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 und Institut für vergleichende Neurologie der Universität Bern

Thalamic Melanosis in Goats¹

by G. Kaliner², K. Frese³, R. Fatzer⁴ and R. Fankhauser⁴

Fankhauser (1963) described melanosis in the left thalamus of a young goat (freemartin). This is the only recorded case of melanosis in the brain of a domestic animal. Five similar cases are recorded in this paper. Goat 1 was an experimental animal of the Veterinary Research Laboratory, Kabete, goats 2–5 were submitted to the Institut für vergleichende Neurologie, Bern. The electron microscopic examinations were performed at the Veterinär-pathologisches Institut in Giessen.

Material and methods

Goat 1: The tissues were from an adult, white male which died six weeks after artificial infection with *Trypanosoma evansi*. Parts of the lungs, myocardium, liver, kidney, spleen, lymph nodes, intestine and the entire brain were fixed in 10% buffered formol-saline. Parablast sections were stained with haematoxylin-eosin; other methods employed are listed in table 1. Some tissue of the thalamus was submitted for electron microscopic (EM) examination after prefixation in formalin for several months. After washing in 0.1 M cacodylatebuffer, pH 7.2 for 24 hours and postfixation in 1% OsO₄ for 2 hours the 1 mm³ tissue blocks were embedded in Durcupan®. Thin sections were examined in a Siemens Elmiskop 101 at 80 Kv.

Goat 2: The right half of the brain of an approximately three year old female of the Toggenburg breed was submitted for histological examination. The left half had been used for rabies diagnosis. Several blocks including the lesion were embedded in paraffin, and 4 μ sections were stained with H & E. Unstained sections were sent to the Kabete Laboratory and the first five methods mentioned in table 1 were applied to this material.

Goat 3: The left half of the brain of a six year old female of the Brienz breed was submitted for histological examination, whereas the other half was again used for rabies diagnosis. The gross sagittal section of the brain was made paramedial, the medial part of the right thalamus was therefore also available

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Table 1 Summary of staining and histochemical results

| Method | Result |
|---|----------------------------------|
| Bleaching in conc. hydrogen peroxide | complete bleaching after 72 hrs. |
| Nile blue technique (Lillie, 1956) | green |
| Masson-Fontana (Pearse, 1960) | black after 18 hrs. |
| Fe ⁺⁺ ion uptake reaction (Lillie, 1965) | green |
| PAS | negative |
| Cone. sulfuric acid | insoluble, yellow |
| Ziehl-Neelsen (180 min.) | negative |
| Prussian blue | negative |
| Von Kossa | negative |
| Gram | negative |
| Sudan black B (on frozen section) | negative |
| Oil red O (on frozen section) | negative |

for examination. Several blocks were embedded in paraffin, and 4 μ sections were stained with H & E. Unstained paraffin sections were sent to the Kabete Laboratory, where the first five methods listed in table 1 were applied to them.

Goat 4: A 4 weeks old male kid of the Brienz breed was autopsied at the Department of Animal Pathology. The brain was removed and examined macroscopically and with conventional microscopy as a routine procedure.

Goat 5: A 5 days old male kid of the Brienz breed was autopsied at the Department of Animal Pathology and the brain was removed and examined macroscopically and microscopically as a routine procedure.

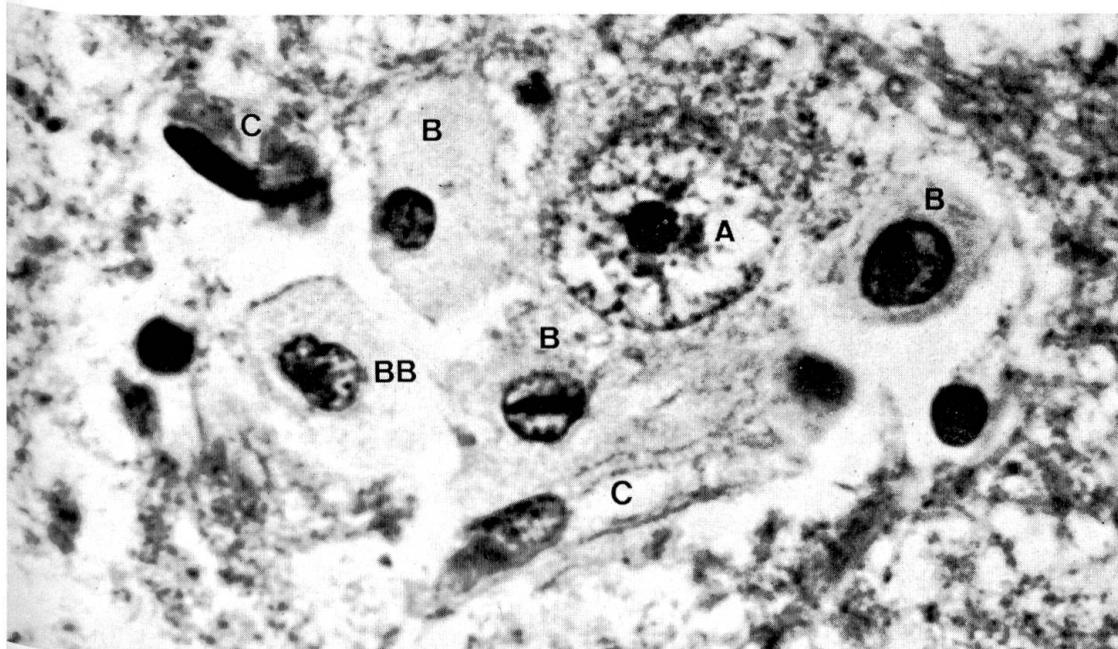
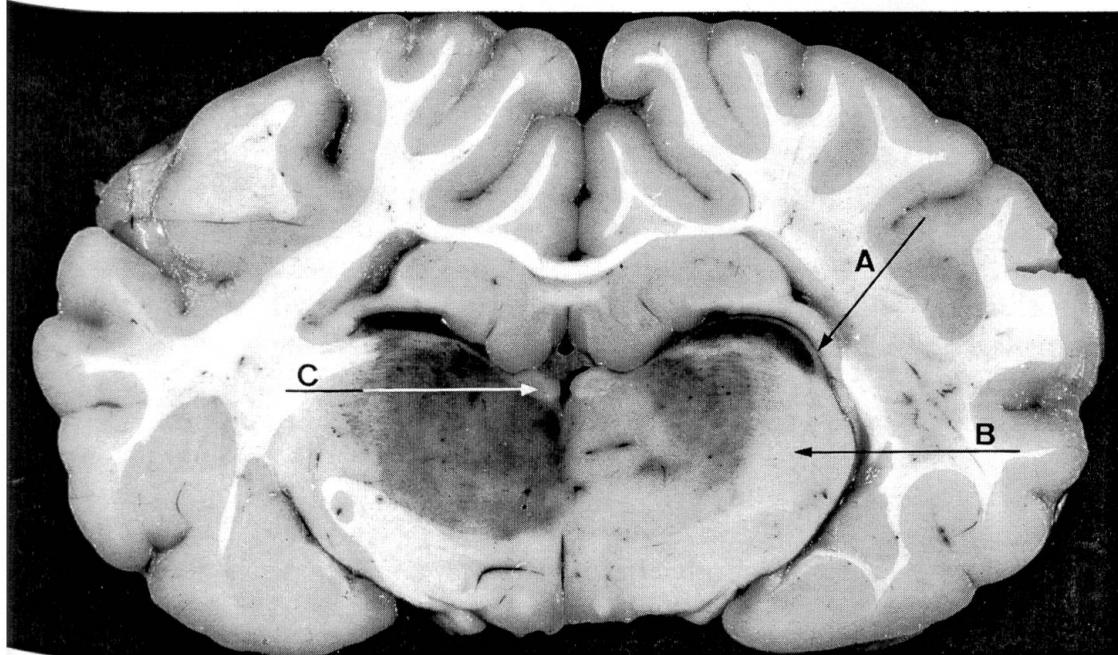
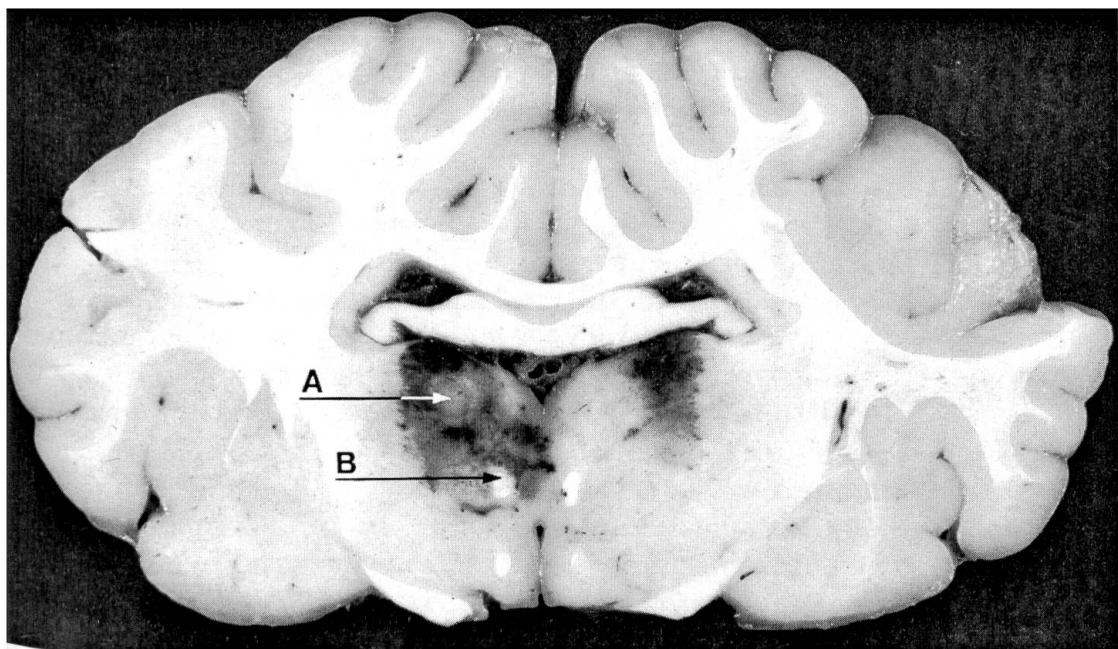
Results

Clinical history: Goat 1 did not show any nervous disorder during the six weeks observation period. Goat 2 had died on pasture in a rabies enzootic area, and because there was some suspicion of a fox bite about three weeks previously, the head was submitted for rabies examination. No neurological signs were mentioned. For goat 3 the following clinical signs were reported: "The animal was unable to masticate food properly, later there was complete inappetence, salivation, slight tympany, paresis of the hind legs, a persistent husky bleat; respiration was slightly accelerated, and pulse and temperature were normal. A tentative diagnosis of rabies was made."

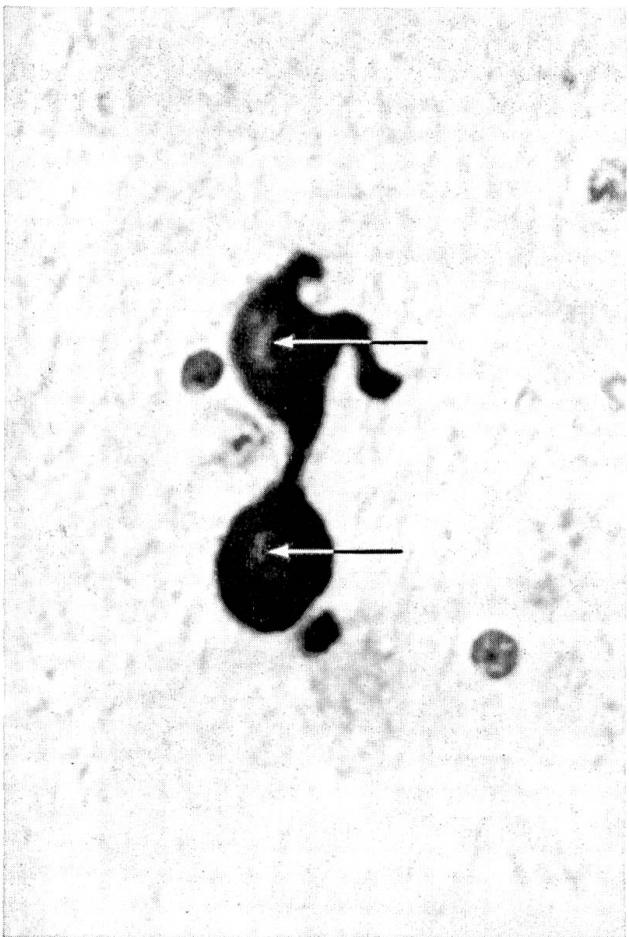
Fig. 1 Cut surface through the melanotic brain of goat 1. A = Nucleus anterior ventralis; B = Fasciculus mamillo-thalamicus.

Fig. 2 Similar to fig. 1. A = Part of the tractus opticus; B = Part of the cortico-thalamic and thalamo-cortical tracts; G = Ganglion habenulae.

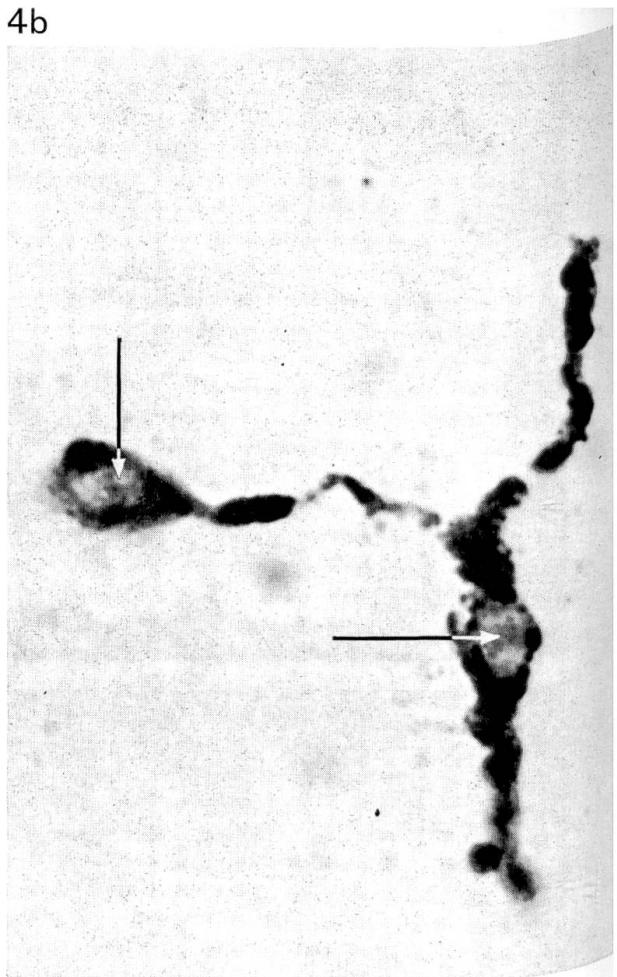
Fig. 3 Thalamus. A = Neuron; B = Incompletely bleached pigment cells; BB = Pigment cell with double nucleus; C = Capillary. H & E, 1000 \times .



4a



4b



4c



4d

Rabies diagnosis: Various tests for rabies on material of goats 2 and 3 gave negative results.

Animal no. 4 was sent for postmortem examination because several young kids had died previously on the same farm. In this case as well as for animal no. 5 which died on its fifth day of life, no clinical history was available.

Gross pathology: In goat 1 an extensive red to greyish hepatisation of the lung was found to be caused by *Pasteurella haemolytica* and *Mycoplasma sp.* Goat 4 had a squamous eczema of the head, and *Demodex sp.* was identified; postmortem findings were inconclusive, and bacteriological examination negative. In goat 5, a diagnosis of septicaemia due to pneumococci was established on morphological and bacteriological grounds. On examining the brain of goat 1, irregularly shaped and distributed areas of the basal leptomeninges were found to be smoky grey to black in colour. During slicing of the brain, an irregularly distributed and intense blackening of both thalami was observed. The left thalamus was more severely involved (figs. 1 and 2). The blackening decreased dorso-ventrally. Whilst no changes could be found in the area of the nucleus anterior ventralis dexter, a moderate blackening of the left nucleus was seen.

In goats 2 and 3 a similar blackening was observed. In goat 2 it was located in the dorsal half of the available right thalamus. It was most marked in the lateral part adjoining the internal capsule, and in a narrow medial part next to the habenular area which itself was spared. In between, there was only a slight greyish discolouration. In goat 3, the affected area of the (available) left thalamus was the same as in goat 2, but the distribution of strongly or less pigmented parts was more irregular. On one level, the pigmentation crossed the midline, but only by a few millimeters. In animal no. 4, the whole dorsal thalamus, pulvinar and lateral geniculate body of the right side were rather evenly pigmented, with the exception of a small dorso-medial area adjoining the dorsal part of the third ventricle. There was also a small blackened area in the left geniculate body. In animal no. 5, a rather even, slate-grey discolouration extended bilaterally from the very rostral end of the thalamus throughout its dorsal half to the pulvinar and lateral geniculate bodies, and faded away in the border area towards the anterior colliculi.

In each animal the ganglion habenulae, tractus-mamillothalamicus (Vicq d'Azyr's tract), brachium colliculi superioris, the fibrils of the cortico-thalamic and thalamo-cortical tracts and fibrils coursing into the tractus opticus appeared to be unchanged. No change in colour was observed in the hypothalamus.

Histopathology: Yellow, dark brown, rarely black, fine to coarse granules were found intracellularly in the thalami. The staining and histochemical reac-

Fig. 4 a = Cytoplasmic process of a pigment cell appears to merge in the other cell. Arrow = Nucleus. ZN, 400 \times .

b = Similar to fig. 4a. Fe⁺⁺ ion uptake reaction, 400 \times .

c = Dendritic pigmented cell. Masson-Fontana, 400 \times .

d = Adventitial pigmented cell, of which one cytoplasmic process incompletely encircles a neuron. Arrow = Neuron, Star = Capillary. Masson-Fontana, 160 \times .

tions of the pigment are listed in table 1. Pigmented cells were oval (fig. 4a) or dendritic (fig. 4c) with cytoplasmic processes of varying length and diameter. In completely bleached sections the cytoplasm of the pigment cells was not stained by eosin. Some pigmented cells possessed indented or double nuclei (fig. 3). Rarely, cells were seen where the pigmented cytoplasmic processes appeared to merge into another pigment cell (figs. 4a and 4b). Pigment cells were found in the adventitia of small blood vessels or like satellite cells around neurons; in their vicinity capillaries were sometimes seen (fig. 3). Single pigmented cytoplasmic processes of adventitial pigment cells of goat 1 incompletely encircled adjacent ganglion cells (fig. 4d). Some pigment cells were found among glial cells of the fasciculi. The distribution of the pigmented cells seems to follow mainly the grey (nerve cell containing) substance (fig. 5). There, many dendritic cells can be seen in a perineuronal position (fig. 6). With Maurer's method, astrocytes are shown to be free of pigment (fig. 7). The leptomeninx above the thalamus was severely pigmented and the adventitia of leptomeningeal blood vessels penetrating into the thalamus, possessed numerous pigment cells. Ependymal cells, the hypothalamus and other organs examined were free of pigment.

Electron microscopy: Despite of the insufficient fixation, two types of pigment cells can be distinguished:

1. Large melanocytes with an indented nucleus and numerous melanosomes distributed diffusely in the cytoplasm (fig. 8). In longitudinal sections the melanosomes are oval and often seem to be membrane-bound. Besides melanosomes, premelanosomes of different maturation can be seen. In longitudinal sections they show lamellae running parallel to the long axis. In cross sections both lamellar or spiral and granular internal structures are visible (fig. 9). Usually the premelanosomes are surrounded by a membrane.

2. Large cells which contain more or less numerous compound melanosomes. Single melanosomes occurring in these cells usually can be found only in lysosomes (fig. 10). These cells are observed especially in the vicinity of small blood vessels. Because of the lack of any melanogenic activity they are supposed to be melanophages.

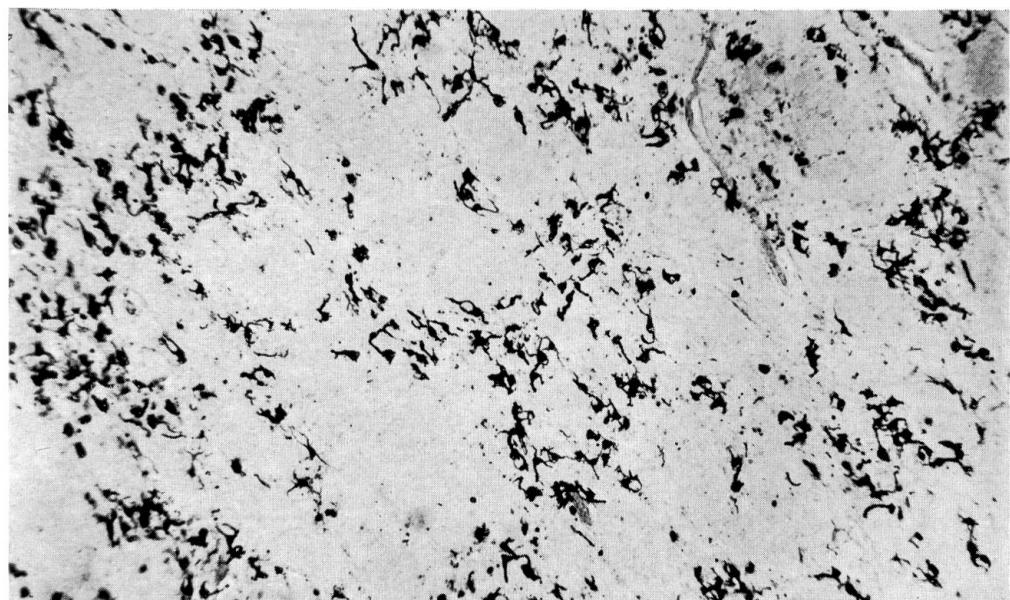
Discussion

Melanin in the brain has to be distinguished from lipofuscin and haematin (Marsden, 1969), although some investigators think that lipofuscin and neuromelanin are closely related (Singer et al., 1974). Haematin appears as crystals (McManus and Mowry, 1960) and lipofuscin has different staining and

Fig. 5 Distribution of the pigment cells along strands of grey matter. Luxol fast blue-cresyl violet, $75\times$.

Fig. 6 Detail of the same area as in fig. 5. Many pigment cells in satellite positions. Luxol fast blue-cresyl violet, $210\times$.

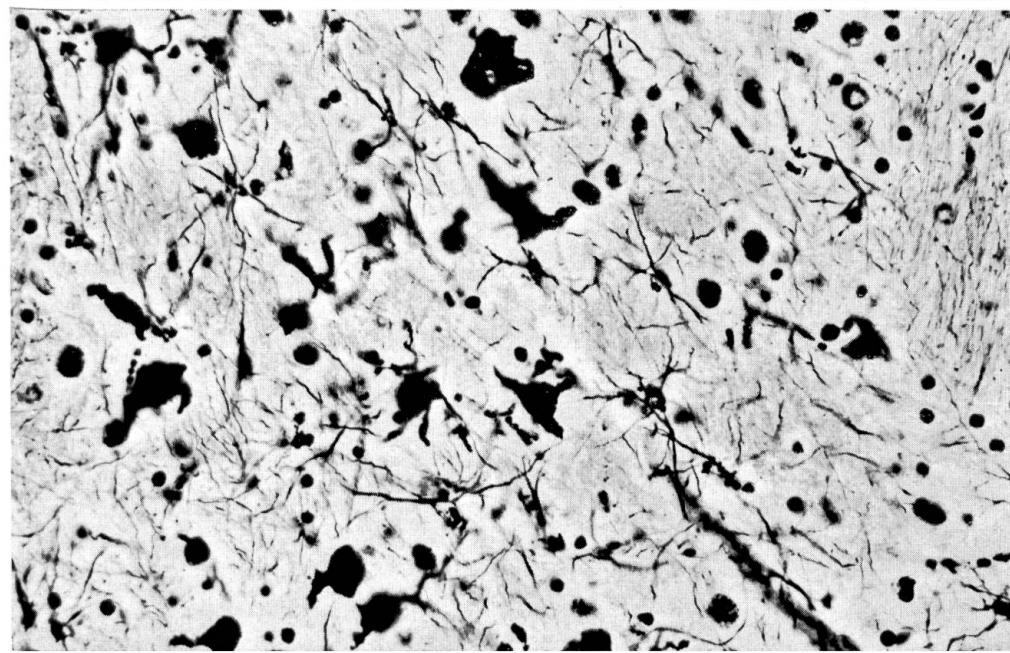
Fig. 7 Normal astrocytes of grey matter between pigment cells. Maurer impregnation, $360\times$. All magnifications refer to the original photographs.



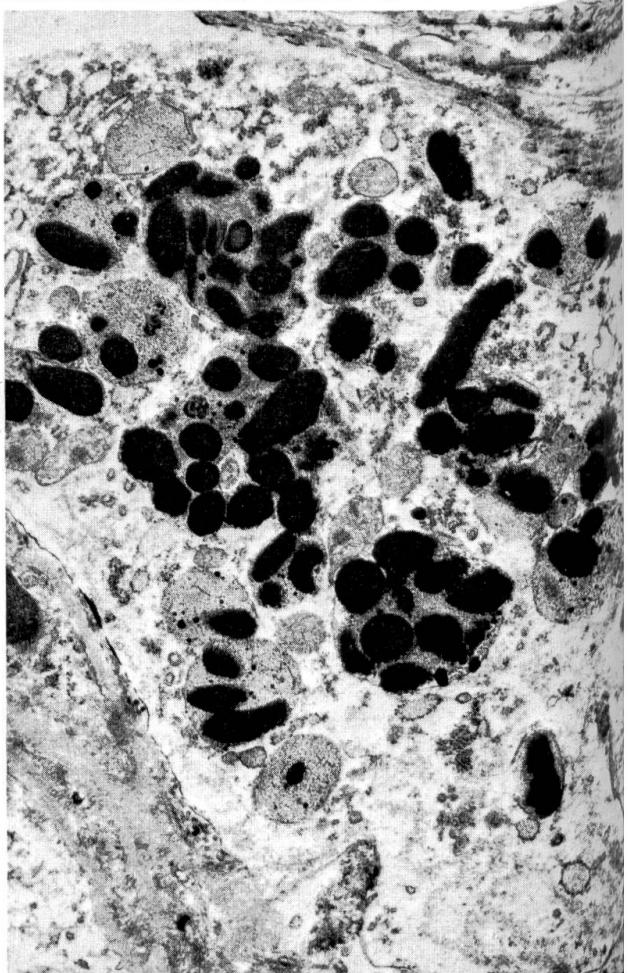
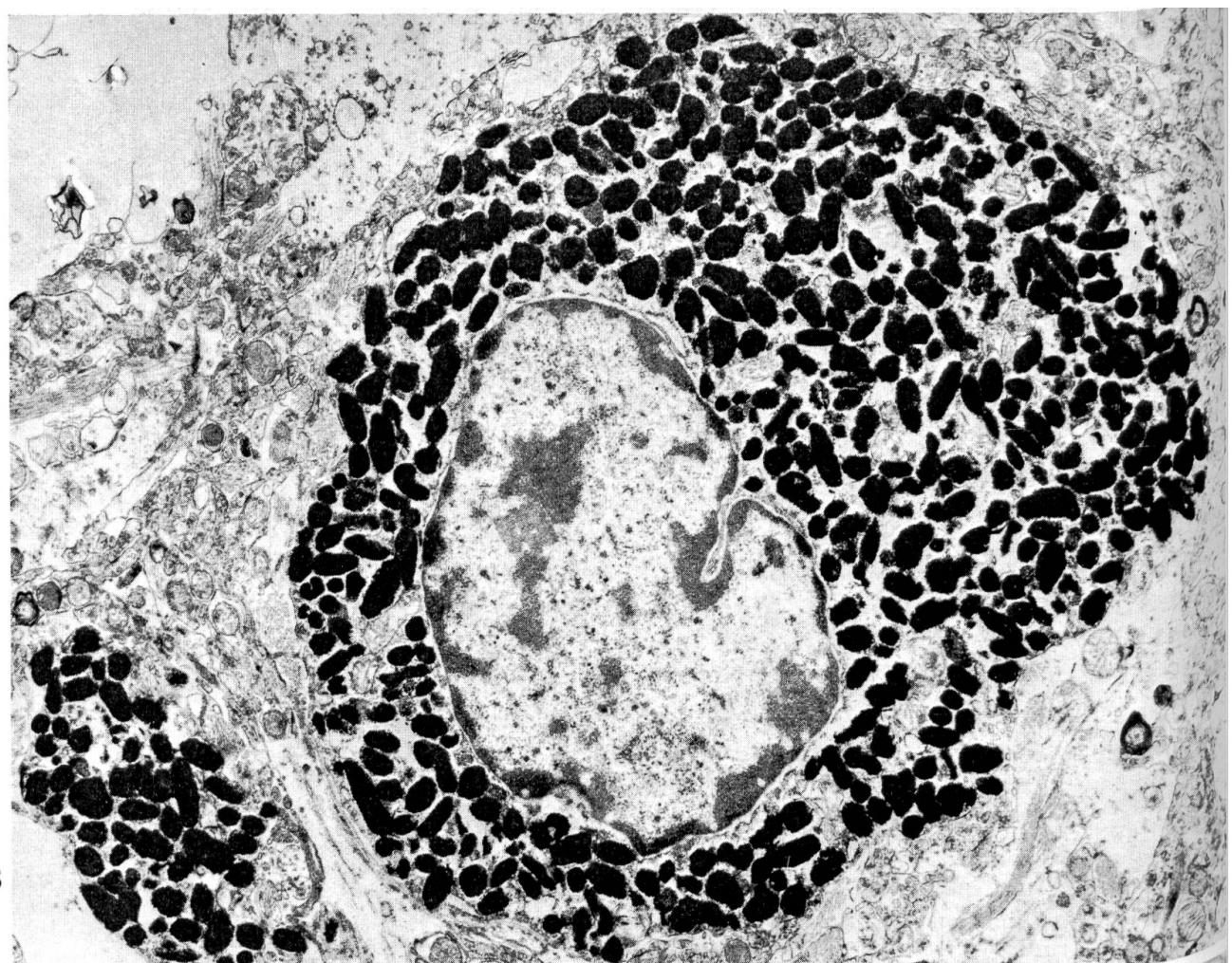
5



6



7



histochemical reactions (Marsden, 1969) from the pigment of these cases. The pigment seen in the thalamus of goats was therefore regarded as melanin. The EM evidence of melanosomes and of the premelanosomes with a typical lamellar inner structure proved the pigment to be melanin. Other pigments (e.g. lipofuscin) do not show this appearance.

Macroscopically, the distribution of the pigmented areas in the thalamus is similar as in the case of Fankhauser (1963).

The location of pigment cells around blood vessels of the thalamus is comparable with Luginbühl's finding (1962), of a melanosis of the blood vessels in the spinal cord of a sheep.

In goat 1 the cytoplasmic processes of adventitial pigment cells passed from capillaries to neurons and incompletely encircled these cells. Fankhauser (1963) using special stains described the cytoplasmic processes of pigment cells of the neuropil passing in the opposite manner. The latter is a morphological behaviour of glia which results in the formation of the *membrana limitans gliae perivascularis*. According to the light microscopic examination, the adventitial pigment cells might be regarded as melanocytes or melanophages; but the pigment cells around ganglion cells behaved topographically like satellite cells (oligodendroglia). The light microscopic morphology of the pigment cells described does not allow a classification as melanocytes or glial cells, since both types of cells are or could be dendritic. The EM examination, however, showed that the cells with diffuse, non-lysosomal-bound melanosomes were pigment producing melanocytes. The other cells with lysosomal-bound melanosomes (compound melanosomes) were macrophages (melanophages). In a case of melanosis of the dentate nucleus in a 62 year old black man, Singer et al. (1974) found the pigment to be predominantly extraneuronal, partly associated with glia, partly in macrophages, but mainly free in the parenchyma. Melanocytes were not present, nor was the pigment in a perivascular position. Therefore, this type of melanosis seems to be different from the one described here.

During embryonic development, melanoblasts migrate from the neural crest to their place of determination (e.g. dermis, eye). Fankhauser (1963) suggested a dysontogenetic development as a possible explanation for the occurrence of melanin in the thalamus (diencephalon), since the optic vesicles originate from the lateral part of the diencephalon.

Our former report (Fankhauser, 1963) is losing some of its uniqueness. The 4 cases of the Bern material from brown coated animals were collected within a one year period. During these 12 months, only 24 goat brains have been examined. Eight of them were dwarf goats, and in none of them thalamic mela-

Fig. 8 Thalamic melanocyte with numerous melanosomes distributed diffusely in the cytoplasm. EM-No. 9126; 13,000 \times .

Fig. 9 Closer view of melanosomes (Σ) and membrane-bound premelanosomes with lamellar or granular internal structure (arrows). EM-No. 9127; 62,000 \times .

Fig. 10 Numerous compound melanosomes in a thalamic melanophag. EM-No. 9119; 21,000 \times .

nosis was observed. Therefore, the 4 observations correspond to 25% of the other goats examined. At Kabete, of approximately 300 goat brains of many breeds examined, the single case shows that the abnormality is not restricted to coloured breeds and goat 5, aged only five days, clearly demonstrates the congenital nature of the disorder. A survey of larger numbers of brains of the Toggenburg and Brienz breeds, as well as embryological studies concerning their early brain development, would be necessary for a better understanding of this phenomenon not reported as yet in other species. Furthermore biochemical, histochemical and electron microscopical investigations of goat brains could possibly be helpful in clearing up the role of catecholamines in the synthesis of brain melanin which is not fully understood and which differs from the pathway of skin melanogenesis (Marsden, 1969).

Summary

A melanosis of thalami in 3 adult and 2 young goats is described. The oval to dendritic pigment cells were found in the adventitia of blood vessels and around neurons. The electron microscopic examination revealed that the pigmented cells were melanocytes and macrophages (melanophages).

Zusammenfassung

Eine Melanose der Thalami bei 3 adulten und 2 jungen Ziegen wird beschrieben. Die ovalen bis dendritischen Pigmentzellen lagen in der Adventitia von Blutgefäßen und perineuronal. Die elektronenmikroskopische Untersuchung zeigte, dass es sich einerseits um Melanozyten und andererseits um Makrophagen (Melanophagen) handelte.

Résumé

Une mélanose de la couche optique chez 3 chèvres adultes et 2 chevreaux est décrite. Les cellules pigmentées, ovalaires ou ramifiées, se trouvent dans l'adventice des vaisseaux sanguins et autour de neurones. L'examen ultrastructural a permis de les identifier comme mélanocytes et macrophages (mélanoctytes).

Riassunto

Viene illustrata una melanosi del talamo osservata in 3 capre adulte ed in 2 capretti. Le cellule pigmentate, ovalari o ramificate, sono state trovate nell'avventizia dei vasi sanguigni ed intorno ai neuroni. L'esame ultrastrutturale ha permesso di rilevare che le cellule pigmentate erano melanociti e macrofagi (melanofagi).

Acknowledgments

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BUCHBESPRECHUNG

Frontiers in Comparative Medicine. Von W. I. B. Beveridge. Vol. 1 der Wesley W. Spink Lectures on Comparative Medicine. University of Minnesota Press, Minneapolis, 1972. 104 Seiten, Dollar 4.75.

Das nach Umfang kleine Werk entstand aus einer Reihe von Gastvorlesungen an der University of Minnesota. Diese, zum ehrenden Andenken an Dr. W. W. Spink geplanten, zweijährlichen, und durch Prof. Beveridge eingeleiteten Zyklen sind dem weiten Gebiet der vergleichenden Medizin gewidmet. Dr. Spink war ein hervorragender Vertreter der komparativen Arbeits- und Denkrichtung und hat sich - als humanmedizinischer Internist - vor allem um die Erforschung der Tier-Mensch-Zusammenhänge bei der Brucellose verdient gemacht. Von ihm stammt die Monographie «The Nature of Brucellosis», der auch K. F. Meyer höchste Anerkennung zollte.

Prof. Beveridge von der University of Cambridge, zugleich Consultant an der Division of Communicable Diseases der Weltgesundheitsorganisation in Genf war in hervorragender Weise qualifiziert, diesen Zyklus zu eröffnen. Als Mikrobiologe und Forscher auf dem Gebiet tierischer, insbesondere virusbedingter Infektionskrankheiten braucht er hier nicht weiter vorgestellt zu werden. Der vorliegende Band tut dies in einem ganz kurzen Anhang (About the Author).

Die Darlegung ist in drei Kapitel gegliedert, wobei das erste wohl nicht zufällig - Prof. Beveridge ist Autor des Buches «The Art of Scientific Investigation» - der Philosophie der vergleichenden Methode gewidmet ist, als deren Hauptwerkzeug die Analogie diskutiert wird. («Der Wert der Analogien liegt in ihrer Suggestivität, nicht darin, dass sie irgendetwas beweisen würden.») Vielleicht hätte der andere Pol, nämlich die bedeutsamen Unterschiede - die sich gerade in der vergleichenden und vor allem klinischen Neurologie aufdrängen - auch eine Erwähnung verdient. Nach einigen Beispielen, wo die Forschung am Tier (mit veterinär-medizinischer Zielsetzung) zu grundsätzlichen und pionierhaften Erkenntnissen für die allgemeine Medizin führte - im wesentlichen auf dem Gebiet der Infektionskrankheiten -, werden gegenwärtige Arbeitsrichtungen und Ausblicke in die Zukunft besprochen: Krebs, Herz- und Kreislaufkrankheiten, Neuropathologie, angeborene Missbildungen, Umweltverschmutzung und Bevölkerungswachstum, psychische und geistige Hygiene. Interessanter- und bezeichnenderweise für den weitgespannten Horizont des Autors wird diesem letztgenannten Problemkreis für die Zukunft überragende Bedeutung beigemessen. Über das vom Autor zur unbezweifbaren biologischen Verwandtschaft (die notwendigerweise auch das Psychische einschliesst) von Mensch und Tier Gesagte hinaus fühlt sich gerade der neurologisch Tätige dazu gedrängt,