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 postfixed 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

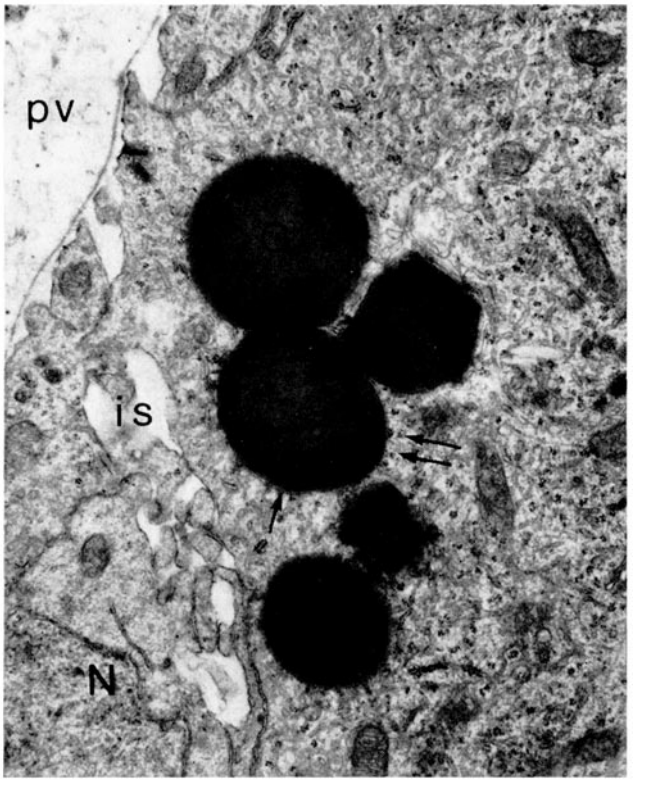

Fig. 1. Pinealocytes of a control rat, showing the characteristic appearance of osmiophilic granules (G). These are surrounded by both cisternae of smooth endoplasmic reticulum (↑) as well as by numerous polysomes (↑↑). The pinealocytes are separated from the perivascular space (pv) by a thin basement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

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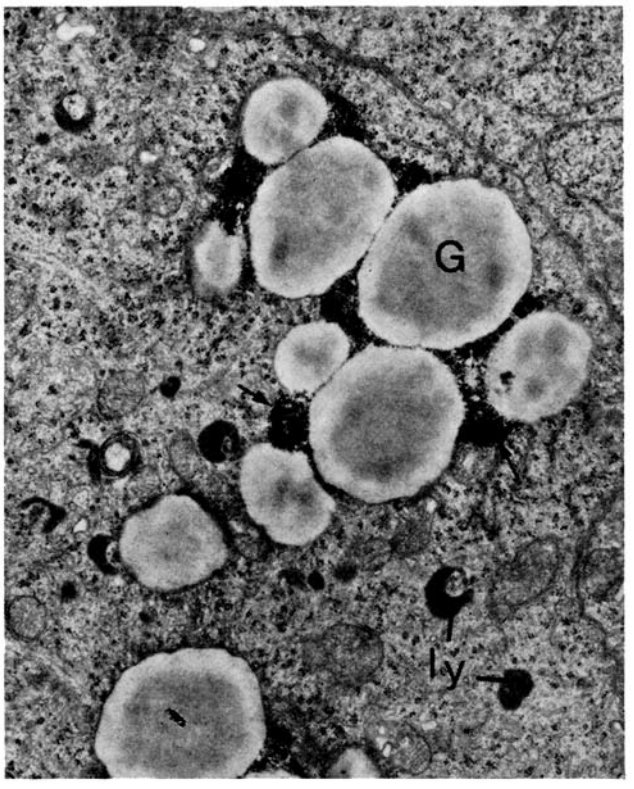

Fig. 2. Pinealocyte cytoplasm of a PCPA-treated rat. The granules (G) are noticeably clarified and several primary lysosomes (ly) can be seen in their vicinity. A dense granular substance is found between the clear granules (↑). × 11,400.

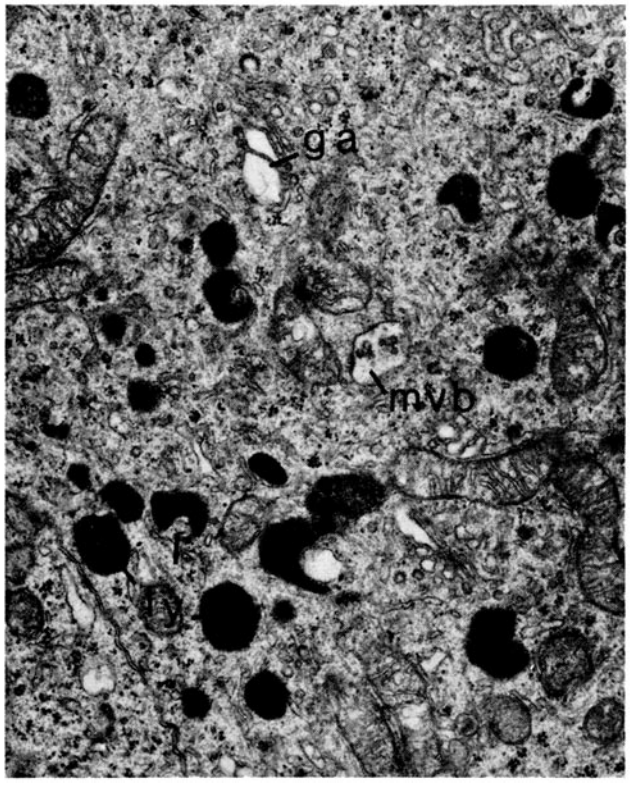

Fig. 3. PCPA-treated rat. Small area of pinealocyte cytoplasm containing a particularly large number of primary lysosomes (ly). ga, Golgi apparatus; mvb, multivesicular body. × 18,000.

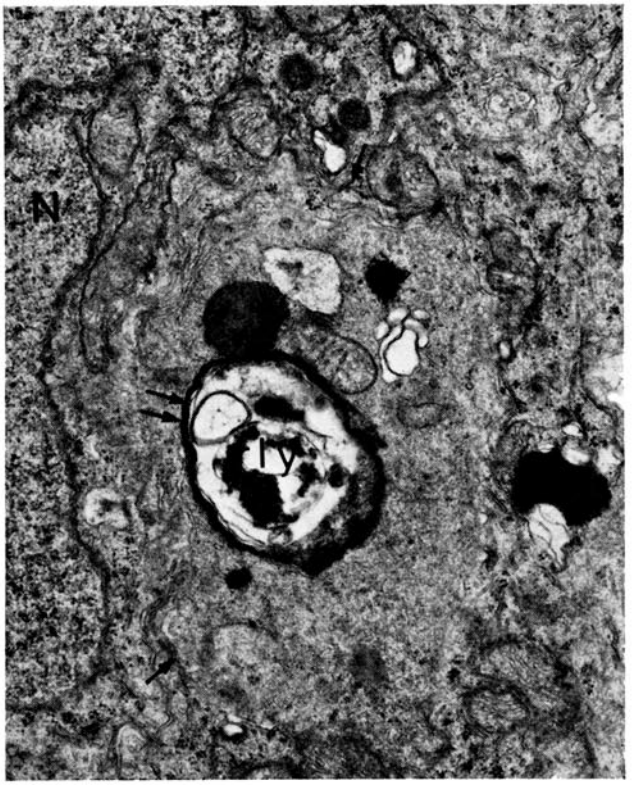

Fig. 4. PCPA-treated rat. Pinealocyte cytoplasm showing an area of focal degenerescence. In this area bound by several layers of membrane (↑), the cytoplasm is characterized by its fibrillar appearance and by its lack of ribosomes. In the centre of this area lies a residual body (rly) also surrounded by several tiers of membrane (↑↑). N, nucleus. ×18,000.

not bound by a unit membrane. The pinealocyte cytoplasm also contains primary lysosomes that are often in close proximity to the granules.

After PCPA-treatment the granules in the pinealocytes no longer have the same appearance. Their opacity is so greatly reduced as to make them electron-lucent, particularly at their periphery (Figure 2). Moreover a dense granular substance, the nature and importance of which remains as yet unknown, can be seen between the cleared granules.

A second characteristic change in the pinealocytes of PCPA-treated rats consists of an increase in the number of primary lysosomes (Figures 2 and 3). These are generally observed near the clarified granules with which they are sometimes in direct contact. The cytoplasm of several pinealocytes shows areas of focal degenerescence, and contains residual bodies whose structure reminds us of granules in the process of digestion (Figure 4).

PCPA is known to inhibit the tryptophane-hydroxylase[8] and consequently serotonin synthesis as well. The production of melatonin too must be considerably reduced, since serotonin is necessary for its synthesis[9,10].

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The clarification of the granules after PCPA-treatment therefore supports the hypothesis that they contain melatonin and serotonin, both known for their osmium affinity[2].

The fact that this clarification is accompanied by an increase in the number of primary lysosomes can be interpreted in several ways: (a) under normal conditions, lysosomes might be involved in the discharge (secretion?) of indolamines from osmiophilic granules where they are stored[11]. Under influence of PCPA, which prevents synthesis of new amines, the stock would be heavily sollicited (clarification of the granules) and the lysosomes therefore in great demand: (b) the increase in lysosomes might be in connection with the areas of focal degenerescence found in several pinealocytes, and may be related to the formation of residual bodies; (c) the increase in lysosomes may be a non-specific reaction to PCPA[12–14].

Résumé. L'administration de PCPA, un inhibiteur de la synthèse de la sérotonine et de la mélatonine, entraîne la clarification des granules osmiophiles de l'épiphyse du rat blanc. Cette observation est en faveur de l'hypothèse selon laquelle la sérotonine et la mélatonine sont des constituants des granules osmiophiles. De plus, plusieurs hypothèses sont proposées pour expliquer l'augmentation des lysosomes primaires constatée parallèlement à la clarification des granules.

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[11] Lysosomes were shown to play a similar part in a 'secretory process' by Wetzel et al. in their study of the colloid resorption in follicular thyroid cells under the influence of TSH[12].

[12] B. K. Wetzel, S. S. Spicer and S. H. Wollman, J. Cell Biol. *25*, 593 (1965).

[13] We are greatly indebted to Dr. W. G. Forssmann for having suggested the use of PCPA, and for providing us with material from his personal experiments.

[14] This work was supported by a grant from the Fonds National Suisse de la Recherche scientifique.

A New Class of Cytostatic Folic Acid Antagonists 1-(3,4-Dichlorophenyl)-5-isopropyl-biguanide and its Boron Compounds

1-(3,4-Dichlorophenyl)-5-isopropyl-biguanide-hydrochloride (Chlorguanil, I) has been found to possess a high antimalarial activity[1,2]. With the following experimental and clinical work we were able to demonstrate the species dependence of the effect of I on hematopoiesis and tumours.

Effect on hematopoiesis. (a) Mouse, rat, rabbit and dog. The highest tolerated doses of 10 mg/kg daily i.p. or 20 mg/kg daily orally had no influence on the hematopoiesis of mice and rats. Rabbits even tolerated doses of 50 mg/kg daily orally for 20 days without developing leucopenia. The dog's hematopoiesis, too, is resistant to I[3]. (b) Guinea-pig and cat. The hematopoiesis of the guinea-pig is very sensitive to I. Guinea-pigs weighing 220–250 g were given daily doses of I in aqueous solution either by the i.p. or the oral route. Groups of 5 animals were used for each dose. The number of leucocytes was determined daily or every other day. The results of the effect of I in different doses on the number of leucocytes in the peripheral blood can be seen from the Table. A dose of 6 mg/kg daily i.p. leads within 7 days to a rapid fall of the leucocytes from a mean value of 6800 to 700 leucocytes/mm³ blood. In lower doses the leucopenia is less marked. In further investigations it was observed that granulopoiesis as well as lymphopoiesis is depressed. Schärer[3] also found that the cat showed a very sensitive response to the administration of I. (c) Man. Patients with neoplastic diseases were given I i.v. or orally. Doses of 1–2 mg/kg daily resulted in marked leucopenia and thrombocytopenia after 1–3 weeks[4].

Effect on tumours. (a) Mouse. The following transplantable tumours were used: Ehrlich carcinoma, solid form, Ehrlich ascites carcinoma and Crocker sarcoma S 180. The maximum tolerated daily doses of 10 mg/kg i.p. or 20 mg/kg orally given from the first day after implantation did not inhibit the growth of these tumours. (b) Rat. 2 rat tumours were used: Walker carcinosarcoma 256 and uterus epithelioma (Guérin) T 8. With the same dose schedule as that administered to mice, the rat tumours were not influenced. (c) Man. In preliminary clinical work[4] it was found possible to achieve some temporary objective remissions in Hodgkins' disease, lymphosarcoma, reticulosarcoma, pleural endothelioma and bronchial carcinoma. An optimal dosage schedule has not yet been worked out.

Mechanism of action. The leucopenia-inducing effect of I in guinea-pigs can be antagonized by formyl tetrahydrofolic acid (THFA)[5]. Not only the bone marrow depression but also the loss of weight and the lethality are reduced or prevented by the prophylactic use of formyl tetrahydrofolic acid. Groups of 5 guinea-pigs were given 6 mg/kg I daily i.p. without or with the addition of different doses of THFA. A dose of 6 mg/kg I daily for 6 days results in the above mentioned leucopenia, in a rapid weight loss and in the death of all animals within 6–9 days. If 12 mg/kg THFA is given i.p. daily 30 min before the administration

Effect of I on the leucocytes in the peripheral blood of guinea-pigs

Daily dose I mg/kg i.p.	Leucocytes			
	Day 1	Day 5	Day 7	Day 14
0.5	5400	3700	3900	4600
1	6500	3600	3700	3900
2	9000	3100	3400	3100
4	6800	3400	1900	No survivors
6	6800	1200	700	No survivors

Each value is the mean of 5 guinea-pigs.

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[4] W. Bollag, unpublished results.

[5] Formyl tetrahydrofolic acid = Leucovorin® (Lederle).