to a value for f'_m of 0.4743. Similarly, treating the 174 and 999 μM azide spectra as references gave $f'_m = 0.4732$. Evidently, it does not make very much difference which of the spectra are treated as fixed references and which as the unknown.

The second point concerns any further analysis to which the f'_m values are subjected. A necessary feature of the procedure we are proposing is that there will be one datum where f'_m is exactly zero and one where it is exactly unity. However, if some binding or other function is then fitted to the entire set of data, these two reference points should not be assigned any special significance. They are treated as if they are no more or less precise than any other datum. This ensures that if one of the reference spectra was faulty resulting in a systematic displacement of all of the other f'_m values, it is the faulty reference which would stand out as a single aberrant point and any further analysis could be adjusted accordingly.

The analysis we are proposing takes an entire series of spectra and describes each of them in terms of two limiting spectra and a single parameter (f'_m) defining the relative amounts of these two limiting spectra. If the dependence of f'_m on the perturbing agent can be accurately described by a theoretical or empirical equation, it is then possible to predict the spectrum at levels of the perturbant which were not examined experimentally. This type of prediction was illustrated by extrapolating the expected spectrum for the protonated form of phenol red (fig. 1 b). A similar type of prediction was carried out for carbonic anhydrase, in this case interpolating a spectrum for 346 μM azide which is shown in figure 2 c (solid circles) together with an experimental spectrum at this concentration of azide, which is shown by the line in figure 2 c. This predicted spectrum imitates the actual spectrum quite well and it must be stressed that this particular experimental spectrum was not used at all in the calculations. The points in figure 2 a are a *conformed* spectrum, deliberately chosen so as to give the best overall match to the experimental observations. By contrast, the points in figure 2 c are a *predicted* spectrum which was calculated independent of the experimental spectrum.

We regard this ability to predict a spectrum as a useful benefit of the procedure described in this report. However, the primary purpose is to characterize the quantitative effect of perturbing agent which promotes a spectral change and our procedure has two important features. First, it uses all the spectral data whereas common practice has been to follow the absorbance at just one or two selected wavelengths. Second, unlike analyses which have been proposed before[1-7], the method described here makes no assumptions about the mathematical form of the relationship between the amount of perturbant and the extent of the spectral change; rather it is this relationship which may be deduced from the analysis.

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Short Communications

Short axon ganglion cells in the chick retina

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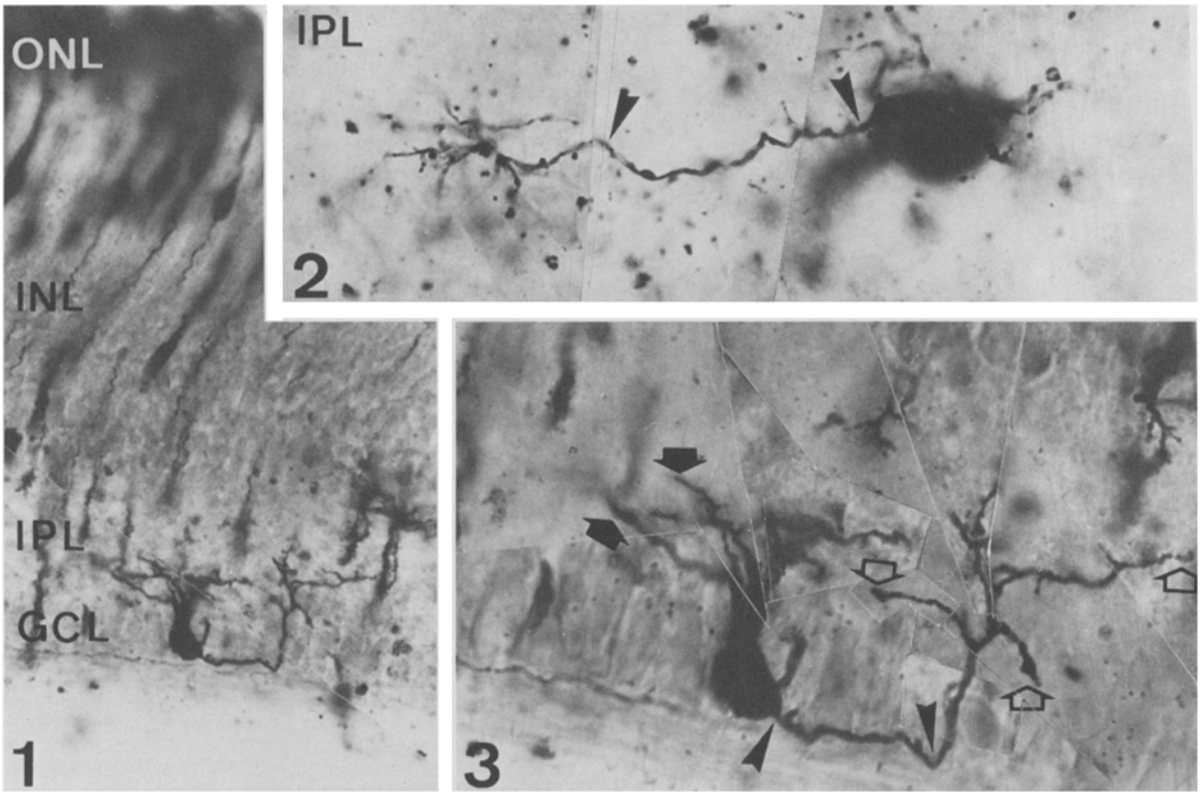
Summary. Using Golgi's staining technique, we investigated some ganglion cells whose axons do not project out of the retina area. These axons, after following a short trajectory through the optic nerve fiber layer or through the 5th stratum of the inner plexiform layer (IPL), change their direction and end in the inner stratum of the IPL.
Key words. Retina; ganglion cells; axon; chick.

The cytological organization of the vertebrate retina is basically considered to consist of five major classes of neurons with different locations and function[1-4]. However, the classic neuronal disposition pattern sometimes presents morphological variations, shown by the displaced location of some of the neurons.

The displacement of retinal cells has been the object of several descriptive studies; for example, of ganglion cells in the



Figures 1 and 3. Vertical section of the retina. Ganglion cell, whose axon (arrow-head) goes through the optic nerve fiber layer in the direction of the IPL, where it ends (open arrows). Solid arrows mark dendritic expansions. ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. ×400 and ×600, respectively.

Figure 2. Flat mount of a ganglion cell with short axon (arrow-head). IPL, inner plexiform layer. ×600.

inner nuclear layer (INL)[1,2,5], displaced amacrine cells[1,6], displaced bipolar cells[1] and displaced horizontal cells[7].

Some studies have found that not only may the location of the cell body be altered, but modifications in the pattern of cell prolongations are also observed. Thus, 'associative ganglion cells' have been described in dog and human retina[8,9] which have a neurosecretory function, due to their relation with blood vessels.

Here, we describe ganglion cells in the chick retina which are termed 'short axon ganglion cells' because their axons end within the IPL.

Material and method. We used 10 1-month-old white leghorn chicks. Eyes were stained following Golgi's technique with Colonnier's[10] modifications. Some of the retinas were flat mounted, but the remainder were embedded in celloidin and vertically sectioned in a sliding microtome at a thickness ranging between 70 and 90 μm.

Observation was carried out using a Zeiss microscope, checking by focusing up and down that all relevant cells were wholly included within the section. The drawing was made using a camera lucida attachment.

Results. The most relevant morphological characteristics of the 'short axon ganglion cells' are the following: they are mostly found in the peripheral retina, and their perikaryons are located in the inner portion of the ganglion cell layer (figs 1, 3, 4).

Three or four main dendritic processes, 2–2.5 μm in diameter, arise from the cell body apical pole and subsequently give rise to secondary and tertiary branches. These dendritic branches form overall a radiate pattern, about 50 μm in span, which extends through the 4th and 5th strata of the inner plexiform layer (fig. 3, solid arrows). Their axons mostly arise from the lateral and inner zones of the cell body. After a short trajectory, 30–60 μm, through the optic nerve fiber layer (figs 2 and 3, arrow-heads, and 4), they change direction from tangential to vertical, reaching the 5th, 4th and 3rd strata of the inner plexiform layer, where they ramify (fig. 3, open arrows). They never ramify in the 2nd or 1st stratum. The axonal branches are scanty and tortuous, covering an area similar to that of the dendritic tree.

The modifications of the axon are the principal defining characteristics and that is why we call them 'short axon cells'.

Discussion. The short axon ganglion cells, such as those described in this study, could be confused with the displaced amacrine cells and ganglionar cells, because of their position and morphological similarities. However, the pattern of dendritic branching of the displaced amacrine cells is different. In addition, the displaced amacrine cells never present axons.

When these cells are compared with ganglionar cells, the morphologic similarities between the cell types are many, both in the dendritic pattern and in the origin of the axon. Nevertheless, the difference from the classically described ganglionar cells is that they always project their axon to the brain.

The finding of the 'short axon ganglion cells' has led us to consider some conceptual questions about the neural organization of the retina.

Our first question is whether the presence of these cells in the retina is ectopic, or whether they are part of a specific functional cellular system. Nowadays, it is known that alterations during the migration process, leading to cellular ec-

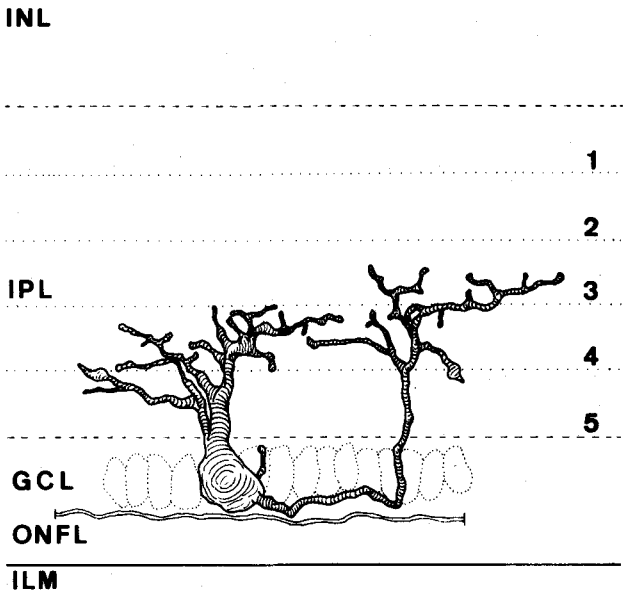


Figure 4. Camera lucida drawing of a 'short axon ganglionar cell', to point out the axonal and dendritic expansions in the different strata of the inner plexiform layer (IPL). × 600.

topies, are common occurrences in NCS development [11, 12]. On the other hand it is also known that the establishment of the retinotectal connection is an important fact in ganglion cells' survival [13]. If ganglion cells do not synapse with optic tectum cells, they degenerate and die [14, 15]. It could be thought that the axon of such a ganglion cell might have been mechanically displaced during its differentiation. But it does not degenerate because it finds a 'guide' or 'signal' [16–18] mechanism which makes possible an effective synaptic connection in the IPL. If this were certain, it would make it obvious that ganglion cells have a clear plasticity during their development, and can subsequently behave as a projection or an association neuron.

Another possibility is that these cells have the genetic information and capability necessary in order to differentiate as association neurons, with the axonal characteristics described. The fact that ganglion cell axons forming synapses with photoreceptors have been found in the retina of the primate [19] supports this hypothesis.

As the Golgi technique is a non-quantitative procedure, it is rather difficult to establish to what extent cells such as those described are a general feature of the cell population of the retina. It is possible that the cells described here represent an isolated case of neuronal ectopia. Since the existence of intraretinal axons is important, in our opinion, in retinal circuitry, we consider that further studies to provide stronger evidence are needed. Electron microscopy using the Golgi procedure is being used at present in our laboratory to study the synaptic connections of these cells.

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Effects of butyrate and insulin and their interaction on the DNA synthesis of rumen epithelial cells in culture

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Summary. Rumen epithelial cells (REC) were incubated in the presence of various concentrations of butyrate or insulin or with both of them, to obtain information on their effect on the DNA synthesis of cultured cells. The 24-h values of ^{3}H-thymidine incorporation into cellular DNA were measured in the presence of butyrate, insulin or butyrate plus insulin. While butyrate reduced DNA synthesis, insulin produced an increase over the control. Combined butyrate plus insulin treatment influenced the incorporation of label in accordance with the relative proportion of these two substances.
Key words. Butyrate; insulin; rumen epithelial cells; DNA synthesis.

Butyrate induces characteristic biochemical changes in cultured cells [1, 2], including rumen epithelial cells (REC) [3, 4]. It increases protein synthesis and alkaline phosphatase activity and inhibits cell growth. In contrast with the last finding, observed in vitro, daily short-term intraruminal infusion of butyrate increases the mitotic index of ovine REC in vivo [5]. It has been suggested that the infused butyrate stimulated mitosis in REC not so much by a direct effect as indirectly, by elevating the insulin level [6]. This hypothetical conclusion has been supported by the experimental facts that intra-