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The Pathology of Radicular Involvement in Angiostrongylosis as Observed in Experimentally Infected Calves and Pigs*

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Die Pathologie des Nervenwurzelbefalles bei experimenteller Angiostrongylosis in Kälbern und Schweinen

Zusammenfassung. 5 Kälber und 5 Schweine wurden mit *Angiostrongylus cantonensis* infiziert, 3, 7, 14, 28 und 56 Tage nach der Infektion getötet und histologisch untersucht.

Alle Kälber, dagegen nur ein Schwein, zeigten morphologische Zeichen der Gehirnerkrankung.

3 Tage nach der Infektion wurden vereinzelt Larven und kleine Blutungen im Gehirn eines Kalbes gefunden. Nach 7, 14 und 28 Tagen waren zahlreiche Parasiten, perivasculäre Infiltrate und Granulome in Gehirn und Rückenmark zu beobachten. 56 Tage nach der Infektion waren nur noch vereinzelte perivasculäre Infiltrate nachweisbar.

7, 14 und 28 Tage nach der Infektion traten in der Umgebung mehrerer Rückenmarkwurzeln epidurale, intradurale und vereinzelt auch subdurale Entzündungsinfiltrate auf. Gleichzeitig fanden sich Parasiten unter der anliegenden Arachnoidea oder Pia.

Diese periradikulären Infiltrate werden als Immunitätsreaktion auf die von den Parasiten in den cerebrospinalen Liquor abgegebenen und entlang der Nervenwurzeln abfließenden Antigene betrachtet.

Die Wurzelsymptome in Fällen von menschlicher Angiostrongylose haben vermutlich dieselbe Pathogenese.

Summary. Five calves and 5 pigs experimentally infected with larvae of *Angiostrongylus cantonensis* were killed 3, 7, 14, 28, and 56 days after infection and histologically examined.

All 5 calves and only one pig showed morphological evidence of cerebral involvement.

Three days after infection, a few larvae and minute hemorrhages were found in the brain of one calf. At 7, 14, and 28 days, numerous parasites, perivascular cuffing, and granulomas were present in the brain and spinal cord. Fifty-six days after infection only a few scattered perivascular cuffs remained.

Surrounding some of the spinal nerve roots, 7, 14, and 28 days post infection, epidural, intradural, and sometimes subdural inflammatory infiltrates with eosinophils and mononuclear cells were found. Their occurrence was associated with the presence of the parasites under the adjoining arachnoidea or pia mater.

These periradicular infiltrates are believed to be an immune response to the antigens released by the parasites into the cerebrospinal fluid and subsequently leaking along the nerve roots. Radicular symptoms in human cases of angiostrongylosis are supposed to have similar pathogenesis.

Eosinophilic meningitis occurring on Pacific islands was first reported by BAILEY (1948) and subsequently studied by others (TRUBERT, 1952; FRANCO *et al.*, 1960; ROSEN *et al.*, 1961). These authors described thoroughly the clinical symptoms of the disease but did not find the causative agent. The rat lungworm,

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Angiostrongylus cantonensis (CHEN, 1935), whose developmental cycle in mollusks and rats was discovered by MACKERRAS and SANDARS (1955), was first incriminated by ALICATA (1961, 1962) as the causative agent of epidemic eosinophilic meningitis, although as early as in 1945, this parasite had been found in the spinal fluid of a man in Taiwan by NOMURA and LIN. The incrimination was subsequently confirmed by (a) detection of the parasite in areas where the disease occurred (ALICATA, 1962, 1963, 1965a; ALICATA and MCCARTHY, 1964), (b) production of eosinophilic meningitis in simian primates following experimental administration of larvae of *A. cantonensis* (ALICATA *et al.*, 1963), (c) reports of cases of the disease following ingestion of raw mollusks (HORIO and ALICATA, 1961; PUNYAGUPTA, 1965) and by (d) findings of the parasite at autopsy in the brain (ROSEN *et al.*, 1962; JINDRÁK and ALICATA, 1965; TANGCHAI *et al.*, 1967).

It is not known whether the clinical manifestations of the disease are mainly related to trauma caused by migratory worms, metabolic products of living worms, or the release of antigens upon the death of worms in the central nervous system. Some patients manifest strong meningeal irritation while others are without it. Signs of transitory involvement of several cranial and/or spinal nerves are frequent symptoms of the disease (BAILEY, 1948; FRANCO *et al.*, 1960; ROSEN *et al.*, 1961; PUNYAGUPTA, 1965; PROMMINDAROJ *et al.*, 1962).

The purpose of this paper is to present observations on the pathology of the central nervous system, and more specifically, on the spinal nerve roots in calves and pigs experimentally infected with larvae of *A. cantonensis*. Since these animals, like man, are abnormal hosts, it is believed that the observations will contribute to the explanation of pathogenesis of some of the clinical symptoms of the disease as observed in man.

Materials and Methods

Five calves and 5 pigs, each 1-month-old, were orally infected with third-stage larvae of *A. cantonensis* (Fig. 1) recovered by artificial digestion of laboratory infected snails, *Achatina fulica*. Each calf and pig received approximately 70,000 and 20,000 larvae, respectively. One animal of each species was sacrificed at 3, 7, 14, 28, and 56 days after infection. After opening the cranial cavity and spinal canal, the brain and spinal cord together with the dura mater, spinal nerve roots, and the spinal ganglia were removed and fixed in Bouin's solution. In order to prevent any possible further migration of the parasites, the fixative fluid together with the tissues were kept cold in a refrigerator for the first 24 hours. Similarly, the contents of both orbits of each animal were preserved. The fixed brains were thereafter cut in frontal sections about 6 to 12 mm in thickness and then dehydrated and embedded in paraffin. The spinal cords together with the dura mater, were cut transversally. The histological sections were cut at 8 microns in complete or incomplete series and stained with 'Harris' haematoxylin and eosin. In several instances, the Luxol-Fast-Blue stain for myelin sheaths was used.

Results

No clinical symptoms which suggested the involvement of the nervous system were observed in the pigs. In three of the calves which were killed 7, 14, and 28 days after infection, uncertain gait was observed at the time of slaughter. This symptom was mostly marked in the calf killed 14 days postinfection. At the time of slaughter, this calf stumbled and fell down and was not able to stand up without help. It was noted also that the spine of the animal was slightly kyphotic in its thoracolumbar portion. This kyphosis disappeared after the death of the animal.

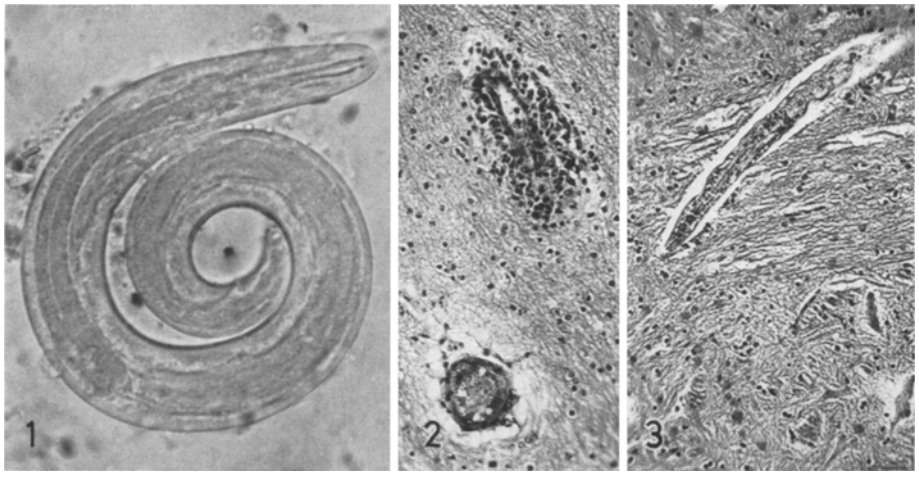


Fig. 1. A coiled infective larva of *Angiostrongylus cantonensis* isolated from snail tissues. (×400)

Fig. 2. A perivascular cuff with a small granuloma in the wall of the blood vessel. From the subcortical white matter of the calf killed 7 days after infection. (H.E., ×70)

Fig. 3. A larva in the spinal cord of the calf killed 7 days postinfection. (H.E., ×70)

All the calves in this experiment and one pig killed 28 days after infection showed pathological changes in the central nervous system. None of the pigs killed 3, 7, 14, and 56 days after infection showed cerebral involvement.

In the brain of the calf killed *3 days after infection*, no pathological changes were seen macroscopically. In the histological sections, very few larvae were found in the white matter just beneath the cortex of the frontal and occipital lobes without any inflammatory reaction. The larvae were coiled and a few minute hemorrhages were present in the white matter not far from the larvae. The spinal cord and the spinal dura mater with the nerve roots were morphologically intact.

In the brain of the calf killed *7 days after infection*, macroscopically, a few hemorrhagic points were found in the white matter of the frontal lobes. Histologically, larvae were present in the cerebellar cortex and in the white matter under the cortex of the cerebral hemispheres. Their presence was associated with minute hemorrhages. Although no inflammatory reaction was evident in the vicinity of most of the larvae, solitary perivascular cuffs consisting of lymphocytes were present on several places in the subcortical layer of the white matter and in the pons. In one of these cuffs, a granuloma affecting the wall of a blood vessels was noted (Fig. 2). Many larvae were found in the spinal cord in the grey as well as in the white substance (Fig. 3). Near one of them, in the cervical spinal cord, perivascular inflammatory cuffs and a small granuloma were found. The granuloma was located in the dorsal horn and consisted of mononuclear cells, eosinophils, and a foreign-body giant cell. Lymphatic cells, plasmocytes, and eosinophils infiltrated the outer and sometimes inner layer of the dura mater in several areas where the spinal nerve roots passed through. This infiltration reached into the adjacent epidural fatty tissue which was edematous. There was no inflammatory reaction on the leptomeninges.

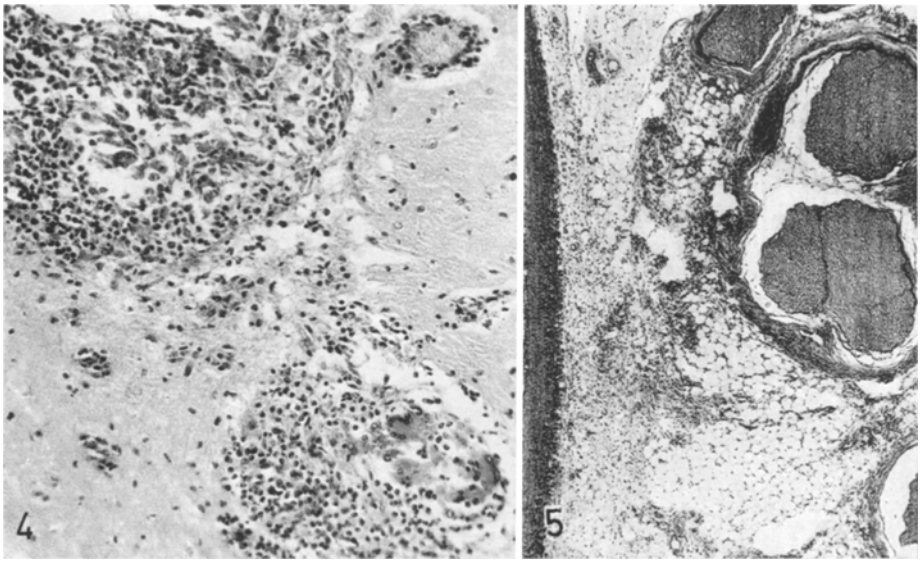


Fig. 4. Two granulomas in the subcortical white matter of the calf killed 14 days after infection. (H.E., ×70)

Fig. 5. Edema and inflammatory infiltration in the outer layer of the spinal dura mater and in the epidural fatty tissue around the nerve roots in calf killed 14 days after infection. (H.E., ×20)

In the calf killed *14 days after infection*, no macroscopic changes were noted in the central nervous system. Microscopically, intact larvae were found in the cortex and subcortical white substance of the brain. There, as well as in the mesencephalon, solitary granulomas with and without dead larvae were present. The granulomas consisted of several foreign-body giant cells and a broad rim of mononuclear cells and eosinophils (Fig. 4). Perivascular cuffing by lymphatic cells and eosinophils was present in the vicinity of the larvae and the granulomas. The pia mater of the cerebellum was slightly infiltrated with mononuclear cells. Granulomas consisting of foreign-body giant cells, mononuclear cells, and eosinophils mostly surrounding dead larvae were present in the spinal cord and on its surface under the pia mater. They were accompanied by perivascular inflammatory cuffs. Leucocytes and fragments of a worm were observed even in the central canal of the cervical spinal cord. Several intact larvae, which were found in the gray substance, measured 30 microns in diameter. The infiltration around the nerve roots appeared similar to that in the calf killed 7 days post infection; however, it was more accentuated in the epidural fatty tissue (Fig. 5). The occurrence of these periradicular infiltrates was associated with the presence of granulomas and parasites under the pia mater of the adjacent segments of the spinal cord (Fig. 6). No inflammatory reaction was found in the arachnoidea which was interposed between the infiltrated periradicular dural sheaths and the subpial granulomas (Fig. 7). However, in several places, the worms were found in the subarachnoid space. Here, the parasites, in process of disintegration, were surrounded by inflammatory cells. This calf was the only one in which some

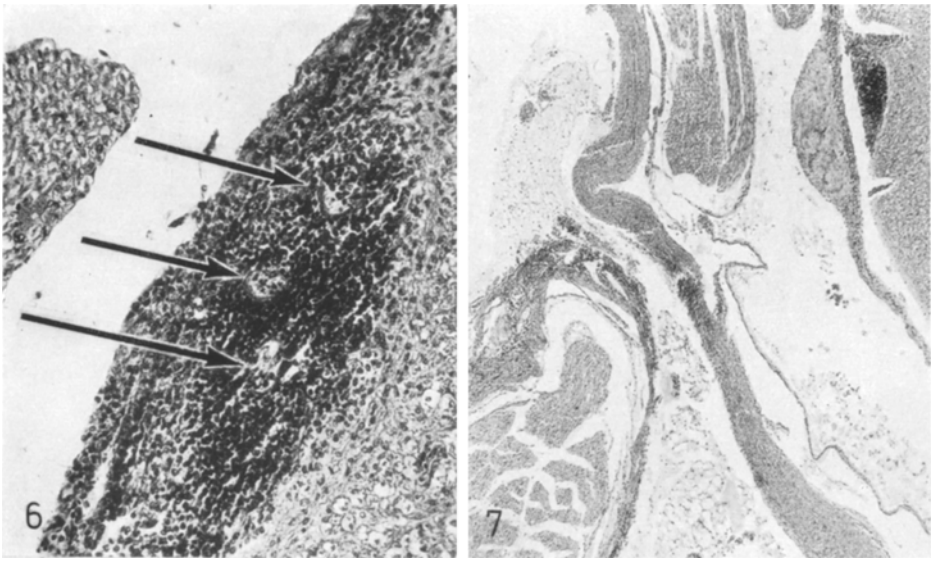
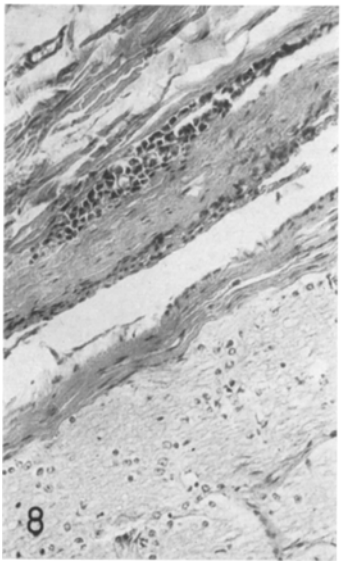


Fig. 6. Subpial granuloma on the spinal cord of calf killed 14 days after infection. Lymphocytes, plasmocytes, and eosinophils surround three transverse sections of a dead parasite (arrows) and fill its body cavity. (H.E., ×100)

Fig. 7. The relation of subpial granulomas to periradicular infiltrates. A granuloma, similar to that in Fig. 6, is located under the pia mater and ligamentum denticulatum of the spinal cord of the same calf. Inflammatory infiltration permeates a portion of the adjacent dura mater and the dural sheath of the spinal nerve root. Absence of inflammatory changes in the arachnoidea. (H.E., ×20)

pathological changes were noted on the tissues surrounding the optic nerves. In the retrobulbar fatty tissue, several small focal infiltrates consisting of eosinophils, lymphocytes, and plasmocytes were present. These infiltrates were situated near the dural sheath of the optic nerve. In a few places, stratified accumulations of eosinophils and mononuclear cells were found between the fibrous layers of this sheath (Fig. 8).

In the calf killed *28 days after infection*, gross examination revealed that the meninges of the cerebellum, pons, and medulla were congested and slightly edematous. A thin subarachnoidal layer of yellow pus could be seen on the base of the third ventricle and in the interpeduncular fossa. Histologically, a few granulomas surrounding disintegrated larvae were present in the cerebral cortex and in the pia mater of the sulci. They consisted of foreign-body giant cells, mononuclear cells, plasmocytes, and eosinophils. A similar granuloma was also present in one of the bracchia conjunctiva. A small focus of rarefied neural tissue with dilated blood vessels was observed in the mesencephalon. All these lesions were accompanied by inflammatory cuffs around the proximal blood vessels. The pia mater on the brain base was thickened and densely infiltrated with lymphocytes, plasmocytes, histiocytes, and eosinophils. The pia mater of the cerebellum contained in many places focal infiltrates of mononuclear cells and eosinophils. In the subarachnoid space of a cerebral sulcus, a morphologically intact worm was found. No inflammatory reaction was noted in its vicinity. The partially collapsed body of the parasite measured in the transverse section about 35 microns



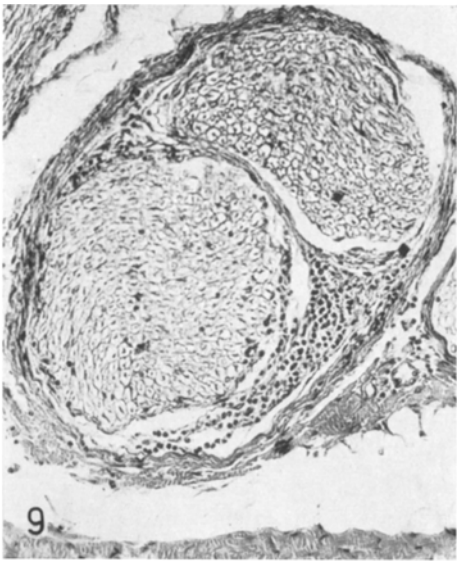


Fig. 8. Fibrous sheath of the optic nerve partially split by a thin layer of eosinophils, lymphocytes, and plasmocytes in the calf killed 14 days after infection. (H.E., ×100)

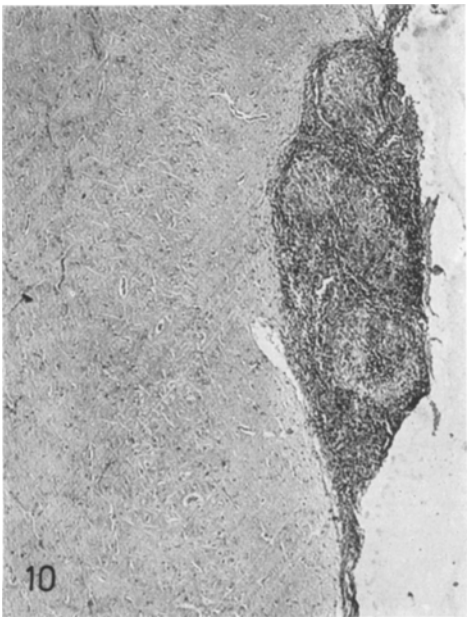


Fig. 9. Spinal nerve root with a layer of inflammatory cells between the nerve root and its fibrous sheath in the calf killed 28 days after infection. (H.E., ×80)

Fig. 10. Granuloma under the pia mater of the brain of the pig killed 28 days after infection. (H.E., ×40)

in minimum width and 100 microns in maximum width. In the spinal pia mater, there was an inconspicuous diffuse inflammatory infiltration. Several granulomas around dead parasites were noted in the same locality. One intact worm was found interwoven in the nerve roots crossing the subarachnoid space. Infiltrates of the dura mater, cuffing the spinal nerve roots, consisted of lymphocytic cells and eosinophils. They were arranged in layers inside the fibrous wall of the dura mater and between the dural sheaths and nerve roots (Fig. 9). Infiltrates in the epidural fatty tissue were perivascular in distribution and consisted almost entirely of eosinophils. Similarly, as it was in the case of the calf killed 14 days

after infection, the presence of periradicular infiltrates was connected with the presence of pial granulomas.

The pig killed *28 days after infection* showed a few granulomas consisting of lymphocytes, epithelioid cells and foreign-body giant cells in the pons and in the white matter and under the pia mater of the parietal and occipital lobes. One granuloma in the hypothalamus contained a disintegrated worm. In the pia mater the granulomas were multifocal (Fig. 10). The arachnoidea on the base of the third ventricle was transformed into a continuous layer of granulomatous tissue with eosinophils and multinuclear giant cells. The choroid plexus in the fourth ventricle contained focal inflammatory infiltrates consisting of lymphocytes and eosinophils. No lesions were found in the neural tissue of the spinal cord. In several places where the spinal nerve roots penetrated the dura mater, intensive infiltration with lymphocytes, eosinophils, and multinuclear foreignbody giant cells was present. This infiltration extended into the pia mater, arachnoidea, and dura mater. In the epidural fatty tissue only small groups of eosinophils were observed. Near one of the dorsal nerve roots which showed a marked inflammatory infiltration of the surrounding tissues, a worm with signs of disintegration was found in the subarachnoid space. Its transverse section measured about 50 microns in diameter. The worm was encompassed with purulent exudate. The adjacent pia mater and arachnoidea were thickened and heavily infiltrated predominantly with lymphocytes.

The lesions found in the central nervous system of the pig 28 days after infection did not differ greatly from those found in the calf killed at the same time after infection. The granulomas in the pig's brain had a marked "pseudotuberculous" appearance, mainly due to the presence of epithelioid cells. In the pig, inflammatory infiltration around the spinal nerve roots was more pronounced in the subdural rather than in the epidural tissues, and a considerably smaller number of nerve roots was involved.

In the calf killed *56 days after infection*, no worms were found in the histological sections. Only minimal perivascular cuffs consisting chiefly of lymphocytes were present in the white matter of both cerebral hemispheres. Despite examination of additional series of sections, no granulomas were found. In sections of the cervical spinal cord, a narrow layer of fibroblasts and fibrocytes between a nerve root and its dural sheath was present. No inflammatory infiltrates were found in the meninges nor in the epidural fatty tissue.

It was estimated that only about one-fifth of all the nerve roots in the calves killed 7, 14, and 28 days post infection showed inflammatory reaction. In each case, usually a dead, disintegrating worm was found near the involved nerve roots. The worms were located either under the arachnoidea, or most often in a granuloma under the pia mater. The involved nerve roots were distributed equally in all parts of the spinal cord and no preference was noted as to involvement of the dorsal or ventral areas.

Discussion

The report on the epidemic of eosinophilic meningitis on Ponape (BAILEY, 1948), records several case histories in which the predominant involvement of the spinal nerve roots, most often thoracic in distribution, was marked. This is evident especially in case No. 4 of the report in which the original complaint of the

patient was that of focal skin hyperesthesia. Other clinical signs, except blood and cerebrospinal-fluid eosinophilia, were minimal. Muscular pain, decreased muscle strength, abnormal reflexes, and ataxia were also described. In Tahiti (ROSEN *et al.*, 1961; ALICATA and BROWN, 1962), different types of paresthesias, asymmetrically localized in various parts of the body have been observed. They have been described as areas exhibiting sensation of burning, numbness, pain or exaggerated sensitivity to touch. Paresis of upper limbs and disappearance of tendon reflexes of the right lower extremity have also been mentioned (FRANCO *et al.*, 1960; PUNYAGUPTA, 1965). All these signs are being described as transitory, lasting from 2 to 31 days and usually from 6 to 8 days. Many of these symptoms may be explained as being due to the involvement of several spinal nerve roots.

However, in man, the morphology of this involvement is unknown. Cellular infiltration with many eosinophils in spinal epidural tissue and surrounding nerve roots was described in cerebrospinal setariosis in sheep in Japan, as quoted by INNES and SAUNDERS (1962), and in experimental pneumostrongylosis in sheep and white-tailed deer (ANDERSON and STRELIVE, 1966, 1967). ISHII *et al.* (1953) succeeded in producing similar lesions experimentally after subcutaneous injection of the infective larvae of *Setaria digitata*. They found eosinophilic infiltrations in the sciatic nerves and spinal nerve roots and theorized, similar to ANDERSON and STRELIVE (1967), about possible direct migration of the larvae through tissues and along the nerves into the spinal canal.

In the present experiment, parasites have been found in the subarachnoid space or under the pia mater next to the inflammatory infiltrations around the nerve roots. The pathogenesis of these periradicular infiltrates cannot be explained by the action of the migrating third-stage larvae. The granulomatous lesion found in the wall of a small blood vessel in the brain of a calf 7 days after infection (Fig. 2), seems to point out that the larvae reached the central nervous system predominantly by blood stream. It was after their emergence from the spinal cord that they reached the proximity of the nerve roots. Here, dead or alive, the worms presumably released antigenic substances into the cerebrospinal fluid. The concentration of these substances should be considerably greater in the proximity of the parasites than in other parts of the subarachnoid space. The ways in which the cerebrospinal fluid is removed from the subarachnoid space could serve also for the absorption of the antigenic substances. According to BOWSHER (1960), relatively large amounts of cerebrospinal fluid are taken up into the veins of the arachnoidea and a certain amount leaks more slowly along the nerve roots into the lymph. This slow leakage of the cerebrospinal fluid with antigenic substances through the thinner portions of the dura mater cuffing the nerve roots and its collision with antibodies outside the dural sac, could be responsible for the periradicular inflammatory reaction. In most cases, non-antigenic products of worms should not be responsible for these lesions because of absence of inflammatory infiltration in the adjacent intercalated arachnoidea as observed especially in the calves (Fig. 7). The locally different concentration of antigens in the cerebrospinal fluid due to the presence or absence of the parasite would perhaps explain the patchy character of the lesions.

The transitory radicular symptoms in cases of human angiostrongylosis are probably similarly caused by inflammatory irritation of the nerve roots and by their compression with thickened dural sheaths. The pathogenesis of this radicular

involvement may be compared to a certain extent with the pathogenesis of other polyradiculitis syndromes of the pseudo-Guillain-Barré type, which are suspected to be allergic or autoimmune in origin (GLANZMANN, 1964). The pathogenesis of the involvement of the cranial nerves might be similar. Also, the impaired vision, an important complication of eosinophilic meningitis (PUNYAGUPTA *et al.*, 1967), might be in part due to the compression of the optic nerve by inflammatory thickening of its sheath.

In the brain of the calves, the larvae and lesions were localized with a certain predilection in the subcortical layer of the white matter and cortex, which is in accordance with previous observations of ALICATA *et al.* (1963) in monkeys.

Observations on the visceral migration of the larvae of *A. cantonensis* in pigs and calves, carried out by JINDRÁK and ALICATA (1968) on the same series of animals, indicated that in the visceral organs of pigs, the parasites were destroyed at the beginning of infection. This confirmed the previous observations by ALICATA (1965b) in the same host. In the calves, however, the capability to arrest and destroy the larvae developed more slowly after the infection and after many parasites had reached and developed in the brain. The present observation demonstrates that a certain number of larvae reached the brain in one of 5 infected pigs. In this pig, the cerebral pathology and growth of the parasites were similar to that observed in the calf which was killed at the same time of infection. This ability of the parasites to survive in the brain and later in the subarachnoid spaces after others had been quickly destroyed in other organs of the same animal, may indicate that in the above localizations, the parasites are protected against the direct action of the existent immune mechanisms of the host. These speculations, however, require further study.

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