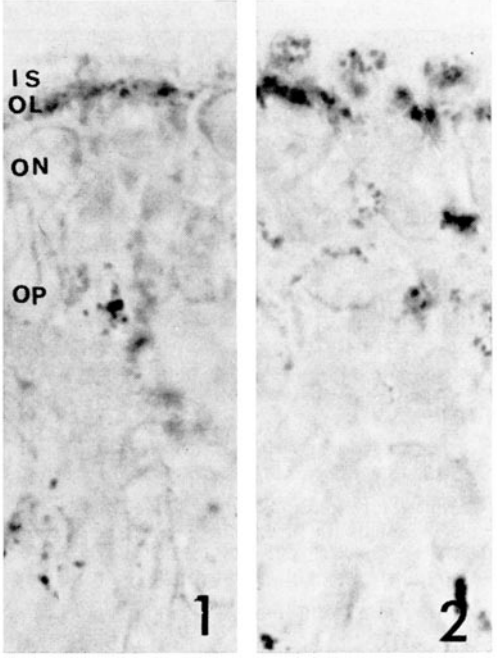
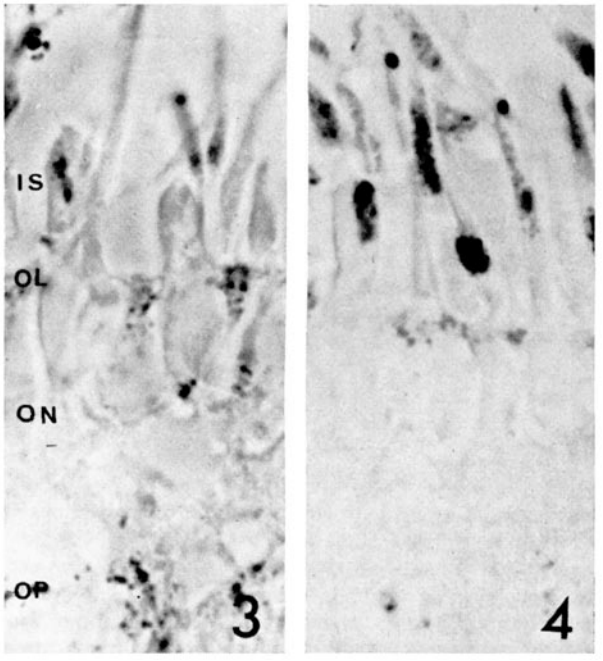
Qualitative and Quantitative Studies of Succinic Dehydrogenase in the Retina of Chick Embryos Treated with Iodoacetate

Because in mammals[1] as well as in the chick[2], iodoacetate (IA) specifically induces a degeneration of the photoreceptors which resembles retinitis pigmentosa, a genetic retinopathy of man, the effect of this inhibitor on the retina of developing chick embryos has been studied. Preliminary results showed that, among histochemically detectable enzymes of the Krebs cycle in the embryonic retina, succinic dehydrogenase (SDH) is the most distinctive, and the reaction, as in the case of the monkey and the frog[3], is confined mostly to the ellipsoids of the rods and cones where mitochondria are aggregated. The visual cell accounted for more than half of the glycolysis in the retina[4].

A single dose of 50 μg of sodium iodoacetate in 1 ml aqueous solution (pH 7.4) was injected into the air chamber of hens' eggs of 'Hyline' breed at 12 days of incubation, and these embryos (E), together with sham controls (C), were sacrificed at 24 h intervals thereafter until hatching at 20 days or later. Although E showed a higher mortality than the C at any given time, neither an external anomaly nor a developmental retardation, according to the embryonic stage[5], was detected. Each observation was made from more than 4 pairs of E and C from different batches of eggs. Throughout the experiment only the strip of retina along the equator of the left eyeball was used. For the histochemical reaction of SDH, the sample was fixed for 10 min in cold 6.25% hydroxy adipaldehyde in phosphate buffer (pH 7.4) before cutting into 4 μ cryostat sections, and incubated for the enzyme[6] using nitro-BT[7] as a hydrogen acceptor. Some samples were processed for electron microscopy of the enzyme reaction by the ferricyanide method[8] after the pre-fixation described above. Spectrophotometric assay of the enzyme[9] using INT[7] was also carried out with 10% water homogenate of the whole retina, and the activity was expressed in terms of protein content[10].

In 13-day-old embryos, 24 h after injection (Figures 1 and 2), the histochemical enzyme activity, in the form of formazan granule deposits, was stronger in E than in corresponding C in 4 out of 5 cases examined. In E the granules were especially conspicuous in the developing inner segments of the rods and cones (IS) which, at this stage, consisted of sprout-like projections extending external to the outer limiting membrane (OL) from the outer nuclear layer (ON). Some deposits were noticed also in the outer plexiform layer (OP). A similar increase of enzyme activity was observed until 4 days after injection. Thus, in 15-day-old embryos, when the rods and cones were barely distinguishable from each other and their ellipsoids were clearly shown, the formazan deposits in E were more intense than in C in all 5 retinas observed (Figures 3 and 4). The cone displayed a more distinct reaction than the rod, which is contrary to the finding that iodoacetate affects mainly the rod in adult mammals[1]. The ultrastructural observation of the enzyme reaction at this age confirmed





Figs. 1–4. Photomicrographs of the inner layers of chick embryo retinas showing SDH activity, representing 13-day-old C and E, and 15-day-old C and E, respectively. × 1200. See text for the keys to abbreviations used in all figures.

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[7] Nitro-BT, 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3-(3,3′-dimethoxyl-4,4′-diphenylene) ditetrazolium chloride; INT, 2-*p*-nitrophenyl-3-*p*-iodophenyl-5-phenyl tetrazolium chloride.
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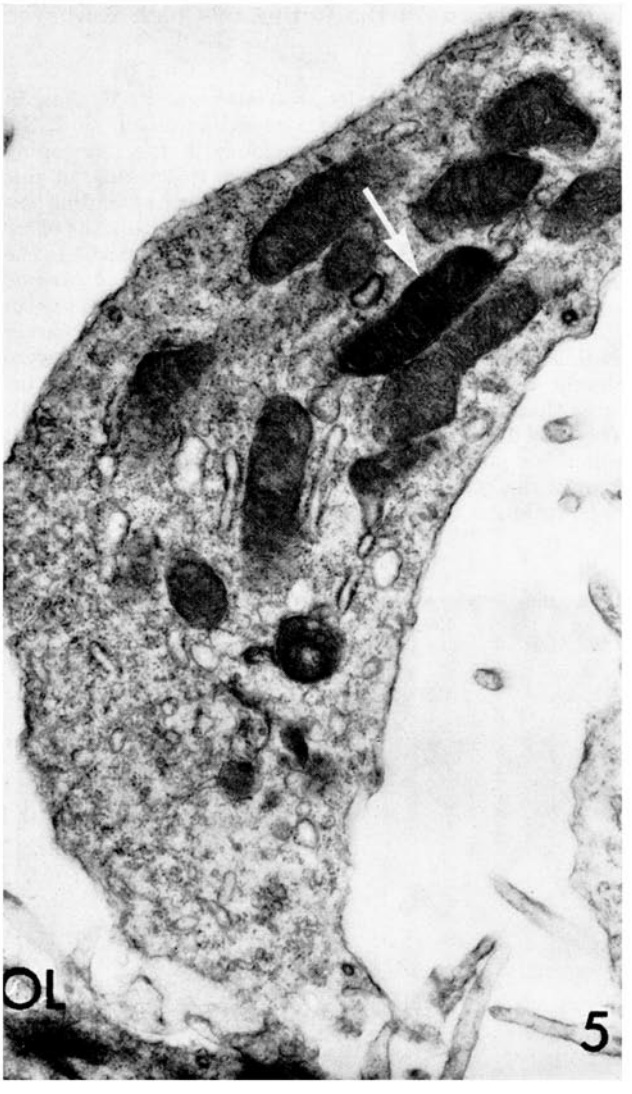

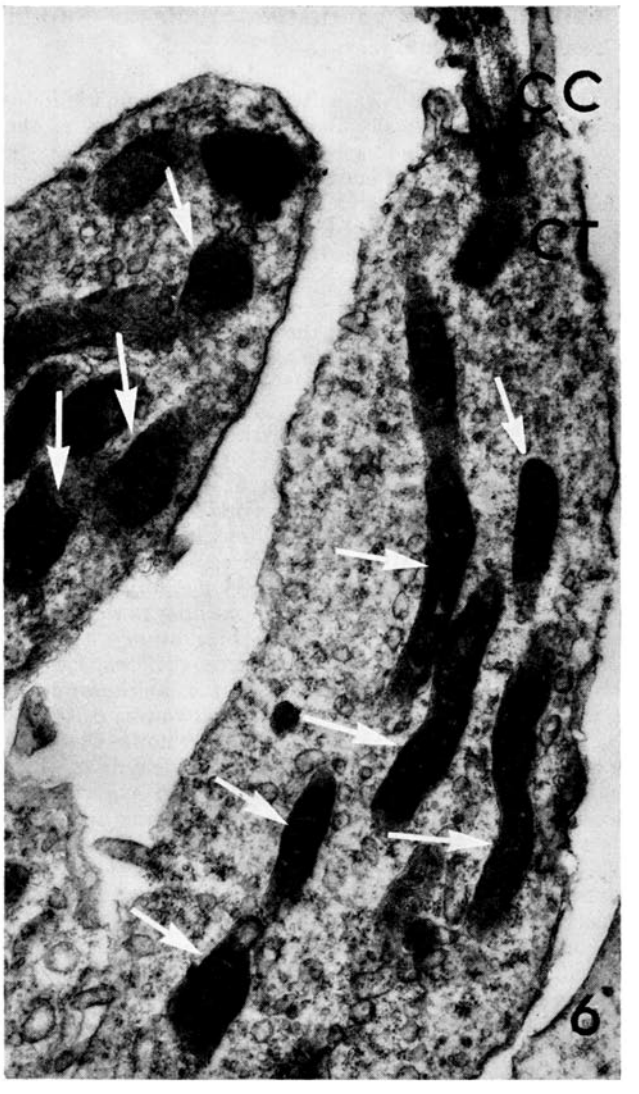

Figs. 5 and 6. Electron micrographs of the developing IS of cones from 15-day-old C and E chick embryos, respectively, showing SDH activity in the mitochondria (arrows). × 27,000. CC, connecting cilium; CT, centrioles.

the histochemical result. In C, only a few mitochondria in a cone ellipsoid showed the reaction product, copper ferrocyanide, within the unit membrane system (arrows in Figures 5 and 6), whereas in E the product was localized in most of them. There appears to be a clear accord between the histochemical and electron microscopical methods employed, each serving to verify the validity of the other. Because no morphological change was evident, the net effect of IA in this experiment can be stated as an increase in the number of reactive mitochondria. One of the earliest ultrastructural changes observable in the IA-treated rabbit was also in the mitochondria[11]. After 4 days of injection, however, the difference in histochemical reaction between E and C was not apparent, suggesting a reversible nature of the effect. In the rat, within certain limits, the metabolic change induced by IA was reversible[4].

The result of enzyme assay (Figure 7b) agreed with that of the morphological findings. When a 6-day-old embryo was treated similarly through the chorioallantois, the recovery after the initial increase also occurred within 5 days after injection (Figure 7a). The primary action of IA is regarded classically as a depression of the metabolic energy production by inhibiting glycolysis at triose phosphate dehydrogenase, and in mammals both anaerobic and

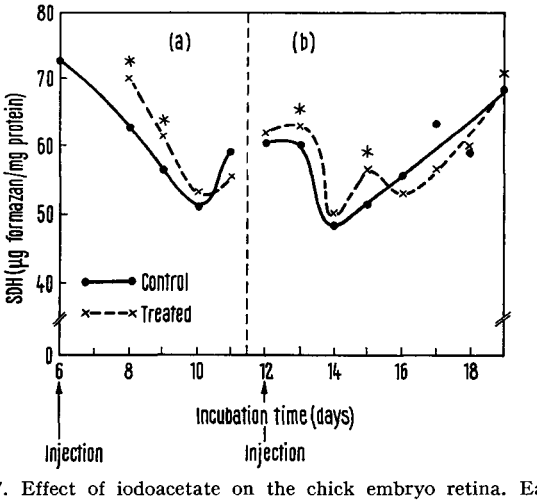

Fig. 7. Effect of iodoacetate on the chick embryo retina. Each observation represents the mean of 4 or more determinations. * statistically significant change (p of 0.05 or less).

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aerobic glycolyses are lowered by IA[4,12]. In view of the importance of retinal glucose metabolism in the chick[13], the SDH increase in this experiment may simply indicate an elevated enzyme accumulation due to this inhibition. On the other hand, it may also indicate the accelerated rate of oxidative activities of the visual cell with such non-glucose substrates as glutamate and aspartate seen in mammals[14]. In the IA-treated rabbit, the significance of the hoxose monophosphate shunt pathway has been emphasized[12], although in chick embryos the histochemical reaction of enzymes of this pathway was negligible[15].

Résumé. Par l'injection d'iodoacétate dans l'œuf de poule, on peut, par l'analyse histochimique et l'usage du microscope électronique, démontrer la présence d'une augmentation de l'activité du succinodéhydrogénase pendant la période embryonnaire.

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The Substantia Gelatinosa of Rolando

Earlier anatomical studies of the terminal distribution of dorsal root axons in the cat and rhesus monkey[1,2] demonstrated terminal fibers of: (1) massive proportions in the nucleus proprius cornus dorsalis, centrobasilar region of the dorsal horn and accompanying zona intermedia (plexus du noyau gris intermédiaire of Ramón y Cajal), Clarke's column, n. cervicalis centralis and its rostral continuation into the caudal medulla (nucleus of Stilling), nucleus intermediomedialis thoracolumbalis, and (2) additional numerous connections around axial and appendicular ventral horn motor neurons. The same studies failed, however, to provide conclusive evidence for synaptic contacts with cells of the substantia gelatinosa, although a terminal plexus was seen around the overlying marginal cells of Waldeyer, and an extremely dense terminal arbor identified in the large underlying nucleus proprius cornus dorsalis (Figure 1). Other studies also have been done in the cat using the selective silver impregnation method of Nauta for degenerated fibers[3-8], yet the results have sometimes been different. Szentágothai[8], and Sprague and Ha[6] reported finding terminal fibers in the gelatinosa of the cat while Liu[3,4] found that the gelatinosa received very few fibers compared with other recipient spinal nuclei such as the nucleus proprius cornus dorsalis, Clarke's column and motor ventral horn neurons. Ralston[5] found that the nucleus proprius cornus dorsalis was a major projection area for dorsal root axons and only rarely did dorsal root terminals appear to end in the gelatinosa of the cat. Sterling and Kuypers[7], using a modified Albrecht-Fernstrom procedure in addition to the Nauta-Gygax method, failed to find terminals in the substantia gelatinosa (Rexed's lamina II of these authors), while confirming, in the same sections, the presence of a massive terminal plexus in the subjacent nucleus proprius. Ramón y Cajal[9], in contrast to most of these results, reported finding, in Golgi preparations of mammalian spinal cord, dorsal funicular fibers forming dense arbors in intimate contact upon gelatinosa dendrites. While lacking good evidence for a dorsal root-gelatinosa link in our own studies[1,2] the possibility could not be excluded that dorsal root terminals might, nevertheless, gain access to the gelatinosa via the ventrally directed gelatinosa dendrites penetrating the n. proprius cornus dorsalis[9]. On the other hand, it appeared equally difficult to ascribe the presence of extremely tiny numbers of degenerated fibers in the gelatinosa as ending exclusively upon gelatinosa cells for this would then rest upon the demonstration that these fibers did not form synaptic junctions with the penetrating dendrites of nucleus proprius neurons[9] or Waldeyer's cells[9].

The current experiments were undertaken with the view of providing new data which overall might explain better the differences prevailing between the Nauta data, or its interpretation, and some of these results with the Golgi findings. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. Rhizotomies were performed in cats and rhesus monkeys. Cats were allowed to survive for 4, 7 and 8 days and monkeys for 1, 2, 3, 4, 5, 7, 8, 10, 13 and 14 days postoperatively. Under deep Nembutal anesthesia, the central nervous system was perfused with physiological saline followed by 10% formalin. Further fixation in formalin was continued by immersion in the fixative for at least 7 days. Horizontal, sagittal or transverse sections were cut above, through and below the lesioned segments. Sections were stained according to the methods of Nauta, Fink and Heimer and, when appropriate, counterstained for cell bodies. Additional companion sections were stained for cell bodies and myelinated fibers in order to facilitate the identification of spinal nuclei and correlate these with the pattern of fiber and bouton degeneration.

Dense fiber and bouton degeneration was found in the substantia gelatinosa of Rolando in sections treated according to the Fink and Heimer techniques and taken from the spinal cords of cats surviving 4 days, and monkeys surviving 3, 4, 5 and 7 days. A dense and rather plexiform arrangement of fiber and bouton degeneration predominates in approximately the dorsal half (Figure 2) of the

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