qu'après un laps de temps variable. Pour faciliter cette adhésion, nous éliminons à maintes reprises le liquide sécrété par le feuillet interne du blastoderme. Le lendemain de l'opération les blastodermes sont fixés au Bouin, analysés in toto et ensuite inclus en paraffine et débités en coupes sériées de 8 μ (Coloration à l'hémalun-érythrosine).

40 expériences ont été faites. Cependant, quelques blastodermes sont morts précocement et dans quelques autres cas, le greffon s'est détaché du millipore. En définitive, notre matériel contient 33 blastodermes.

L'examen histologique a révélé que l'élongation et la différenciation de nos greffons ont été plus ou moins inhibées. Ces greffons n'ont jamais dépassé les limites du millipore et se terminent par une masse cellulaire compacte. Plus en avant, ils se sont pourtant différenciés normalement et ont fourni une chorde, des somites, du mésenchyme et une gouttière neurale. Les greffons sont fortement attachés au millipore, en particulier de nombreuses cellules mésenchymateuses adhèrent à lui étroitement. En revanche, l'ectoblaste, revêtant la face opposée du filtre, est toujours séparé du millipore par un interstice qui semble vide au microscope optique. Cependant, à l'exception d'un seul cas, cet ectoblaste a toujours réagi à la présence du greffon situé de l'autre côté du millipore. Cette réaction, toujours localisée au territoire correspondant à l'emplacement du greffon, est en général la plus marquée à la hauteur de sa région antérieure. Dans 9 cas, il s'agit de la formation d'une petite, mais typique plaque neurale aux bords plus ou moins soulevés (Figures 1 et 2). Dans 7 cas, nous avons affaire à une différenciation plutôt neuroïdale que neurale. L'ectoblaste est épaissi en plaque, mais ses cellules sont moins allongées et moins régulièrement disposées que dans l'ébauche neurale normale du même âge. Dans les autres cas, l'ectoblaste situé vis-à-vis du greffon est moins épaissi et se présente soit sous la forme d'un épithélium aux cellules cylindriques dont les noyaux sont disposées en 2 ou 3 rangées (11 cas), soit d'un épithélium cubique, identique à l'épiblaste céphalique d'un jeune embryon (5 cas).

Insistons encore sur le fait qu'à part la zone correspondant à l'emplacement du greffon, l'ectoblaste sur toute l'étendue du millipore est composé de cellules fortement applaties et garde son caractère périphérique. Il est donc évident que l'action inductrice du greffon sur l'ectoblaste a dû se réaliser à travers le filtre millipore. Quoique l'analyse au microscope optique ne nous permette pas d'affirmer d'une façon définitive que cette réaction ne s'exerce pas par l'intermédiaire de microvillosités transversant le millipore, cette éventualité nous paraît peu probable. En effet, comme nous l'avons déjà mentionné, l'ectoblaste n'adhère jamais au millipore. D'autre part, aussi bien chez les Amphibiens que chez les Mammifères (voir GROBSTEIN et DALTON[6]), le millipore, même aux pores plus larges que dans le nôtre, intercalé entre les systèmes inducteur et réagissant n'a pas été traversé par de tels prolongements plasmatiques. Enfin, BELLAIRS[7], en soumettant de jeunes embryons de Poulet à l'analyse détaillée au microscope électronique, n'a jamais observé des microvillosités reliant le neurectoblaste à son substratum chordo mésoblastique.

Pour clore cette note préliminaire, soulignons l'analogie frappante entre nos résultats et ceux obtenus par SAXÉN chez les Amphibiens. Aussi bien chez le Poulet que chez le Triton, l'action inductrice peut s'exercer à travers un filtre millipore, bien que ce dernier représente un obstacle sérieux. Dans les deux cas, les réponses de l'ectoblaste au flux inducteur plus ou moins freiné par le millipore s'ordonnent comme suit: épiblaste épaissi, structures neuroïdales et, enfin, neurales typiques.

Summary. In the avian embryo, as in the amphibian, we can even obtain a neural induction, if we interpose a millipore filter (thickness 25 μ, size of pores 0.45 μ) between the inductor and the competent ectoblast.

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Electron Microscopical Aspects of Myelin Ultrastructure

At present, the most widely accepted view on myelin structure is the one that each lamella is the result of the apposition of 2 'unit membranes'[1]. However, the unit membrane theory does not provide a convincing explanation for at least 2 well-established facts: the greater thickness of the major dense line in comparison with the intraperiod line and the existence of double intraperiod lines.

Moreover, as reported elsewhere[2], the results of high-resolution electron microscopical observations on thin sections of glutaraldehyde-fixed, post-osmicated, Epon-embedded tissues suggested a structural organization of biological membranes more complex than the one described by the 'unit membrane' concept. Indeed, in preparations of frog brain cortex, the membrane of synaptic vesicles appeared to consist essentially of a single layer of globules (diameter about 40–45 Å) having a thin electron-dense contour and a light core and bound to each other directly or through an osmiophilic granule. In face-on views, the membrane appeared to be a mesh of polygonal or round areas (diameter 90–110 Å) of unstained material limited by osmiophilic lines, containing in their centers an osmiophilic granule (diameter 20–30 Å). In these unstained areas it was often possible to

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resolve thin radially arranged septa dividing the area into 6 electron-lucid globules. Such bidimensional images were thought to suggest that the membrane of synaptic vesicles is formed by elementary building blocks with a polyhedric shape (probably not too different from the shape of a hexagonal prism), each of the blocks being composed of 6 osmiophobic globules surrounding an osmiophilic granule. Other membranes, including the membranes of mitochondria and the axolemma, also displayed the above described ultrastructural features. These images were probably not due to artifacts, since results obtained by other investigators using different techniques were in substantial agreement. Thus, SJÖSTRAND and co-workers observed a globular structure in some biological membranes which were either chemically fixed or frozen-dried[3]. Hexagonal patterns with a central pit were found on the surface of negatively stained isolated cell membranes by BENEDETTI and EMMELOT[4] and by MURRAY[5]. Hexagonal structures with a central dense spot were described by ROBERTSON in the synaptic discs of Mauthner's cells of the goldfish[6]. Finally, observations by WHITTAKER and SHERIDAN[7] on negatively stained isolated synaptic vesicles appeared to be in disagreement with the classical model of the 'unit membrane'; but we believe they could be easily explained on the basis of the proposed model of membrane structure. The results presented here suggest that the myelin of frog and rat sciatic nerves, whether fixed in glutaraldehyde[8], dehydrated in ethanol and embedded in Epon[9], or fixed in osmic acid[10], dehydrated in ethanol and embedded in Maraglas[11], or fixed in osmic acid, dehydrated in acetone and embedded in Vestopal[12], appears to consist of an orderly mesh of the same kinds of osmiophobic globules and osmiophilic granules, in which an arrangement in polyhedric-globular units can frequently be distinguished.

Nerve specimens were removed from the thighs of frogs and of rats anesthetized with amobarbital and immediately fixed. In other experiments, sciatic nerves were isolated from pithed frogs and kept at room temperature for specified periods of time immersed in frog Ringer solution to which different concentrations of trypsin[13] (or no trypsin) had been added. A Porter-Blum ultramicrotome equipped with a Ge-Fe-Ri diamond knife and a Hitachi HU 11-B electron microscope were employed. Most sections were stained with lead monoxide[14]. Photographs, which were often through focal series, were taken at instrumental magnifications between 70,000 and 140,000, at either 50 or 75 KV.

In our preparations of normal nerve, the spaces between the major dense lines of myelin are occupied by unstained globules limited by a delicate osmiophilic sheath (Figure 1). Size and shape of such globules are similar to those observed in the frog brain preparations. Very frequently, they appeared to be arranged in 3 rows parallel with the major dense lines. In this case the space between consecutive major dense lines measures about 120 Å. Less frequently only 2 rows of clear globules are present and the space measures only about 90 Å. Often,

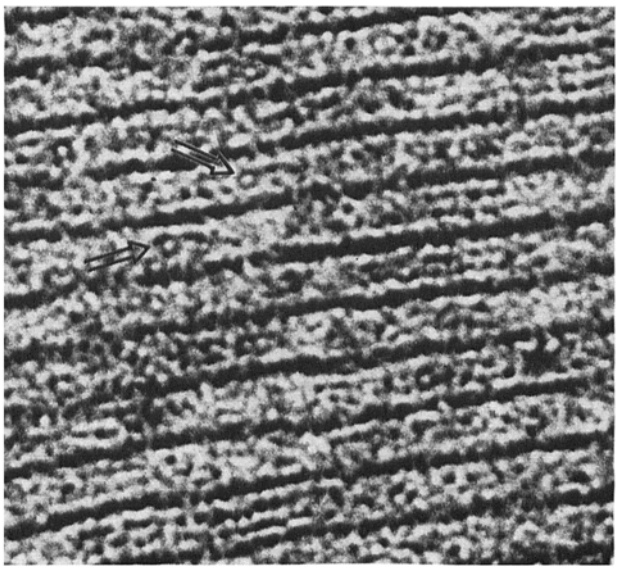

Fig. 1

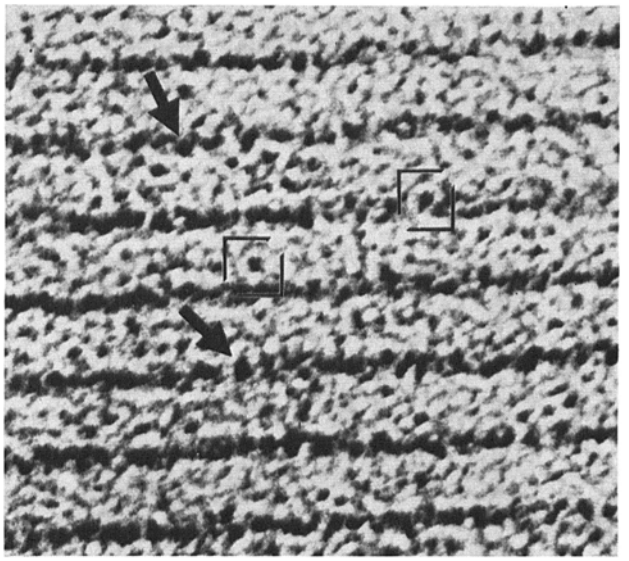

Fig. 2

Fig. 1. (× 550,000) and Fig. 2. (× 910,000). Myelin of rat sciatic nerve (fixation in osmic acid, embedding in Maraglas). 3 rows of osmiophobic globules occupy the space between consecutive major dense lines. The outlined arrows point to osmiophobic globules; the black arrows indicate some osmiophilic granules which constitute myelin's major dense lines. Polyhedric-globular units in face-on view can also be seen (in the squares).

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the rows of globules are interrupted by one or more polygonal or round structures (diameter around 90 Å) which have a central osmiophilic granule surrounded by an unstained area (Figure 2). In other words, polyhedric-globular units in face-on view can be seen in the lamellae of myelin. Similar designs occupy more or less completely the space between major dense lines in oblique sections of myelin (Figure 3) and are practically the only kind of structure visible in the areas where no major dense lines can be observed.

The major dense lines themselves appear to consist mainly of a sequence of osmiophilic granules measuring 20–25 Å in diameter and set at a distance of about 20 Å from each other (Figures 1, 2 and 4).

The intraperiod lines can assume different aspects in different areas. They can be double and symmetric; in this case the myelin lamella consists of 3 rows of osmiophobic globules, the contour lines of which produce the double-line pattern. They can also be double and asymmetric; in such a case, one line consists of a series of arcs, and the other of a series of granules, each granule being situated in the center of the surface limited by an arc (Figure 5). The center-to-center distance between consecutive granules can vary from about 60 to about 90 Å,

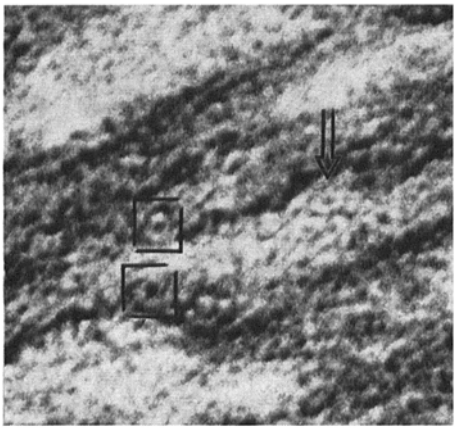

Fig. 3. In this oblique section of myelin lamellae, the space between 2 consecutive dense lines shows the presence of polyhedric-globular units in oblique view (arrow). 2 more of such units are singled out in the squares. (× 440,000).

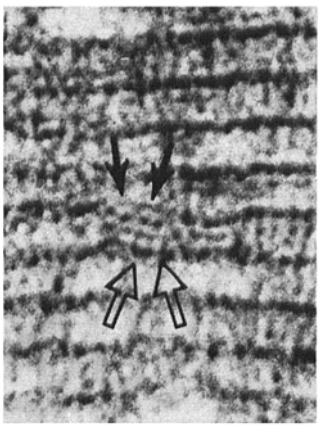

Fig. 4. Osmiophilic granules bound to osmiophobic globules (black arrows) can be seen in an area of myelin in which the structure is slightly disarranged. Notice also some well defined osmiophobic globules (outlined arrows). (× 440,000).

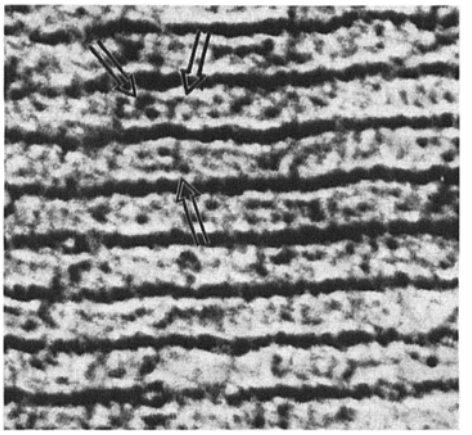

Fig. 5. 'Arc and granule' pattern of asymmetric double intraperiod lines, due to oblique view of polyhedric-globular units (arrows). (× 440,000).

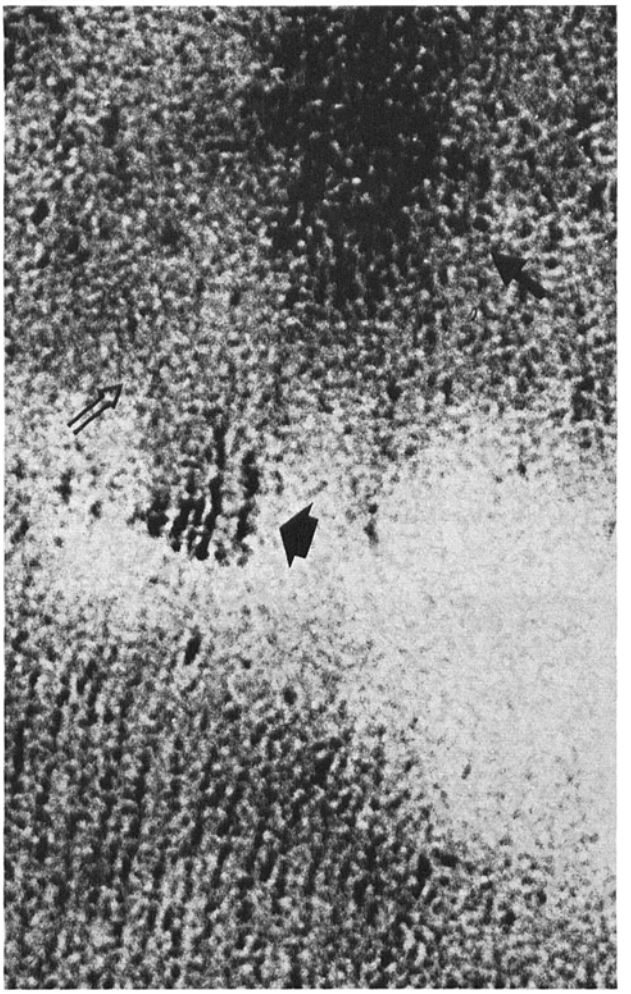

Fig. 6. Trypsin-treated frog sciatic nerve (fixation in osmic acid, embedding in Vestopal). An area of myelin in which the lamellar structure can still be recognized (on the bottom) is contiguous to an area in which a profound disruption has occurred. Notice a cluster of osmiophilic granules (long black arrow), a conglomeration of osmiophobic globules (outlined arrow) and a fragment of myelin lamella with its content of osmiophobic globules (short black arrow). (× 500,000).

and, accordingly, the arcs can span the same length. The arcs are clearly part of the peripheral contour, and the granule is the central osmiophilic granule of polyhedric-globular units as seen in oblique view. When the intraperiod line is single and continuous, the myelin lamelia is constituted by 2 rows of globules only. Finally, the intraperiod line can consist of a sequence of osmiophilic granules set at a distance of about 90–120 Å one from the other, each appearing to be, more or less clearly, the center of a polyhedric-globular unit seen in face-on view.

When the myelin lamellae show splits, they are usually found to occur at the level of a continuous intraperiod line, giving the impression that the bonds between the granules of the major dense line and the adjacent clear globules are firmer than the bonds between rows of clear globules.

Since the described findings agree with those obtained on frog[2], rat and guinea-pig[15] brain cortex, they seem to justify the conclusion that membranes of amphibian and mammalian central and peripheral nervous system, fixed, dehydrated and embedded in various ways, consist essentially of osmiophobic globules and osmiophilic granules, and to suggest the hypothesis of the arrangement of these globules and granules into polyhedric-globular units. This hypothesis is attractive in that it furnishes an explanation of the presence of osmiophilic granules in the lamella of myelin on the basis of a particular orientation of polyhedric-globular units with respect to the section plane.

It is difficult to say what such electron microscopic appearance may mean in terms of real structure of the biological membrane. When very high magnifications are employed, one must take into consideration how much the embedding process may contribute to the structural organization of the cellular constituents, as well as the effects of fixation and dehydration on the tissue as sources of artifacts, even if, as in our case, one is dealing with results consistently obtained with 3 different embedding media. With this problem in mind, we began to study the effects upon myelin ultrastructure of different chemical and physical treatments applied before fixation. In short segments (about 2 mm) of isolated frog sciatic nerves kept immersed in 0.1% trypsin in Ringer for 16 h at room temperature, the myelin showed areas of structural disorganization in which the lamellae appeared disrupted and substituted by agglomerates of osmiophobic globules irregularly interspersed with osmiophilic granules (Figure 6). These preliminary results seem to indicate that, in the experimental conditions to which the frog nerve fibers were subjected, not only the disruption of myelin lamellae but also the scission of the hypothesized polyhedric-globular units into subunits were obtained[16].

Zusammenfassung. Das Myelin peripherer Nerven von Amphibien und Säugetieren scheint, mit Hochleistungs-elektronenmikroskop untersucht, aus einer regelmässigen Anordnung von osmiophilen Granula und osmiophoben Globula zu bestehen. Solche Untereinheiten scheinen häufig polyedrische Gebilde zu formen, in welchen zentrale Granula jeweils von 6 osmiophoben Globula umgeben sind. Es ist beim Froschischiadicus möglich, die Struktur des Myelins zu zerstören und die Granula von den Globula durch Behandlung mit Trypsin zu trennen.

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[15] Unpublished results.

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Role of the Nuclear Membrane in Smooth Endoplasmic Reticulum Formation in White Rat Pinealocytes

Although both granular and agranular endoplasmic reticulum have been described in rat pinealocytes[1], the agranular one is much more abondant[1]. The presence of a well-developed agranular reticulum is also a characteristic of pinealocytes in other species[2] This structure is present mainly in the form of vesicles, the amount of which may vary in different physiological or experimental situations[3] The present work was undertaken in order to obtain further information on the origin and nature of this cytoplasmic component

Materials and methods. Adult white rats of both sexes of about 200 g were used Their pineal glands were dissected under ether anesthesia and immediately fixed in Palade's fixative[4] (1% osmium tetroxide in a 7.4 veronalacetate buffer at 0 °C) and embedded in buthylmetacrilate. Thin sections were cut with a Porter-Bloom microtome, and examined with a Philips EM 100 electron microscope.

Results. A well-developed smooth endoplasmic reticulum was observed in pinealocyte cytoplasm. This was mainly formed by characteristic cysternae and small vesicles with a diameter varying between 250 and 1500 Å (Figure 1).

Nuclear membrane showed the typical double-layered constitution; its pores did not appear to be a simple membrane discontinuity since they appeared closed by a thin diaphragm to which a mass of electron dense material had joined (Figure 2). Some ribosomes were observed in close contact with the external surface of the outer layer (Figure 1). Blebs of this outer layer were frequently seen; they varied in size from one zone to another (Figures 1 and 3). At the site where blebs

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