periment (see Table), NA and 5-HT in the high-speed sediment accounted for 94% and 70% of total ^{3}H and ^{14}C respectively, considerable amounts of both isotopes appeared in the first 4 or 5 fractions of the gradient. This observation suggests that binding of the amines is not sufficiently firm to prevent some loss during resuspension and centrifugation in a sucrose gradient.

Résumé. Après injection i.v. de 5-hydroxatryptamine-C^{14} et de noradrénaline-H^{3} à des rats, on a préparé et centrifugé des homogénates de leurs poumons dans un gradient linéaire de sucrose. Après sacrifice immédiat ces deux amines sont retrouvées dans une grande fraction de particules pulmonaires sédimentant dans 1 *M* de sucrose. Après 30 min les poumons des rat offrent un pic additionel de noradrénaline-H^{3} correspondant à une densité de sucrose de 0.4 à 0.5.

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Neuronal Types in Long-Term Culture of Avian Retina

Tissue culture of nervous system has provided an ideal model system for experimental studies in the various disciplines of the neurosciences. For understanding and interpretation of any experimental studies, precise definition of neuronal types in a given culture is most crucial, since no valid conclusion can be drawn without such information. Although there have been a few reports on culture of mammalian and avian retina, little is known about the neuronal types differentiated in culture[1–3]. The present communication is primarily concerned with the identification of neuronal types in silver impregnated chick retinal cultures.

The eyes of 10–14-day-old chick embryos were dissected out under sterile conditions and the globes were incised anterior to the equatorial plane. The retinae were detached gently and cut in small pieces for explantation. The explants were placed on rat tail collagen coated coverslips (11 by 22 mm rectangular), and then sealed in Maximow's slides[4] or in roller tubes[5]. The nutrient fluid consisted of equal parts of horse serum, medium 199, Hanks' balanced salt solution and supplementary glucose giving a concentration of 600 mg per 100 ml nutrient. The cultures were incubated at 36 °C. At various time intervals (12–34 days in vitro) cultures were taken out and fixed for silver impregnation by a modification of Bodian's protargol method[6,7].

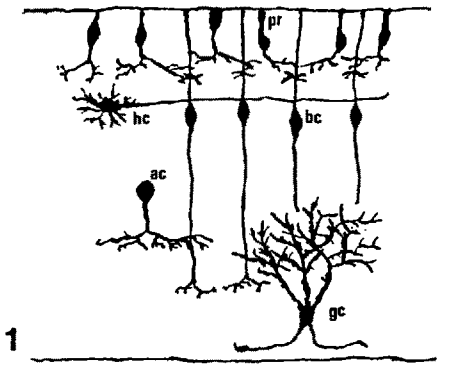

Fig. 1. Neuronal elements of 13–14-day-old chick embryo retina. Pr, photoreceptors (rods and cones); bc, bipolar cells; hc, horizontal cell; ac, amacrine cell; gc, ganglion cell (modified from Ramon y Cajal[8]).

The normal developmental stage of the retina from 13–14-day-old chick embryos is depicted in Figure 1, which is modified from the sketches of Ramon y Cajal[8]. The cell elements illustrated are photoreceptor cells (rods and cones), bipolar cells, ganglion cells, and two types of association cells, horizontal and amacrine cells. Neurons demonstrated in silver impregnated cultures were closely correlated morphologically to the neuronal types shown in Figure 1.

The development of chick retinal cultures in general was similar to the descriptions made by previous authors in cultures of new-born rat retina such as the rosette formation of photoreceptors[1,2]. Amongst the heavy population of photoreceptors and glial cells (including Müller cells), various neuronal types were observed. Although nerve cells in culture were irregularly oriented, 4 principal types of neurons were identified as such by characteristic features of their morphology and size. Ganglion cells were the largest among the neurons observed in retinal cultures (14–20 µm soma), with multipolar orientation of dendrites. They closely resembled typical neurons demonstrated in cultures of other regions of the chick central nervous tissue[9,10] (Figure 2). Bipolar cells were distinguished from other neurons by their smaller size (6–10 µm soma) and 2 long processes extending from the cell body (Figure

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[6] The cultures were fixed in Ramon y Cajal's formol-ammonium bromide solution for 24 h at room temperature, then transferred to 95% ethyl alcohol to extract lipids to improve the stainability of neuronal elements for 48 h at 36 °C. After a brief wash in distilled water, the fixed cultures were incubated in a Columbia staining dish containing 0.7% protargol solution and copper fragments (0.2 g per 10 ml solution) for 24 h at 36 °C. After the incubation in protargol solution, the cultures were processed in a reducing bath, 0.5% gold chloride (without acetic acid added), 1% oxalic acid, and 5% sodium thiosulfate, for 5, 3, 3 and 3 min respectively.
[7] When the protargols (silver proteinate) from various manufacturers were tested in our cultures, the quality of staining was rated in the following order: Prewar Beyer, Roque (French), Winthrop (American), Chroma (German), Merck (German), Gurr (British), British Drug House.
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3). Two types of association neurons, horizontal and amacrine cells were of an intermediate size (10–14 μm soma). A peculiar feature of amacrine cells enabled us to distinguish this type of neurons. The processes of amacrine cells pointed to one side of soma. This feature seemed to be a reflection of the morphology in vivo (Figure 4, also ac in Figure 1). On the other hand, horizontal cells had a typical multipolar configuration with 4 to 6 processes extending from the soma (Figure 5). There also was a strong indication of de novo formation of the interneuronal connections evidenced by the demonstration of the terminal boutons which were closely applied to the surface of neuronal soma and dendrites (Figure 6). These terminal boutons appeared as delicate neurofibrillar rings with clear central cores.

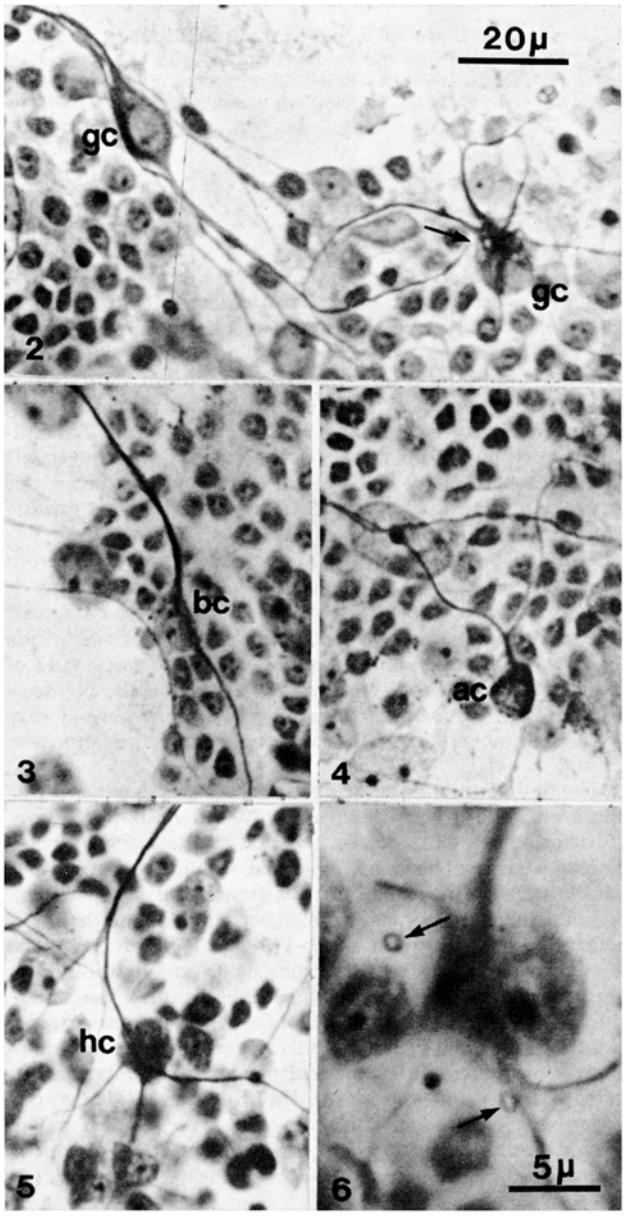

Fig. 2. Two ganglion cells (gc) are shown. An axon from the left ganglion cell terminates on the soma of the ganglion cell at the right (arrow).
Fig. 3. Bipolar cell with a thicker dendritic process heading to upward and the thinner axon heading to downward.
Fig. 4. Amacrine cell with its processes originating from one pole of the soma.
Fig. 5. Horizontal cell with multipolar projections of dendrites and an axon.
Fig. 6. Two terminal boutons (arrows), fine neurofibrillar rings, localize on and nearby the neuronal soma possibly a horizontal cell. Figures 2 through 6 are light micrographs of Bodian silver impregnated nerve cells in chick retinal cultures fixed after 12–34 days in vitro. Figures 2 through 5 are at the same magnification.

Previous investigators have attempted unsuccessfully to identify neuronal types differentiated in retinal cultures[1–3]. BARR-NEA and BARISHAK[3] maintained that in chick retinal cultures all neurons except horizontal cells degenerated with the process of aging. This explanation for their failure to define neuronal types in culture is unwarranted: their oldest cultures were merely 3 weeks in vitro, in that stage we had scores of cultures with well silver stained neurons. Their claim of the identifying horizontal cells is also questionable, since their illustration only demonstrates network of nerve fibers without showing nerve cell itself.

KIM[5,11] has reported that synaptic connections were newly formed in cultures of central nervous tissue, demonstrating ring-shaped terminal boutons in silver impregnated cultures of kitten cerebellum. Subsequent reports[12,13] have shown similar synaptic configurations in silver impregnated cultures of central nervous tissue. Fine structural accounts of such synaptic connections in cultures have also been reported[14–17]. Further evidences of the functional operation of interneuronal connections in cultures were given by electrophysiological methods[18]. The terminal boutons shown in the present communication are morphologically identical to those observed by previous authors. Recently there has been an abstract reporting an electron microscopic observation of synapse in rat retinal cultures[19]. Thus it is evident that the interneuronal connections are present in the retina explants developed in long-term tissue culture.

We defined 4 principal types of retinal neurons in silver impregnated cultures. We believe that such study will serve as a base line study for the interpretation of oncoming experimental studies using retinal cultures.

Zusammenfassung. Morphologische Befunde an Nervenzellen der Retina von Hühnerembryonen werden mitgeteilt. Mit der Bodian-Silberimprägnation in Langzeit-Gewebekulturen konnten multipolare Ganglienzellen, bipolare und Horizontalzellen, Amakrine und Synapsen identifiziert werden.

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