Retinal synaptogenesis in the primate

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Morphologic observations on early retinal synaptogenesis of Macaca mulatta fetuses of known gestational ages are described. Attempt is made to correlate the first appearance of synaptic vesicles, lamellar material, and the establishment of presynaptic contacts in both the inner and outer plexiform layers and in both types of synapses developing in these zones. Possible origin of lamellar material and synaptic vesicles are postulated, based on morphologic evidence derived from electron micrographs. Attention was focussed on fetal gestational stages from the earliest appearance of the outer plexiform layer to past mid-term in gestation, just prior to the development of outer segment discs. Synaptic connections occurred in both layers nearly simultaneously and before the appearance of outer segment discs. Cone synaptogenesis preceded that of rods.

Key words: retina, primate, synapses, synaptic vesicles, synaptic ribbons, inner plexiform layer, outer plexiform layer, rods, cones.

The problem of the differentiation of the retina has intrigued investigators for many years. The classical period began with Cajal.1 The result of these and other studies with the light microscope have been well reported and interpreted in various texts (Mann,2 Duke Elder and Cook,3 and Barber4). Only in recent years has electron microscopy been applied to this problem. The results of these investigations have been very exciting, particularly those of Meller5-6 on the chick retina, Olney,7 and Weidman and Kuwabara8,9 on rodents and scattered investigations on monkeys by Keefe, Ordy, and Samurajski.10 These have cumulated in the reports by Hollenberg and Spira11 and Spira and Hollenberg12 on the developing retina of human fetuses which has recently appeared. We were intrigued with the possibility of adding information to that gathered by our predecessors by a study of the fetuses of Macaca mulatta of precisely known gestational ages, which allows us to choose the stages to study. The adult retina of this species is very nearly identical to the human. In the present paper we have confined our attention to describing the appearance of synaptic complexes in the inner and outer plexiform layers of the developing retina. Particular attention has been placed on the
morphology of early chemical synapses, those equipped with synaptic lamellae, the differentiation of the membranes facing the synaptic space and the material within it. Attention was directed to the relative time of appearance of the synapses in rod and cone cells and to the degree of development of the inner and outer segments of the photoreceptors at this time.

The area of the retina studied was the fundus, but not necessarily restricted to that in which the fovea presumably would develop. Our study is morphologic and qualitative in nature.

Methods

The fetuses studied were of *Macaca mulatta* and the stage of gestation was accurately known by determination of the day of ovulation. The fetuses were delivered by aseptic surgery, the eyes carefully and immediately enucleated, and fixed in 3.5 per cent glutaraldehyde in 0.05 M phosphate buffer, pH 7.4, for approximately 20 hours. They were then postfixed in 1 per cent osmic acid in 0.05 M phosphate buffer at pH 7.4 for 1 hour. After a few minutes in glutaraldehyde, the eyes were opened by an equatorial section. The larger eyes were subsequently cut into smaller pieces just prior to osmication. They were then dehydrated in a series of ethanol and embedded in Epon. After the preparation of 2-μ thick sections for light microscopy, the area to be studied by electron microscopy was selected. Thin sections were then cut and examined in a Siemens Elmiscope 1.

Observations

At the sixty-seventh day of gestation the layer of Chievitz could no longer be identified as such. This disappearance resulted from the outward migration of some of the inner neuroblastic cells through the layer of Chievitz. The neuropil which was left between the definitive ganglion cells and the inner nuclear layer becomes the inner plexiform layer. At this stage the outer plexiform layer had not made an appearance. The tangled processes of bipolar and presumably amacrine and ganglion cells still had a loose texture. There were spaces between some of the processes; however, in a number of instances attachment bodies could be seen uniting them. These appeared to be punctate desmosomes, but in others there was a densification of the apposing cell membranes between which some material was found in the intercellular space.

Asymmetrical junctional specializations without synaptic vesicles were seen in the newly established inner plexiform layer. A coated vesicle or coated pit, confluent with the plasmalemma of the neural process was present at the edges of such a junctional differentiation (Fig. 1*) or was seen approaching the differentiating membranes, usually in the postsynaptic process. Some instances could be found in which a single or perhaps two or three round vesicles were associated with one of the thickened membranes. The contents of these vesicles had a slight electron density greater than that of the cytoplasm surrounding them. Such junctional adhesions had the appearance of early bouton synapses.

During the interval from 67 to 76 days gestation, the outer plexiform layer made its appearance (Figs. 2, A and B). It is

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*Explanation of figures: A, amacrine, C, cone, CN, cone nucleus, Cap, capillary, CH, chorio-capillaris, cv, coated vesicle, D, dendrite, DB, denser bodies, ELM, external limiting membrane, GC, golgi complex, Ge, ganglion cell, He, horizontal cell, INL, inner nuclear layer, IPL, plexiform layer, M, mitochondria, Me, Müller cell, NVB, nerve-fiber layer, S, synaptic lamella, SV, synaptic vesicles, OPL, outer plexiform layer, P, pedicle, PE, pigment epithelium, R, rod, RN, rod nucleus, S, synaptic lamella, and SV, synaptic vesicles.
Figs. 2, A and B. Light micrographs of a 76-day fetal retina. A, is more central than B. Note the alignment of the cone cells; there are no outer segments. A, narrow outer plexiform layer is present. ×752.

very narrow at this stage as is the layer of photoreceptor nuclei. Fig. 2, A shows this layer to be composed almost completely of presumed cone cells, whereas in Fig. 2, B they are associated with a number of presumed rod cells. The section showing only cones is thought to be in the area of the future fovea. The inner plexiform layer was much more advanced in development than that in the 67-day fetus. Many interneuronal junctions were found. These appeared to be desmosome-like, however, their differentiation to form synapses had progressed sufficiently so there could be no doubt that a synaptic junction was in the process of development. If the section was cut precisely at right angles to the neuronal plasma membrane, a dense line could be seen lying in the intercellular space which was 360 Å to 380 Å wide, and was filled with a somewhat electron-dense material (Fig. 3, insert). The opposing membranes were asymmetrical in thickness and the thinner presynaptic membrane had synaptic vesicles associated with it. These ranged in any given section from a few to many vesicles of 505 Å to 580 Å in diameter and thus all of the criteria of an ordinary or chemical synapse were present in the inner plexiform layer of the 76-day fetus.

In addition to the chemical synapse, others could be identified which possessed either short or well-developed synaptic lamellae (Fig. 3, insert). These have synaptic vesicles associated with them. Oc-
Fig. 3. Terminal bulb in the inner plexiform layer of a presumed bipolar axon. An early chemical synapse (arrow) and several synaptic lamellae are present in the postsynaptic process. Note the coated vesicle (short arrow) at the site of a presumably forming synapse. Fetus of 76-day gestation. \( \times 48,000 \). Insert: early bipolar synaptic bouton showing desmosome-like junctional differentiation with the intercellular contact substance represented by the dark dense line bisecting the synaptic cleft. A synaptic lamella and vesicles are shown; 76-day gestation. \( \times 48,000 \).

 Occasionally, cross-sections of these lamellae were seen in which the vesicles were arrayed radially around them, thus giving a concept of their three-dimensional arrangement (Fig. 4). The synaptic lamellae, however, were seldom oriented in the adult manner in respect to the dense neuronal membrane. They often appeared to be floating freely in the cytoplasm of the presumed bipolar terminal. In some instances the lamellar material was in the form of a very short mass and seemed thicker than in the adult, thus leading us to the supposition that this material was in the process of being assembled. Some were very clearly lamellated with a central, very dense line (Fig. 5). All of these synapses were between processes, therefore, were axodendritic in nature. No axosomatic synapses were identified.

The outer plexiform layer of the same monkey fetus was a neuropil composed of intertwined processes of the inner nuclear layer. Some of the elements in this layer appeared to be horizontal cells on the basis of light microscopy (Fig. 2, A). The photoreceptors were all very undeveloped. The cones consisted of rather large short cylindrical cells attached to the outer-limiting membrane. Their apex bulged past their junctional specialization and impinged upon the inner surface of
the pigment epithelium. This bulge was an early stage in the development of the inner segment (Fig. 6). Mitochondria, ribosomes (polysomes), and a Golgi apparatus occupied the space apical to the nucleus. In some fortunate sections a cilium could be discerned on the apex of the rounded protruding inner segment. No traces of formation of membranes of the outer segment were observed. The basal cytoplasm of these cells was filled with profiles of smooth endoplasmic reticulum, polysomes, and a few scattered and irregularly arranged neurotubules. The bases of the cones were somewhat flattened where they were opposed to the outer plexiform layer which was invaded by blunt processes from the growing cone cell cytoplasm. Numerous synaptic vesicles and synaptic lamellae were observed in the perinuclear cytoplasm which constituted the primitive, or beginning pedicle. There was only a small volume of cytoplasm in the basal aspect of these cells. Premature synaptic lamellae, which resembled those found in the adult with synaptic vesicles covering each surface, were present. Such structures were only occasionally arranged at right angles to the cell membrane facing the outer plexiform layer.

Most of the premature synaptic lamellae were dispersed through the basal cytoplasm. Some of them were near the nucleus, unassociated with synaptic vesicles and, in many cases, were thicker and their substance was less dense than the more mature lamellae. Those presynaptic bodies which appeared to be most immature had a somewhat granular structure and there was evidence in a few instances of a longitudinal arrangement of these granules. The axes of the lamellae had no particular orientation, but in a few cases, they were arranged near the nuclear membrane with their long axis parallel to it (Fig. 7). In other instances, they were similarly parallel to the cytoplasmic membrane facing the outer plexiform layer. Only seldom were they positioned at right angles to this membrane, and in no instance were they arranged in a typical triad formation. Often the synaptic lamellae and associated vesicles faced a dense cytoplasmic membrane opposite a slightly intruding process from the outer plexiform layer (Fig. 8). In one or two cases, two processes had pushed side by side into the cytoplasm of the base of the cone. These processes could be of horizontal cells with a bipolar cell inserting either between them, or the inserting process may be out of the plane of section.

There were no rod cells with synaptic organelles or indeed a recognizable spherule in fetuses of this age.

A four-day-older fetus (80 days gestation)
had a more frequent occurrence of presynapses in the inner plexiform layer. These were exclusively axodendritic and they occurred more frequently in the outer aspect of this layer. They ranged from asymmetrical junctional specializations with one or two approaching or attached synaptic vesicles, or typical early synaptic knobs, to floating or already adhering synaptic lamellae, surrounded by 6 to 8 synaptic vesicles. Other synaptic lamellae were not arranged at the confluence of axodendritic processes but only at right angles to one.

Some spaces were still to be found between neuronal extensions in the inner plexiform layer and coated vesicles or coated pits were frequently prominent in the vicinity of such spaces.

The layer of photoreceptors was dominated by large cells which we interpreted as developing cones. The apex of the cells was filled with mitochondria, rough-surfaced endoplasmic reticulum, and a prominent Golgi apparatus. The space between the cones and the pigment epithelium was largely occupied by villi of the pigment epithelium.
Fig. 7. Higher magnification of presumed early synaptic lamellar material near the nucleus of a cone cell in a 76-day gestational fetus. Arrows show synaptic vesicles. x48,000.

Fig. 8. Synaptic ribbons and vesicles near the basal plasma membrane of a pedicle at 76-days gestation. x54,000.

Fig. 9. Low-power electron micrograph of an 80-day gestation fetal retina, with immature cone cell. Dense bodies, presumably presynaptic lamellar material are seemingly progressing from the Golgi site along the nucleus toward the basal portion of the cell. Long arrow points to external limiting membrane. Short arrow to presumed lamellar material. x9,000.
Fig. 10. Basal part of a cone cell of fetus of 80-days gestation. The cytoplasm has many clumps of synaptic vesicles, mostly surrounding the several lamellae not yet in position near the plasmalemma. \( \times 30,000 \). Insert: portion of a red nucleus and developing spherule showing close relationship of synaptic vesicles to the nuclear membrane and the synaptic lamella. The outer plexiform layer is visible below. \( \times 48,000 \).

epithelium. There was no more than a potential cleft between the two cell layers. The nuclear membrane contained many blebs which we interpreted as demonstrating activity of this structure in the formation of membranous components of the cytoplasm. Lateral to the nucleus were a number of dense bodies which could be synaptic lamellae in formation. They are of the correct size and electron density (Fig. 9). The basal portion of the cone cytoplasm was filled with polysomes, profiles of smooth endoplasmic reticulum, and was pressed against the outer surface of the outer plexiform layer. Extensions of their substance reached into and between the mass of outer plexiform processes. The general form of this base was an irregularly flattened surface. There were few or no indentations into the cone cell cytoplasm by neuronal processes of horizontal and bipolar cells. Synaptic lamellae
associated with synaptic vesicles were found in the cytoplasm, but not always arranged at right angles to the cell surface (Fig. 10). Many more were floating unattached in the photoreceptor cytoplasm surrounded by synaptic vesicles. Maturing identifiable rod cells were very rare at this stage (mid-gestation). The insert (Fig. 10) illustrates a portion of such a cell with synaptic lamellae and synaptic vesicles in the very narrow basal karyoplasm.

The eighty-sixth-day fetus demonstrated a continued increase in the number of synapses in the inner plexiform layer. This layer was rapidly increasing the thickness of the complicated neuropil (Fig. 11). There were still many gaps between the processes so that at this age the intercellular space in the retina as shown by our electron micrograph is considerable. Synaptic lamellae were frequently associated with vesicles. The synaptic space between processes was usually filled with a filamentous electron-dense material. Some neural processes were replete with synaptic vesicles and some also contained lamellae (Fig. 12). Large, presumably bipolar, terminal processes were quite filled with vesicles and one had, in addition, four synaptic lamellae directed at membrane densifications applying to two other processes. In one instance synaptic lamellae without vesicles were present, apparently floating quite freely in the cytoplasm of the process. Some lamellae were thicker than the definitive ones and may be particularly young forms.

The photoreceptor layer consisted of two types of cells (Fig. 11): an outer early cone layer; and, just between the cones and internal to them, a number of nuclei of cells which presumably will form rods. The shape of the cone cell had changed somewhat and could now be called cylindrical, slightly lengthened in its basal aspect. The apical aspect was, as before, filled with mitochondria. There was still no evidence of an outer segment. Between the somewhat swollen mitochondria were dilated vesicles of smooth endoplasmic reticulum which were grouped together to form a Golgi apparatus (Fig. 13). The nuclear membrane, as in earlier stages, was scalloped as if the blebs were forming cytoplasmic membranes. In fact, evidence that they were doing so could be seen in this figure, where rough-surfaced endoplasmic reticulum was budding off from the nucleus into the apical cytoplasm. The basal cytoplasm of cones was filled with numerous polysomes, some profiles of smooth endoplasmic reticulum, vesicles, and synaptic lamellae in various stages of development (Fig. 14). At this stage the synaptic lamellae were mostly grouped toward the basal surface which had not as yet dilated in the typical pedicle form but was somewhat squared off facing the outer plexiform layer. This surface has been by now indented by a few processes from the outer plexiform layer. Some of the
lamellae appeared to be free in the cytoplasm of the developing pedicle. It is difficult to assert this with assurance in the absence of serial sections, however, the orientation of the sections is such that it appears most likely that the synaptic lamellae were not yet attached to the plasma membrane of the photoreceptor. The immature synaptic lamellae were irregularly elongated oval structures. It was difficult to resolve any internal detail, however, there was a suggestion that these lamellae were at this early stage surrounded by a membrane and the dense material within them was linearly positioned (Fig. 14, insert).

**Ninety-two-day gestation.** The light micrograph has all the layers of the adult (Fig. 15). Very immature horizontal and amacrine cells were recognizable and capillaries have reached the ganglion cell layer. The inner plexiform layer had areas of dense synapse formation throughout its thickness, but no axosomatic junctions could as yet be detected electron microscopically. All stages of early synapse formation could be observed. These stages occurred in all zones and were not confined to either the outer or inner area of the inner plexiform layer. Thus, asymmetrical junctional specializations with one or two vesicles nearing the presynaptic membrane and with no synaptic vesicles in other places were still occurring. Often coated pits confluent with the plasmalemma of the postsynaptic neuron were seen (Fig. 16). Synaptic knobs, always with spherical vesicles, were present. In such synapses no synaptic lamella was obvious. In other sections, synaptic lamellae were well-developed and surrounded by a halo of synaptic vesicles. All were encased in a dilated neural process. Presumably such sections represent a terminal bouton of a bipolar cell. In some of these the synaptic lamellae and associated vesicles appeared to float freely in the cytoplasm of the terminal process, unattached to the cell membrane (Fig. 17). We did not observe
Fig. 13. Low-power electron micrograph of a cone cell in an 86-day gestation fetus. The pedicle cytoplasm is filled with lamellar material, polysomes, vesicular, and tubular endoplasmic reticulum (arrow). Extensions of its basal cytoplasm reach between neural processes constituting the outer plexiform layer. ×6,000.
sections of typical dyad arrangement at this stage.

Rods and cones were easily distinguishable from each other in the photoreceptor layer. Neither had as yet developed an outer segment. Early stages of these consisted of a cilium which was slightly dilated, but no membranous sacs had as yet formed. The inner segment of the cone cells was filled with mitochondria and bulged outwardly, passing the external limiting membrane more markedly than in the preceding stages. The greatest change in the shape of the cells was a definite elongation of the axonal or basal portion. It was still filled with polysomes and a few profiles of smooth endoplasmic reticulum. A pyramidal-shaped pedicle had not yet developed. The cone axon terminated bluntly and with a rather flattened profile from which a few finger-like processes penetrated the outer plexiform layer. Some extensions of this layer also indented the future cone pedicle but these indentations were not yet organized into the tuft characteristic of the adult. Well-formed
Fig. 15. Light micrograph of a 92-day gestation fetus. x752.

Synaptic lamellae and their associated synaptic vesicles were in contact with the cell membrane but in most cases were not oriented between two invading processes (Fig. 18). For the most part, they appeared to have moved through the cytoplasm to the cell membrane where they stationed themselves without specific relation to the invading dendrites from the outer plexiform layer. An exception (Fig. 19) shows a well-developed triad. Formation with two lateral invading processes, presumably of horizontal cells, with a central process lying between them but not touching the cone cell membrane in this section. The synaptic lamella has an arciform density. In contrast to this highly developed synaptic apparatus, other presumed presynaptic lamellae may be found which were nearly ovoid in shape and had no accumulation of synaptic vesicles (Fig. 20).

Rod cells could be seen with their nuclei lying adjacent to the cone pedicle. The
Fig. 18. Synaptic lamellae in various locations in an immature pedicle in a 92-day gestational fetal retina. Horizontal cell processes barely indent the pedicle substance. x54,000.

axon of the rods had not started to form and the scant cytoplasm lay close to the nucleus. Synaptic lamellae, however, were seen occasionally in the basal cytoplasm of the rod cells (Fig. 21). The lamellae were quite mature, surrounded by numerous synaptic vesicles and most of them were nearer the nucleus than the cell membrane applied to the outer plexiform layer (Fig. 22). There was some indication of an invagination of this scant rod perikaryon by processes from the outer plexiform layer.

Some sections illustrate the close relationship of the synaptic lamellae and vesicles to the nucleus. Such pictures suggest that some synaptic vesicles were derived from the nuclear membrane (Figs. 20 and 23). An additional point of interest may be the occurrence of the multivesicular bodies. The contents of these closely resemble synaptic vesicles in size, structure, and density. They were found in both the inner plexiform layer (Fig. 12) and in the cytoplasm of the rod cell (Fig. 24). The average diameter of vesicles in the multivesicular bodies of rods was 574 Å.

Observations were made of the presumptive origin of the synaptic lamellar material. Such observations apply to the various ages of pre- and mid-term fetuses.

Fig. 19. Synaptic lamella in the definitive location at the base of a pedicle, between two presumed horizontal cell processes, which reach into the pedicle's cytoplasm. The central, presumed bipolar terminal, is almost in the adult position. Note arciform condensation (arrow). x54,000.
Fig. 20. Base of a pedicle of a 92-day gestational monkey fetus. Tilted section shows structures which have the size and appearance of synaptic vesicles within the outer nuclear membrane and another just outside this membrane (arrows). ×18,000.

Fig. 21. Early synaptic lamellae surrounded by vesicles in the basal perikaryon on an immature rod in a 92-day gestation fetus. Adjacent is a growing cone pedicle. ×24,000.
which have been studied. The definitive mature synaptic lamellae are slender, dense structures often with a central, more electron-dense line. In many instances, larger elongated dense bodies were found in the cytoplasm of photoreceptor and in the terminal boutons of presumable bipolar processes which appeared to be immature lamellae. These presumptive lamellae were "sausage-shaped" sometimes slightly bent, were not associated with synaptic vesicles, and were occasionally seen between the large nucleus and the cell membrane in the center of the cell of photoreceptors (Fig. 25). A few examples were found in the apical cytoplasm near the Golgi apparatus or profiles of smooth endoplasmic reticulum related to the Golgi complex. We were unable to observe a definite morphologic relationship which would allow us to conclude that this presumptive lamellar material was being produced by the Golgi apparatus. The early presumptive material was more granular than the definitive lamella. It was, however, enclosed in a membrane (Fig. 26). These observations lead to the presumption that the lamellae may arise as dense bodies in the apical cytoplasm near the Golgi complex, pass around the nucleus through the rather narrow rim of cytoplasm surrounding the nucleus, and migrate gradually to the base of the cell. During the migration through the basal portion of the cell synaptic vesicles, which were in the cytoplasm, join the lamellae. Perhaps in contrast to this postulated mode of appearance was an occasion in which the lamellar material was found in close association with the nuclear membrane on the lateral aspect of the nucleus (Fig. 27). It is as if the lamellar material and the vesicles were drawn to the basal aspect of the cell especially at loci where
bipolar or horizontal cell processes commence to indent it. The vesicles appeared to be attracted to the lamellar material and then the entire complex seems to move to the cell membrane, against which dendrites of the outer plexiform layer were pressed. Later the synaptic lamellae and their vesicles became oriented with respect to the invaginating horizontal and bipolar cell dendrites.

At this stage short segments of neurotubules were observable in the apical cytoplasm of developing cones (Fig. 26). Cross-sections of such neurotubules, however, were present in the large processes of the inner plexiform layer at 67 days gestation and increased in the later stages.

Discussion

Our observations indicate that the synaptic complexes in both the inner plexiform layer (IPL) and outer plexiform layer (OPL) begin to form at about the same time and almost as soon as these layers appear. Since the IPL develops first, perhaps synaptic complexes in this layer may precede those in the OPL, but the difference in time is not very great. This finding is in contrast to those on rodents\(^7\) where synapses formed in the OPL slightly earlier than in the IPL, but is similar in our material to the time of formation in the human.\(^1\) The pedicles of the cone develop at an earlier age than do the spherials of the rods. It is also clear that the rod synaptic complexes with their respective bipolar and horizontal fibers form a little later than do those of the cones.

It is worthy of comment that both photoreceptors establish their synapses with the second neuron before either develop their outer segments, also observed in humans as well as rodents.\(^7\)\(^-\)\(^9\)\(^-\)\(^1\)\(^3\)

The time sequence in which various parts of the synaptic apparatus forms is of interest. It would appear that the first indication of a synapse in the IPL is a...
membrane thickening at the future synaptic site. Some of these may be maculae denseae diminuta and never develop into a synapse. Others, however, appear as desmosome-like densifications of apposing membranes. Appearance of synaptic contacts in mid-gestation was noted by Korniguth, Anderson, and Scott in the cerebellum of Macaca mulatta. This suggests that synaptogenesis in brain and retina are approximately synchronous. They interpreted the development of synaptic specializations on the dendritic processes of neurons in the deep cerebellar nuclei to indicate that the potential for the regulation of energy output was appearing in these species between 75 and 100 days of gestation. Initiation of axodendritic synaptic contacts, and thus neuronal circuits was demonstrated very early (8.5 weeks gestation) during gestation in the human fetal cerebral cortex. This is earlier than they could appear in the retina where the relevant neuroblasts have not yet migrated to their permanent locations, however, it is interesting to note that in the chick the formation of inter-retinal synapses takes place after the junctions between retinal fibers and those of the optic tectum have been established.
Fig. 27. Higher power electron micrograph of a dense lamellated body in the narrow perikaryon of a rod cell at 86-days of gestation. x72,000.

Presumed developing retinal synapses are not accompanied by synaptic vesicles at first. A slightly later stage is characterized by an asymmetry of the opposing dense membranes. At this stage the first synaptic vesicles may appear. It is difficult to state that there are no vesicles in early synapses because they are few and vary in numbers and very thin sections which pass through a differentiating synaptic junction need not include a vesicle. This was Bodian's view expressed in his study of synaptogenesis in the monkey spinal cord. Such a situation holds true in the developing retina also. Synaptic specializations can be characterized with more certainty when observing at least one synaptic vesicle in their close vicinity. In such an event there are usually a few such vesicles in the neuroplasm of the process. All the synaptic vesicles in our material were round, which, according to Bodian would indicate that they contained noncholinergic material. Even the earliest formed vesicles enclosed material of medium electron-density, probably a precursor of the neurosecretory substance, which is presumably stored within the vesicles.

Glees and Sheppard observed in the chick spinal cord that membrane thickenings were the first indication of a synaptic site followed later by the addition of several dense segmented presynaptic projections directed toward the cytoplasm; synaptic vesicles and mitochondria were present in the elements. Meller noted that membrane thickenings were laid down first, followed somewhat later by the appearance of synaptic vesicles at the presynaptic sites only, in chick retinal receptors. Our data differs from that of Kevan and Hervonen on the development of synapses in ganglia. They observed that the accumulation of synaptic vesicles preceded or occurred at the same time as the formation of pre- and postsynaptic thickenings. Whenever such early thickenings were noted by us in axodendritic junctions, there were some synaptic vesicles in the neuron, but they were still scarce and widely scattered. This is in general agreement with the succession of events in the central nervous system.

Adherence of synaptic vesicles to the presynaptic membrane takes place immediately upon formation of the junctional specialization in the mouse cerebellum. Larramendi contends that material composing the presynaptic densification entraps the synaptic vesicles floating down the axon. This may indeed be so in the retinal synapses, as vesicles near synaptic sites invariably had fine spoke-like fibrillar protrusions extending from their membrane and merging into the densities.

The synaptic clefts that had formed in both the IPL and OPL contained a slightly electron-dense material. This material and the dense synaptic membranes
may have resulted from a contribution of coated vesicles. Stelzner, Martin, and Scott\textsuperscript{23} noted the presence of many coated vesicles in the perikarya and dendritic processes of the embryonic chick spinal cord. They derived these vesicles from the Golgi substance and found them confluent with the plasmalemma near the postsynaptic membrane specialization. Numerous such coated vesicle fusions were present in our material and are interpreted as possibly contributing to the increase in the densification. The presence of coated vesicles opening into the synaptic cleft was frequent in early synaptic junctional specializations of the IPL: Stelzner, Martin, and Scott\textsuperscript{23} and also Altman\textsuperscript{24} suggest that these vesicles transport material for dense membranes to such developing synaptic sites in the cerebellum and spinal cord. This would seem a plausible hypothesis, as the retinal nuclei and cytoplasm were engaged in very active membrane elaboration during the stages studied. Many of the coated vesicles in our specimen contained granules, which, according to Altman,\textsuperscript{24} would indicate transport of some chemical(s) into the intercellular space.

Synaptic lamellae characterize the synaptic complexes of the photoreceptors and bipolar cells. In both instances, these lamellae appeared to be synthesized at some distance from the definitive synaptic site. This was particularly easily observed in the developing rod and cone cells. Dense bodies, presumptive immature lamellae, were found in the apical cytoplasm in close association with the Golgi apparatus. In other sections they were observed in the narrow zone of cytoplasm lateral to the nucleus apparently passing toward the base of the cell. It was easy to find examples of these dense bodies near the basal aspect of the nucleus of both rods and cones. These dense bodies were membrane-bound and their contents appeared to consist of granules arranged in a linear pattern. In a few instances a central dense row of granules or a line was observed (Fig. 5). Such presumptive immature lamellae were thicker or more oval than the definitive ones and were frequently curved in a "banana"-like configuration. While they were still in this immature form and while approaching the future synaptic site, vesicles clustered around them as a halo when observed in cross-section. This complex (lamellae and synaptic vesicles) floated freely in the primitive pedicle or the cytoplasm of the rod and cone cells to the base of the cell against which processes of the OPL were pressed. These processes did not, at first, indent the pedicle or spherules and the lamella-vesicle complex seemed to attach to the cell membrane more or less at random where it was opposed by processes from the OPL. They were not, at first, necessarily oriented between two invaginating dendrites. Certainly, they were not clearly oriented between two (presumed horizontal cell) processes with a third process (presumably bipolar) pressing between them. This configuration, although it might have been missed in our nonserial sections, was not obvious until later in gestation.

Sheffield and Fischman\textsuperscript{25} reported on an early ribbon synapse in the inner plexiform layer of the 14-day chick retina. Early synapses, in this species, lacked pre- and postsynaptic membrane thickenings, but contained the ribbon-like cytoplasmic inclusion composed of two unit membranes in close apposition. Such lamellae were associated with clear vesicles 200 to 300 Å in diameter.

Our observations are in agreement with those of Olney\textsuperscript{7} and Weidman and Kuwabara\textsuperscript{8, 9} who also saw dense bodies free in the cytoplasm of the photoreceptors. Weidman and Kuwabara\textsuperscript{8, 9} noted amorphous dense bodies in the growing photoreceptor axon which coalesced to form synaptic bars with vesicles accumulated around them. In our material we could show dense bodies, membrane bound and lamellated, in close approximation to the nucleus and in some instances seemingly coming off the nucleus (Figs. 26 and 27).
The origin of synaptic lamellae and their vesicles is not as easily studied in the bipolar as in the photoreceptor cells. However, they were found separately and in association with each other in the axon of bipolar cells.

The genesis of synaptic vesicles has intrigued numerous investigators of a variety of neurons. Meller in the 16- and 17-day-old chick embryo retina showed synaptic vesicles and lamellae without attached vesicles near the basal part of the nucleus, whence they later migrated distally to the presynaptic loci. Though no accumulation of synaptic vesicles was observed in the monkey cytoplasm by us near the basal pole of the nucleus, as was by Meller in the chick, synaptic lamellae, with or without attached synaptic vesicles, could be seen very close to the basal aspect of the nucleus, indeed organelles having the appearance of synaptic vesicles were seen between the two nuclear membranes and immediately adjoining the outer membrane at the basal pole of both kinds of immature photoreceptors (Figs. 22, 23, and 24).

According to Stelzner in the immature rat spinal cord, synaptic vesicles are intimately associated with the Golgi apparatus and smooth endoplasmic reticulum and the number of these vesicles decreases after synaptogenesis has terminated. This interpretation is based on differential staining techniques. Our purely morphologic evidence seems to involve the nuclear membranes in producing at least some of the synaptic vesicles and indicates that the smooth endoplasmic reticulum, originally derived from the nucleolmma, is responsible for the majority of these early vesicles.

We observed several occurrences of multivesicular bodies in both the IPL and OPL during the period when synaptic vesicles were rapidly forming. These bodies contained formations with the same semi-electron-dense content, thus closely resembling synaptic vesicles. Kilarski and Jasinski in their study of the gas gland of perch found multivesicular bodies to result from intense nuclear activity and to represent projections of both nuclear membranes. They interpret the vesicles in such bodies as originating from the infoldings of the inner membrane and their contents to be filled with nuclear (not cytoplasmic) elements and they believe that multivesicular bodies might disintegrate and their vesicles be dispersed in the cytoplasm. As most morphologic data provides purely circumstantial evidence, we can only speculate that in our material multivesicular bodies might be one of the sources of immature synaptic vesicles.

It is important to note that chemical synapses characteristic of amacrine cells and the lamellar synapses characteristic of bipolar cells in the IPL appeared at almost the same time. All of these synaptic complexes were axodendritic not axosomatic. Other students of developing synapses in nonocular tissues have noted that they occurred between processes prior to axon-to-soma attachments.

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