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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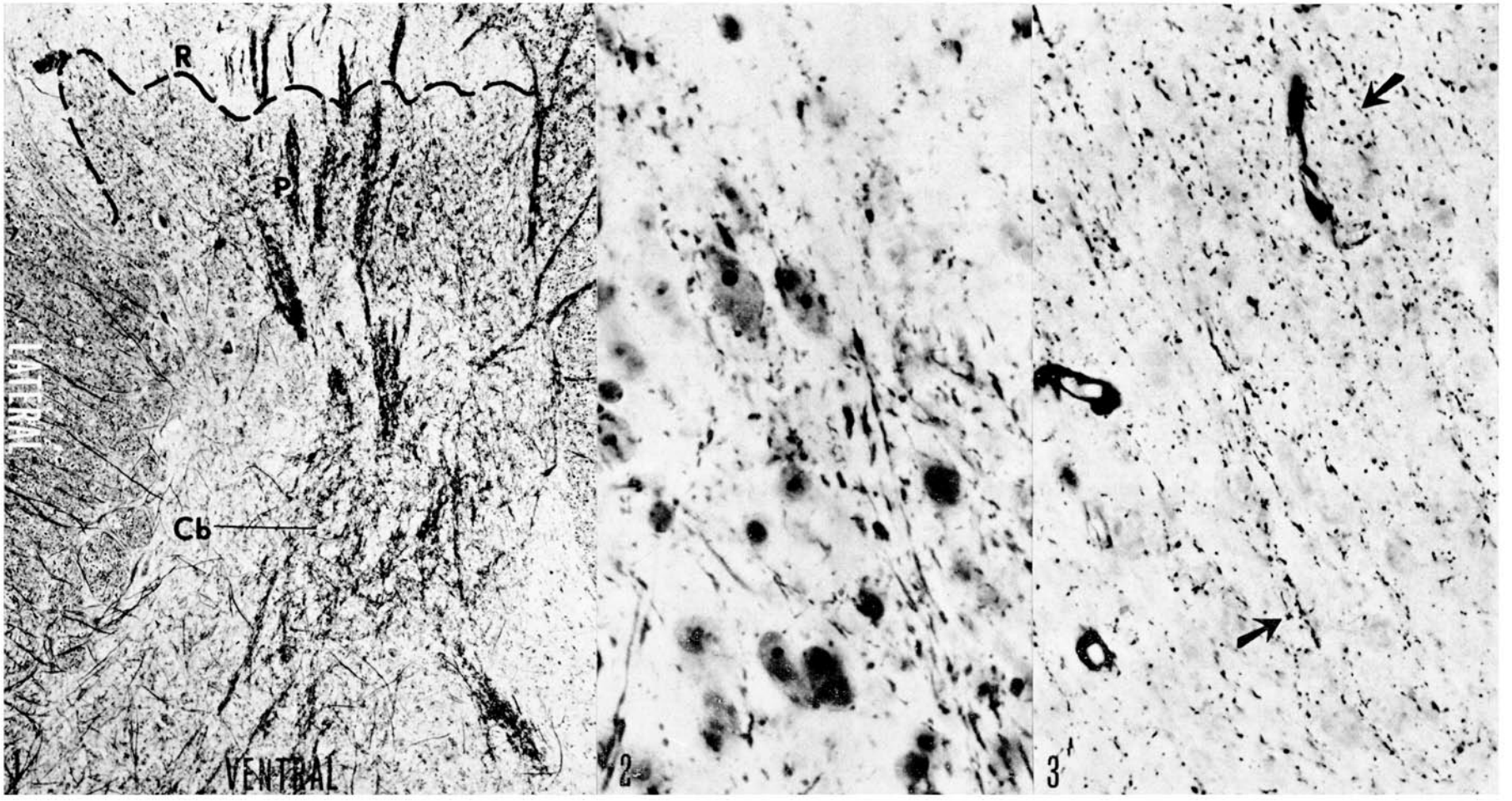

Figs. 1–3. Massive fiber degeneration (Figure 1) in the nucleus proprius cornus dorsalis (P) and centrobasilar region of the dorsal horn (Cb) revealed with the NAUTA method following dorsal rhizotomy in a cat surviving 8 days postoperatively. Fiber degeneration in the nucleus proprius follows extremely closely the border (approximated by the broken line) shared with the overlying substantia gelatinosa of Rolando (R). Terminal endings upon gelatinosa cell bodies and dendrites are not easily demonstrated in this case or in similar rhesus monkey cases. Abundant fiber and bouton degeneration upon gelatinosa cell bodies and dendrites are clearly demonstrated with the FINK and HEIMER methods (Figure 2, procedure I; Figure 3, procedure II) and are illustrated in the case of a rhesus monkey sustaining a rhizotomy for 3 postoperative days. Figures 2 and 3 are high-power photomicrographs of the substantia gelatinosa in this case. Vertical and lobularly arranged arbors predominate in the ventral part of the nucleus (Figure 2 and lower arrow in Figure 3), whereas, a somewhat more 'plexiform' arbor (upper arrow in Figure 3) is seen to predominate in the dorsal part of the same nucleus.

gelatinosa, whereas, a vertical disposition of fibers and boutons abounds (Figure 3) in the ventral half of the nucleus. Sparse terminal degeneration in the gelatinosa can also be demonstrated in these cases with the NAUTA method. It is important to recall that SZENTÁGOTHAI's and SPRAGUE and HA's report of gelatinosa afferents were made in cats surviving 4, and 3 and 5 days, respectively. Our present results are in accord with their findings. We have also seen in a few selected sections of the monkey, degenerated fibers coursing in close apposition to the penetrating dendrites of Waldeyer's and nucleus proprius cells. Comparable parallel studies of dorsal root degeneration have been performed recently in the rat and cat by HEIMER and WALL (personal communication). Their results also demonstrate dense bouton degeneration in the gelatinosa following the application of the FINK and HEIMER methods to the problem.

In summary, with appropriate selection of survival times and the application of the NAUTA, FINK and HEIMER methods, dorsal root terminal fibers and boutons can be demonstrated upon the perikarya and dendrites of gelatinosa neurons, and upon the penetrating dendrites belonging to Waldeyer's and nucleus proprius neurons. A richer terminal plexus is evident with the FINK and HEIMER methods. The present results serve to corroborate the GOLGI data, explain the diversity of findings using the same experimental method for degenerated fibers, and confirm and extend by further example the advantages afforded by use of the methods of FINK and HEIMER[10-12] for the study of fiber connections in the central nervous system[13].

Zusammenfassung. Es wird mit Hilfe von Degenerationsversuchen bei Katzen und Rhesusaffen gezeigt, dass Nervenfasern aus der dorsalen Wurzel an den Zellen der Substantia gelatinosa, des Dendriten auf Nucleus dorsalis und von Waldeyer in der Substantia gelatinosa enden.

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Clarification of the Osmiophilic Granules of the Rat Pinealocytes by *p*-Chlorophenylalanin

The mammalian epiphysis has long been suspected of performing an endocrine function (cf. review by WURTMANN and AXELROD, 1965), and many authors: BUSS and GUSEK[1], DE MARTINO et al.[2,3], PROP[4], for example, have tried by various experimental means to acquire morphological evidence that will confirm this supposition.

Our present purpose is to put forward the tentative results of our studies in favour of the hypothesis stated by DE MARTINO et al., who suspect the osmiophilic granules in the rat epiphysis of containing serotonin and melatonin. We also wish to suggest how these 2 substances could be secreted.

Adult albino rats are given 2 i.p. injections of 100 mg *p*-chlorophenylalanin (PCPA) at 24 h intervals. 24 h after the second injection the urethane-anaesthetized rats undergo i.v. glutaraldehyde-perfusion[5]. The epiphysis are post-fixed in osmium tetroxide and embedded in Epon[6]. Ultrathin sections are doubly stained with 2% uranyl acetate and with lead citrate[7].

The pinealocytes of control rats fixed in similar conditions contain osmiophilic granules that are either isolated or grouped in small clusters (Figure 1), and whose electron-density varies from black to grey. Several cisternae of smooth endoplasmic reticulum, as well as numerous polysomes were observed surrounding the granules. These are

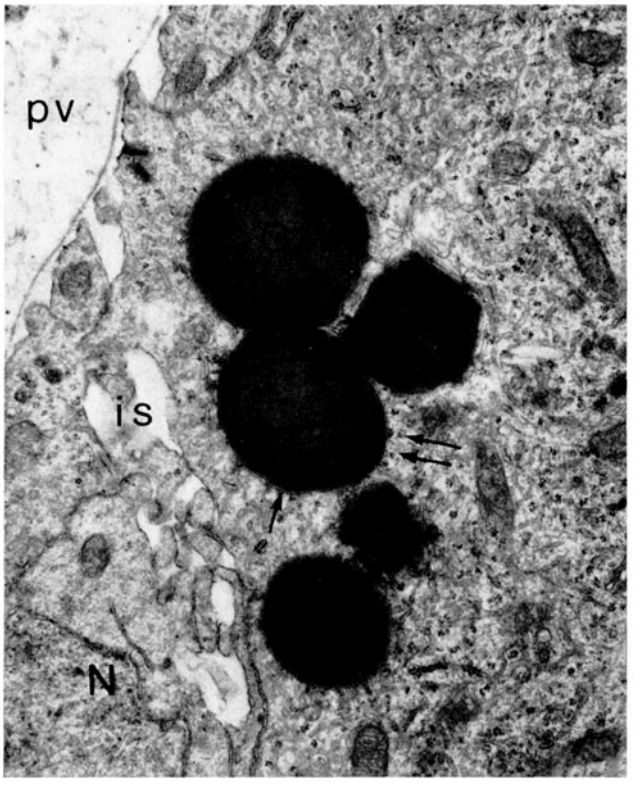

Fig. 1. Pinealocytes of a control rat, showing the characteristic appearance of osmiophilic granules (G). These are surrounded by both cisternae of smooth endoplasmic reticulum (↑) as well as by numerous polysomes (↑↑). The pinealocytes are separated from the perivascular space (pv) by a thin basement membrane. N, nucleus; is, intercellular cleft. × 11,900.

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