve terminals, and acetylcholine receptors, which are thought to be represented by P face particles in the motor end plate membrane. E face particle accumulations occur in certain postsynaptic membranes, but these particles have not been identified as yet.

In conclusion, the distinctive particles that appear in freeze fracture replicas of the nodal axolemma are realistic candidates for the voltage-sensitive sodium channels known to be concentrated there. These particles can be localized with considerable precision, and changes in their distribution and concentration under experimental or abnormal conditions can be followed. Such particles can be detected not only at nodes, but also in 'concealed' positions beneath the myelin sheath. Studies of axolemmal particle distribution under experimental conditions may be useful in clarifying axonal pathophysiology and may bear also on broader questions concerning the relationship between cell-to-cell interaction and cell membrane differentiation.

Acknowledgment. This work was supported by grant NS-07495 from the NIH.

1 Barchi, R.L., Weigele, J.B., and Cohen, S.A., Isolation of sodium channels from excitable tissues. Adv. Neurol. *31*, (1981) 377–390.
2 Black, J.A., Waxman, S.G., and Foster, R.E., Spatial heterogeneity of the axolemma of nonmyelinated fibers in the optic disc of the adult rat. Cell Tissue Res. *224* (1982) 239–246.
3 Bostock, H., and Sears, T.A., The internodal axon membrane: electrical excitability and continuous conduction in segmental demyelination. J. Physiol. *280* (1978) 273–301.
4 Bray, G.M., Cullen, M.J., Aguayo, A.J., and Rasminsky, M., Node-like areas of intramembranous particles in the unsheathed axons of dystrophic mice. Neurosci. Lett *13* (1980) 203–208.
5 Cartaud, J., Benedetti, L., Cullen, J.B., Meunier, J.C., and Changeux, J.P., Presence of a lattice structure in membrane fragments rich in nicotinic receptor protein from the electric organ of *Torpedo marmorata*. FEBS Lett. *33* (1973) 109–113.
6 Chiu, S.Y., Asymmetry currents in the mammalian myelinated nerve. J. Physiol. *309* (1980) 499-519.
7 Gross, H., Kuebler, O., Bas, E., and Moor, H., Decoration of specific sites on freeze-fractured membranes. J. Cell Biol. *79* (1978) 646–656.
8 Hong, K., and Hubbel, W.K., Preparation and properties of phospholipid bilayers containing rhodopsin. Proc. natl Acad. Sci. USA *69* (1972) 2617–2621.
9 Kristol, C., Akert, K., Sandri, B., Wyss, U.R., Bennett, M.V.L., and Moor, H., The Ranvier nodes in the neurogenic electric organ of the knifefish *Sternarchus:* a freeze etching study on the distribution of associated particles. Brain Res. *125* (1977) 197–212.
10 Kristol, C., Sandri, B., and Akert, K., Intramembranous particles at nodes of Ranvier of the cat spinal cord: a morphometric study. Brain Res. *142* (1978) 391–400.
11 Levinson, S.R., and Ellory, J.C. Molecular size of the tetrodotoxin-binding site estimated by irradiation inactivation. Nature New Biol. *245* (1973) 122–123.
12 Livingston, R.B., Pfenninger, K., Moor, H., and Akert, K., Specialized paranodal and interparanodal glial axonal junctions in the peripheral and central nervous system: a freeze etching study. Brain Res. *58* (1973) 1–24.
13 Margaritis, L., Elgasaeter, A., and Branton, D., Rotary replication for freeze etch. J. Cell Biol. *72* (1977) 47–56.
14 Neumcke, B., and Stämpfli, R., Sodium currents and sodium current fluctuations in rat myelinated nerve fibers. J. Physiol. *329* (1982) 163–184.
15 Peracchia, C., Gap junctions: structural changes after uncoupling procedures. J. Cell Biol. *72* (1977) 629–641.
16 Pinto da Silva, P., and Branton, D., Membrane splitting in freeze etching. Covalently bound ferritin as a membrane marker. J. Cell Biol. *45*(1970) 598–605.
17 Rasminsky, M., Kearney, R.E., Aguayo, A.J., and Bray, G.M., Conduction of nervous impulse in spinal roots and peripheral nerves of dystrophic mice. Brain Res. *143* (1978) 71–85.
18 Ritchie, J.M., and Regart, R.B., Density of sodium channels in mammalian myelinated nerve fibers and nature of the axonal membrane under the myelin sheath. Proc. natl. Acad. Sci. USA *74* (1977) 211–215.
19 Rosenbluth, J., Intramembranous particle distribution at the node of Ranvier and adjacent axolemma in myelinated axons of the frog brain. J. Neurocytol. *5* (1976) 731–745.
20 Rosenbluth, J., Aberrant axon Schwann cell junctions in dystrophic mouse nerves. J. Neurocytol. *8* (1979) 655–672.
21 Rosenbluth, J., Freeze-fracture approaches to ionophore localization in normal and myelin-deficient nerves. Adv. Neurol. *31* (1981) 391–418.
22 Sigworth, F.J., The variance of sodium current fluctuations at the node of Ranvier. J. Physiol. *307* (1980) 97–129.
23 Smith, K.J., Bostock, H., and Hall, S.M., Saltatory conduction precedes remyelination in axons demyelinated with lysophosphatidyl choline. J. neurol. Sci. *54* (1982) 13–31.
24 Stolinski, C., and Breathnach, A.S., Freeze fracture replication of mammalian peripheral nerve: a review. J. neurol. Sci. *57* (1982) 1–28.
25 Tao-Cheng, J-H., and Rosenbluth, J., Nodal and paranodal membrane structure in complementary freeze fracture replicas of amphibian peripheral nerves. Brain Res. *199* (1980) 249–265.
26 Tao-Cheng, J-H., and Rosenbluth, J., Development of nodal and paranodal specializations in amphibian peripheral nerves. Devl. Brain Res. *3* (1982) 577–594.
27 Waxman, S.G., and Quick, D.C., Cytochemical differentiation of the axon membrane in A- and C-fibers. J. Neurol. Neurosurg. Psychiatr. *40* (1977) 379–386.

0014-4754/83/090953-11$1.50 + 0.20/0