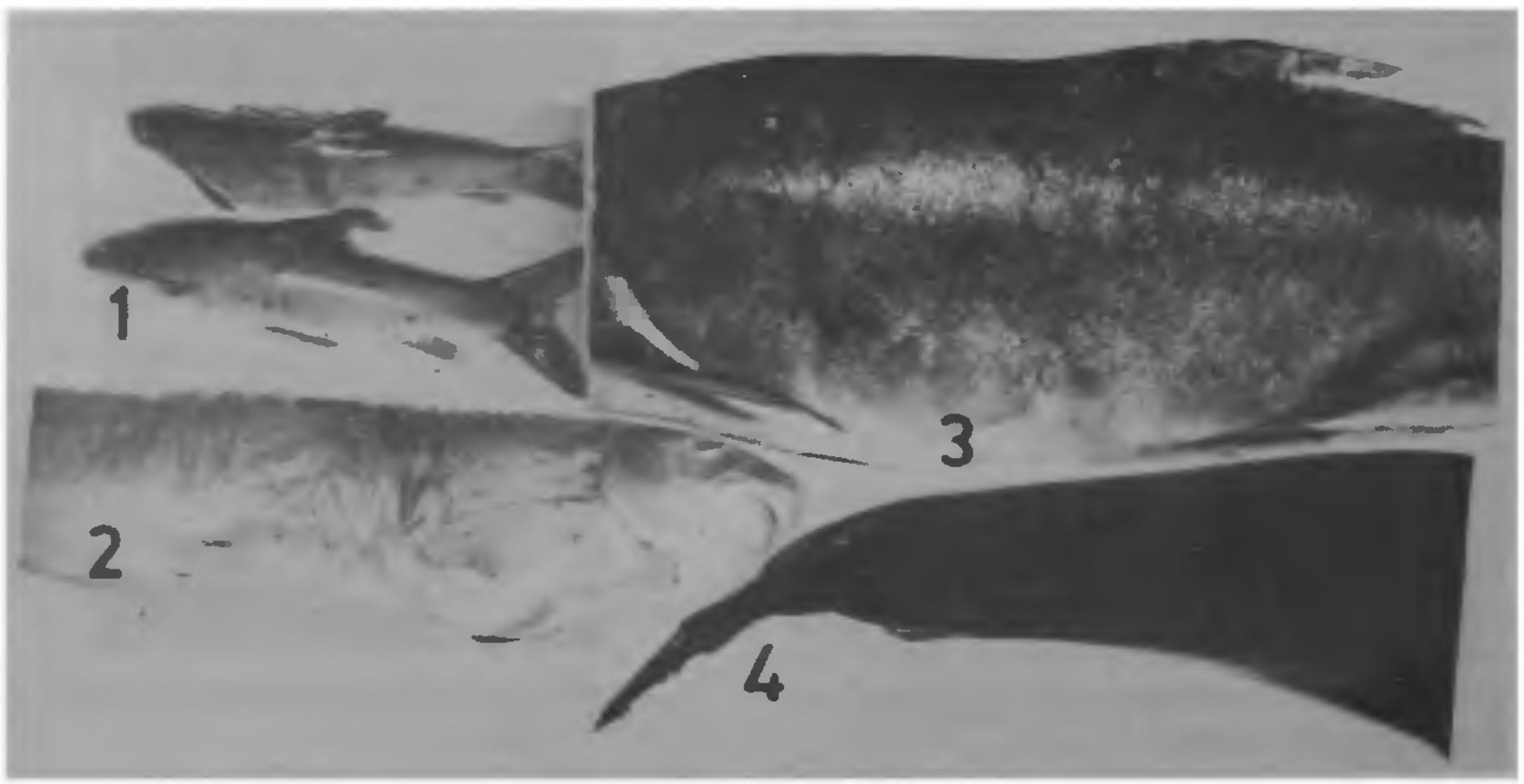
16 August 1988

1. Pal, A. K., *Progress Report of GSI for 1981–82*, 1984, (Unpublished).
2. Rama Rao, L. and Pia, J., *Mem. Geol. Surv. India*, 1936, No. 21, p. 4.
3. Sastry, M. V. A., Rao, B. R. J. and Mamgain, V. D., *Mem. Geol. Surv. India*, 1965, No. 2, p. 10.
4. Narayan Rao, S. R., *J. Indian Bot. Soc.*, 1943, **22**, 179.

BIOLOGY AND PATHOGENIC POTENTIAL OF BLACK SPOT TREMATODES IN HIGH ALTITUDE FISHES OF INDIA

SHAMPA BANERJEE and SANDEEP K. MALHOTRA
Department of Zoology, University of Allahabad, Allahabad 211 002, India.

METACERCARIAE of *Neascus vetestai*[1–3] are known to cause black spot disease (figures 1–4) in Indian hill-stream fishes but explicit host responses induced by them, including pigmentation patterns on skin and viscera, have not been reported[2,4]. This investigation records the maximum outer host cyst size ever reported[5] (Meyer, pers. commun.). In a total sample of 3000 *Schizothorax richardsonii* inhabiting the rain-fed river Nayar in Garhwal Himalayas at 750 ± 100 MSL during 1982–1988, we recorded host cysts 0.13–3.7 mm in diameter encircling the parasite cyst (figure 5), 0.3–0.56 mm in diameter. Cysts consist of an outer thick cellular layer (host origin) and an inner thin non-cellular layer (parasite origin). Several parasite cysts occurred collectively engulfed in a host cyst during periods of extensive invasion, and their presence underneath the skin evoked extensive pigmentation response from the hosts. Details of *in vitro* excystment and cyst morphology have been reported only for *N. pyriformis*[6] till date. Though cyst and larval morphology of *N. vetestai* appeared to be similar to those of *N. pyriformis*[6] and another black spot trematode, *Uvulifer ambloplitis*[7], the larvae infrequently occupied the parasite cyst completely and the forebody was folded back on itself (figure 5). In early stages of infection, very small host cysts 0.13–1.73 mm in diameter were observed scattered all over the body surface in appreciably high frequency (figure 3). Each small host cyst usually contained one parasite cyst. In later phases of extensive invasion (figures 1, 2), frequency of cyst occurrence was low



Figures 1–4. **1** and **2.** Black spots in neasciasis of *Schizothorax richardsonii* showing low frequency of congregated cyst pattern; **3.** Black spots showing high frequency of low intensity on *S. richardsonii*; **4.** Black spot disease showing "skin burns" on tail of *S. richardsonii*.

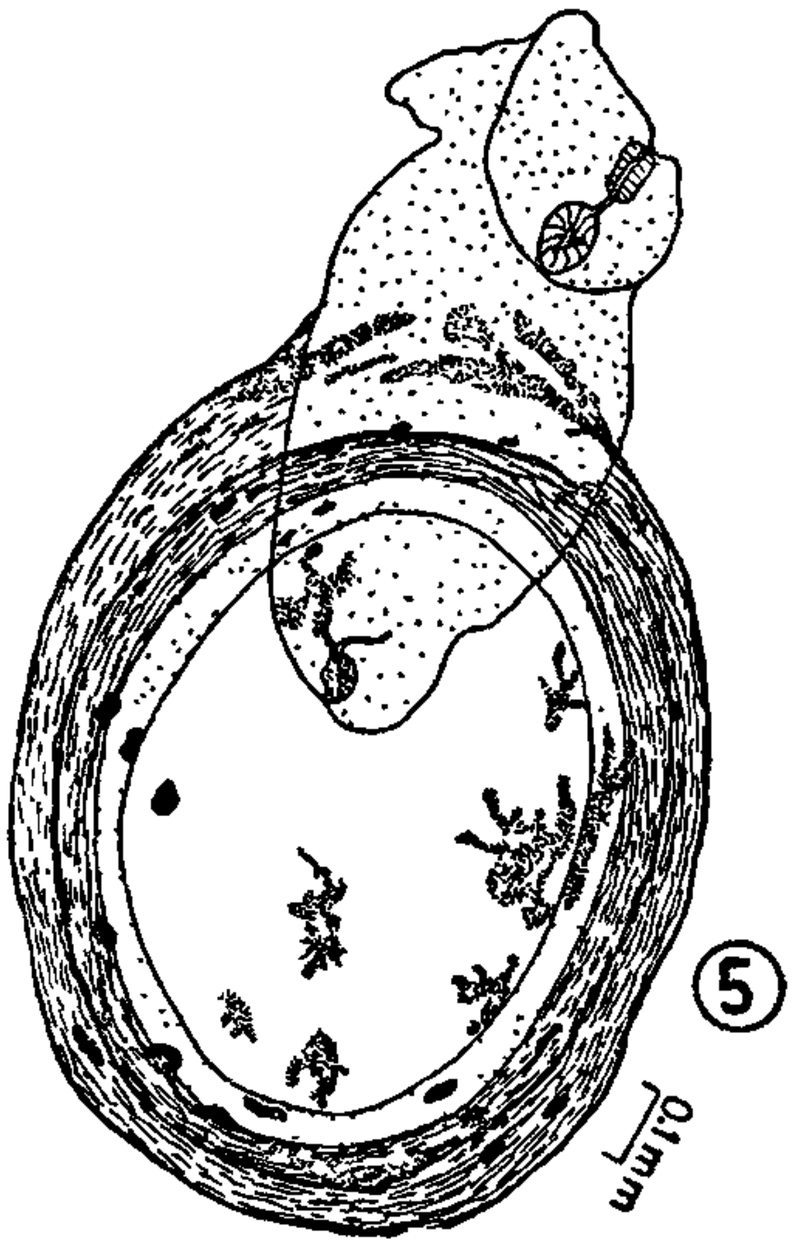
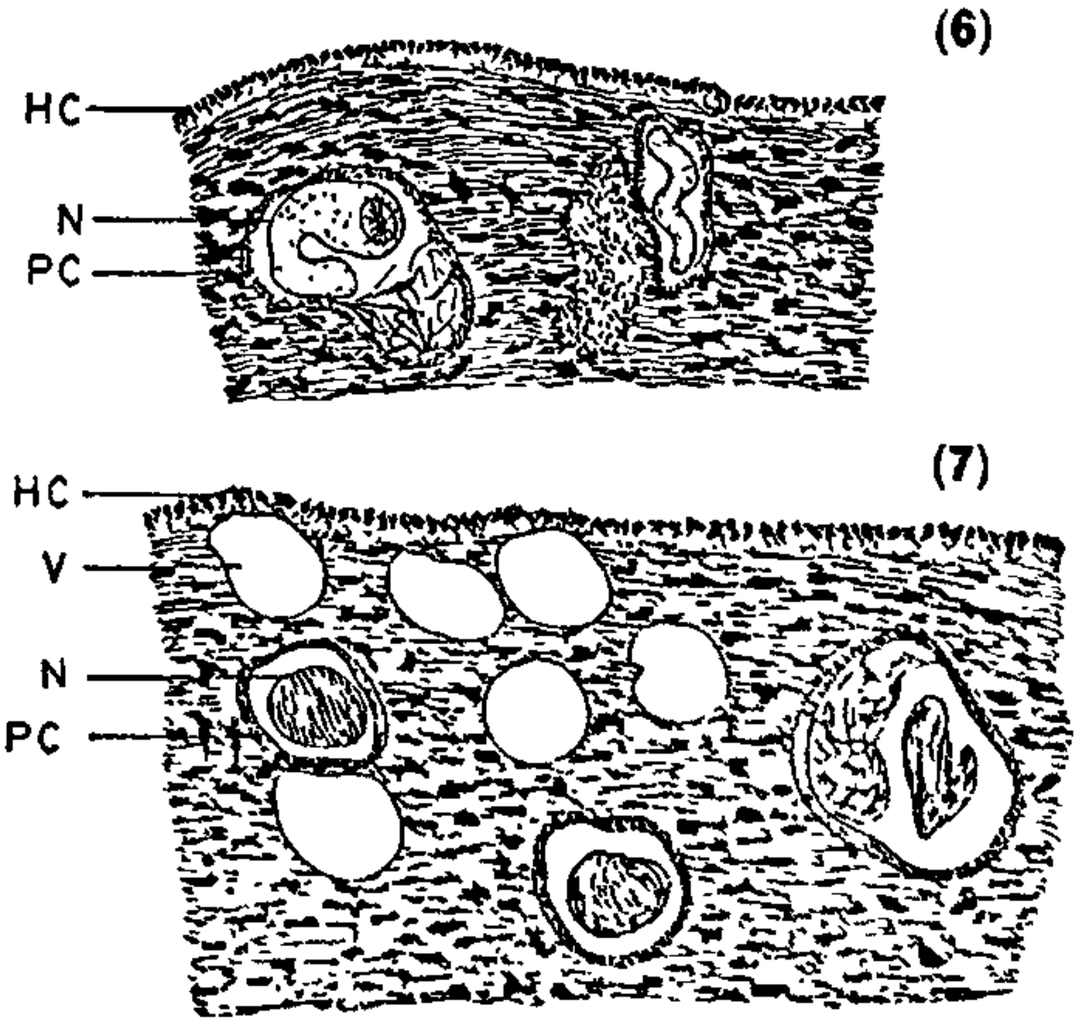


Figure 5. Whole mount of black spot trematode cyst with metacercaria coming out.

but the cysts appeared to be congregating randomly on the skin, mostly on the lateral or latero-ventral surface, thus giving the appearance of tubercles[8] (up to about 3.7 mm in diameter). Tumorous lesions[9] of the musculature could also be observed. This may be suggestive of cyst migration and clumping underneath the scales of the skin. Cases of helminth migration from the sites of infection[10] due to changes in pH levels[11] within the host environment are not uncommon in Indian fishes, but reports of similar instances of cyst migration are scarce in the literature. This raises a new question regarding locomotor processes in helminths and their cystic stages and points to the need for extensive research to explore the role of chemotaxis[12] in such migration.

Sublethal infection may lead to pigmentation response that is directly proportional to the extent of invasion, gill tissue damage that may interfere with gaseous exchange, reduced reproductive potential, physiological dysfunctions, abnormal behaviour or disorientation, and loss of weight[13] in the affected fish. There is also the increased visibility of diseased fish to predators.

On certain parts of the body, such as the tail and latero-ventral parts (figure 4), uncontrolled spread of melanocytes in larger areas was sometimes observed. These areas gave the appearance of burns (8.67–19.5 mm in diameter) on the skin surface (figure 4). They suggest bursting of host cysts under pressure of hyperplasia in cases of severe infestation. There are records of pathological symptoms like skin erosion of this type in the literature[14,15]. Histopathological studies (figures 6, 7) of tuberculated cysts revealed simple hyperplasia, excessive melanocyte distribution in scattered and aggregated pigmentation patterns, and multiple vacuole formation within host cysts. Pigmentation was dense in the parts of the host cyst in the immediate vicinity of parasite cysts, while scattered pigmentation and relatively uneven distribution with occasional clumping was observed in other parts of the host cyst (figures 6, 7). The vacuolation patterns observed are similar to the damaged and vacuolated submucosa of *Channa gachua* and *Heteropneustes fossilis* infected with *Genarchopsis goppo* and *Procamallanus* (*monospiculus*) *devendri* respectively[16,17]. Subepidermal vacuolation in muscle of penetrating miracidia of *Fasciola hepatica*[18] and *in vitro* cultured miracidia of *Schistosoma mansoni*[19] have also been observed and these were considered to indicate loosening of the epidermal cells and some futuristic transition in larval development. These vacuoles were not observed in free-swimming *F. hepatica* miracidia[18]. Interestingly, this vacuolation was observed in the oldest miracidia, and rendered



Figures 6–7. TS of skin surface of infected *S. richardsonii* showing multiple cysts in a single host cyst (6); and vacuolation (7) in host cysts. HC, host cyst; N, *N. retestai*; PC, parasite cyst; V, vacuole.

them most susceptible to osmotic stress[20]. There is evidence[21] to conclude that trematode cuticle is not only very permeable to ions but also to water in either direction during osmotic exchange. Though the possession of an alimentary canal greatly lessens the necessity for a permeable outer covering, the physiological needs of parasites have forced them to evolve osmotic processes involving cuticular exchange[22], but their tolerance limit to osmotic stress varies. Parasite-induced osmotic stress beyond the tolerance limits of the host and the parasites within host cysts may be postulated to explain the unusual bursting of the oldest cysts in fish skin.

Funding for the project to SKM and a fellowship to SB by DST, New Delhi, are acknowledged.

28 April 1988; Revised 27 September 1988

1. Kaw, B. L., *Indian J. Helminthol.*, 1950, **2**, 67.
2. Malhotra, S. K. and Banerjee, S., In: *Recent trends in Indian zoology*, Meerut, 1989, (in press).
3. Pandey, K. C., *Proc. Indian Acad. Sci.* (*Anim. Sci.*), 1971, **72**, 162.
4. Abraham, F. G. and Anantaraman, M., *Nature* (*London*), 1955, **175**, 127.
5. Hoffman, H. L. and Sindermann, C. J., *Common parasites of fishes*, US Dep. Int. Fish Wildl. Ser. USA., 1969, p. 17.
6. Schroeder, D. J., Johnson, A. D. and Mohammad, K. H., *Proc. Helminthol. Soc. Wash.*, 1981, **48**, 184.
7. Spellman, S. J. and Johnson, A. D., *Int. J. Parasitol.*, 1987, **17**, 897.
8. Petrushevski, G. K. and Shulman, S. S., In: *Parasitology of fishes*, Leningrad University Press, 1958, p. 299.
9. Kearn. G. C., *Int. J. Parasitol.*, 1988, **18**, 313.
10. Kirmse, P. D. and Ferguson, H. W., In: *Wildlife diseases*, Plenum, New York, 1976, p. 561.
11. Bose, K. C. and Sinha, A. K., *Curr. Sci.*, 1980, **49**, 683.
12. Smyth, J. D., *The physiology of trematodes*, Oliver & Boyd, UK, 1966, p. 279.
13. Malhotra, S. K., *Ecological and karyological segregation of helminths of vertebrate hosts in Garhwal Himalayan ecosystems*, 1st Annu. Rep. submitted to DST, 1988, p. 96.
14. Wootten, R., Smith, J. W. and Needham, E. A., *Proc. R. Soc. Edinburgh*, 1982, **B81**, 1.
15. Wootten, R., *ICES CM 1985/F7*, 1985, p. 4.
16. Bose, K. C. and Sinha, A. K., *Curr. Sci.*, 1979, **48**, 747.
17. Bose, K. C. and Sinha, A. K., *Curr. Sci.*, 1979, **48**, 275.
18. Southgate, V. R., *Parasitology*, 1970, **61**, 177.
19. Basch, P. F. and Diconza, J. J., *J. Parasitol.*, 1974, **60**, 935.
20. Dunn, T. S., Hanna, R. E. B. and Nizami, W. A., *Int. J. Parasitol.*, 1987, **17**, 885.
21. Rogers, W. P., *The nature of parasitism*, Academic Press, New York, 1962.
22. Siddiqi, A. H. and Lutz, P. L., *Exp. Parasitol.*, 1966, **19**, 348.

ISOLATION OF CHLAMYDIA FROM CEREBRAL TISSUE OF BUFFALO CALF

CHARAN KAMAL SINGH, P. N. DHINGRA and NARENDRA KUMAR
Department of Veterinary Pathology, College of Veterinary Science, Punjab Agricultural University, Ludhiana 141 004, India.

THE present work on isolation of chlamydia was undertaken as serological studies carried out by various workers[1-3] amply suggested the occurrence of chlamydial infection in domestic ruminants. Since chlamydia were isolated from a variety of tissues and not from nervous tissue, it was considered necessary to attempt isolation of chlamydia from nervous tissue of buffalo calves.

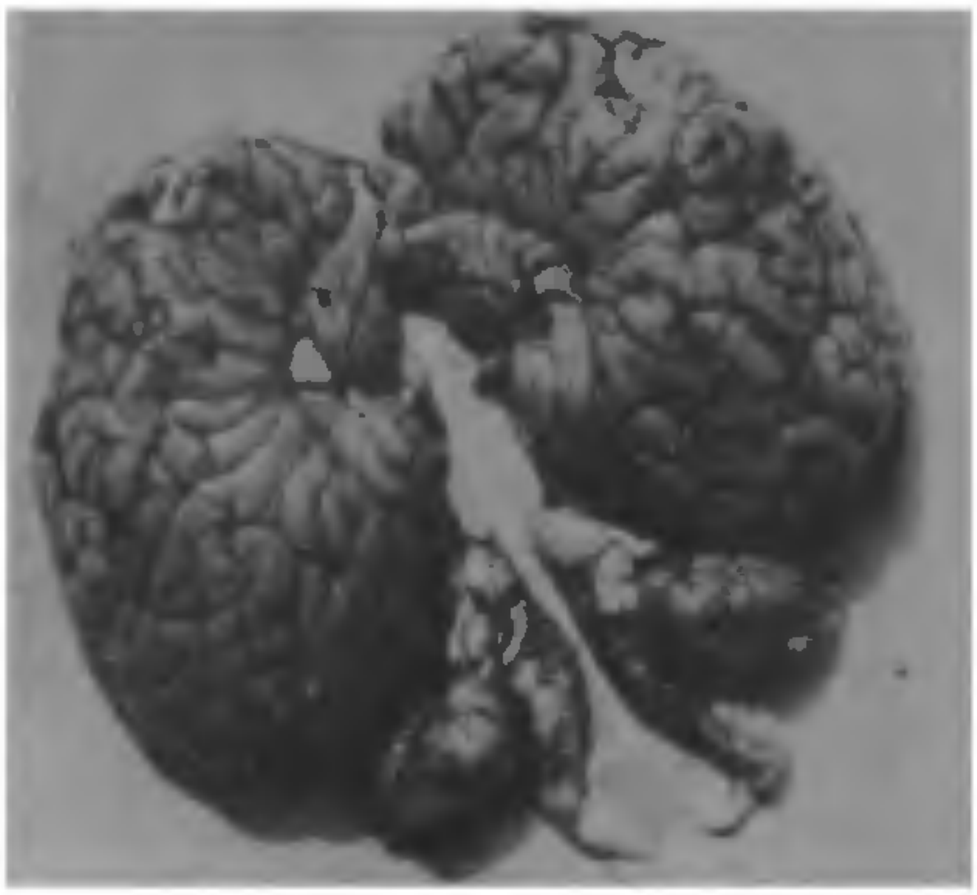


Figure 1. Severe congestion and edema of cerebrum and cerebellum in brain of buffalo calf.