An Ig-A-like substance in the chicken's pineal

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Summary. Immunohistochemistry revealed an Ig-A-like substance on the luminal surface of the pineal follicles and in the parafollicular layer. This substance was observed around 1 week of age and disappeared by 8 weeks at the time when the transformation of the follicular pattern leads to an adult-type pineal tissue.
Key words. Chicken; Ig-A; pineal; immunocytochemistry; immuno-endocrine relationship.

During the past 50 years several authors have observed lymphoid accumulation in the pineal gland [1–4]. The transmission microscope provided evidence that lymphocytes enter the pineal follicles forming a transitory lymphopineal tissue in the chicken [5]. Following the demonstration of T and B cells and antibody producing cells in the avian pineal, Cogburn and Glick [6] concluded that the pineal was a peripheral lymphoid organ.

We have studied the pineal lymphoid tissue with a panel of monoclonal antibodies (manuscript in preparation). These immunohistochemical examinations revealed the presence of an α-heavy chain positive substance on the luminal surface of the pineal follicles. In this manuscript we discuss the evidence of an IgA-like substance in the pineal.

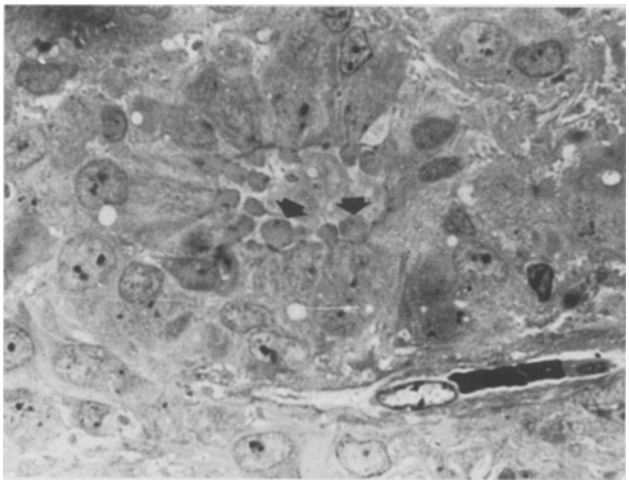

Figure 1. A 1-μm thick section of pineal follicles. The B type pinealocytes form club-shaped processes in the lumen (arrow). 930×.

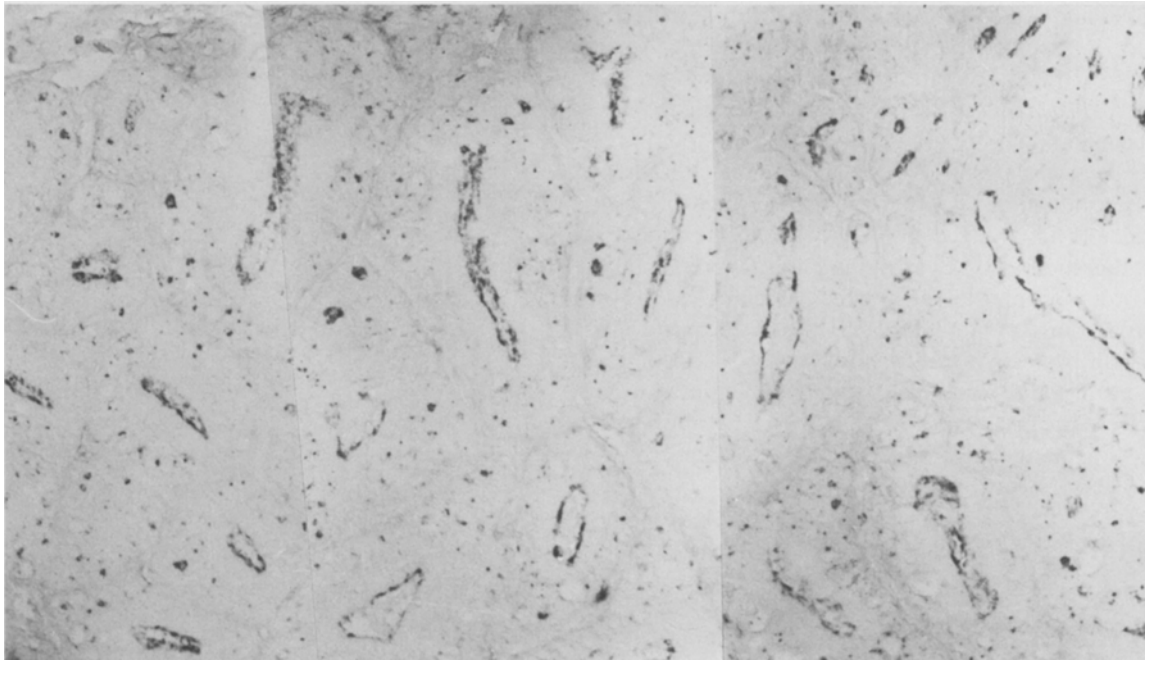

Figure 2. 23-day-old chicken pineal stained with anti-μ-mAb. IgM positive cells are in the connective tissue between the follicles, but are not present in the follicles. 65×.

Materials and methods

Small bursa line (SBL) chickens were used [7]. Immunohistochemical observations of 4, 8 and 4 SBL chickens were made at 7, 23 and 53 days of age, respectively.

Monoclonal antibody (mAb) α-(I-A) and μ-chains (M-4) were gifts from Drs Max Cooper and Chen-Lo-Chen (Birmingham, AL). The anti-lambda light chain was purchased from Sigma (St. Louis, MO). The biotinylated anti-mouse IgG and the ABC Vectastain Elite Kit were purchased from Vector Laboratories (Burlingame, CA). The pineals were frozen in liquid nitrogen and 10–12-μM sections made with a cryostat. The endogenous peroxidase either was quenched by 0.3% H_2O_2 in methanol for 30 min or developed by diaminobenzidine (DAB). The exogenous horse-radish peroxidase was visualized by using 4-hydrochloronaphthol as a substrate.

Following quenching or developing of endogenous peroxidase with DAB, the sections were overlaid with primary antibody (30 min, 20 °C), washed 2 times with phosphate buffered saline (PBS) and then flooded with biotinylated second antibody (30 min, 20 °C). Following two washings with PBS the sections were covered with the ABC complex (30 min, 20 °C), rinsed with PBS and then developed with 4-hydrochloronaphthol. Control sections were covered with M-4 mAb.

Results and discussion

The pineal follicles are made up of A and B-type pinealocytes which form follicular and parafollicular layers [8–10]. After hatching, the pineal possessed distinct follicles with pinealocytes exhibiting club-shaped processes (fig. 1). At this time, the luminal surface of every follicle stained with the I-A mAb (fig. 2) but not with anti-μ (fig. 3). The reaction product varied in thickness, continuity and density (figs 4 and 5). This immunological reaction may be associated with B type pinealocytes, which are of photoreceptor origin, since the B type pinealocytes are more frequent and occupy a larger surface area of the follicles than the A type supporting cells. Also, the I-A positive

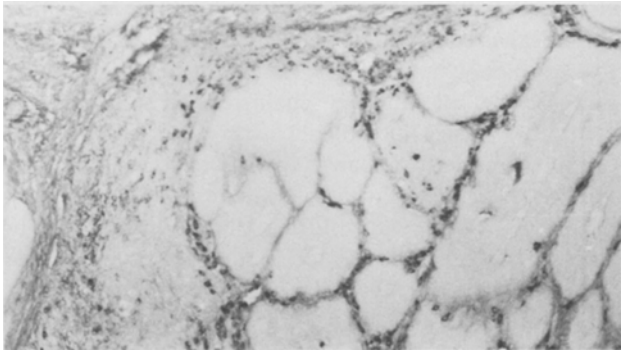

Figure 3. The pineal gland of a 23-day-old SBL chicken stained with I-A mAb. The lumen of the follicles are outlined by I-A positive staining. Scattered, dotted reaction products can be seen in the parafollicular layer of the follicles. 90 ×.

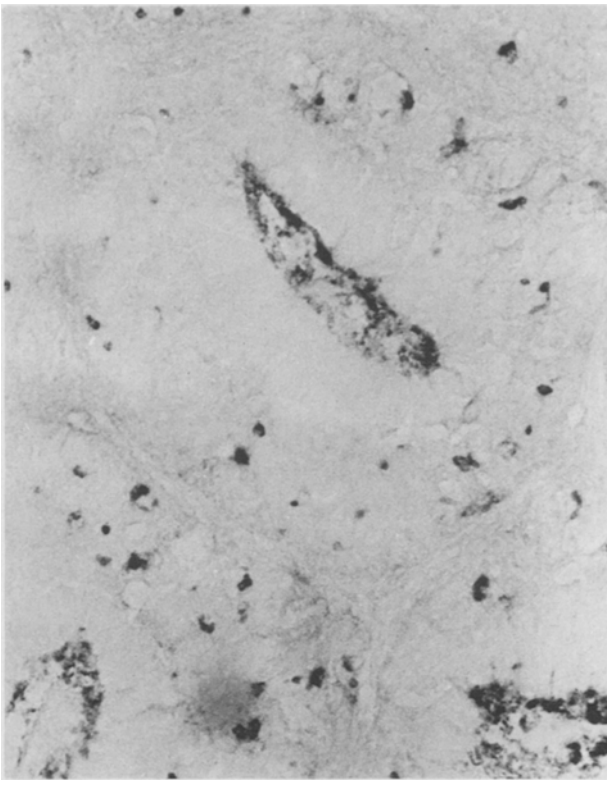

Figure 4. The I-A positive substance is uneven in density and thickness in the lumen of the follicles. Peripheral to the follicular cells scattered I-A positive reaction appears in the parafollicular layer. 300 ×.

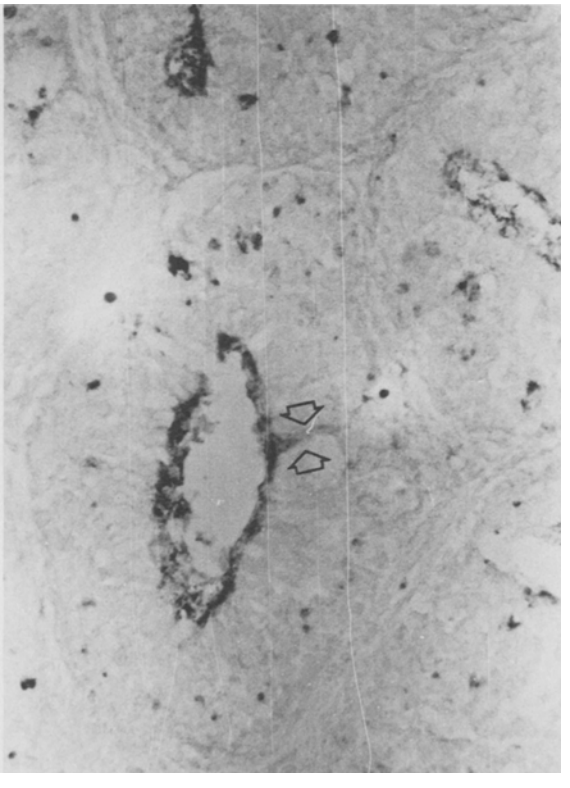

Figure 5. One weakly positive pinealocyte shows continuity with an I-A positive reaction area (arrow). 300 ×.

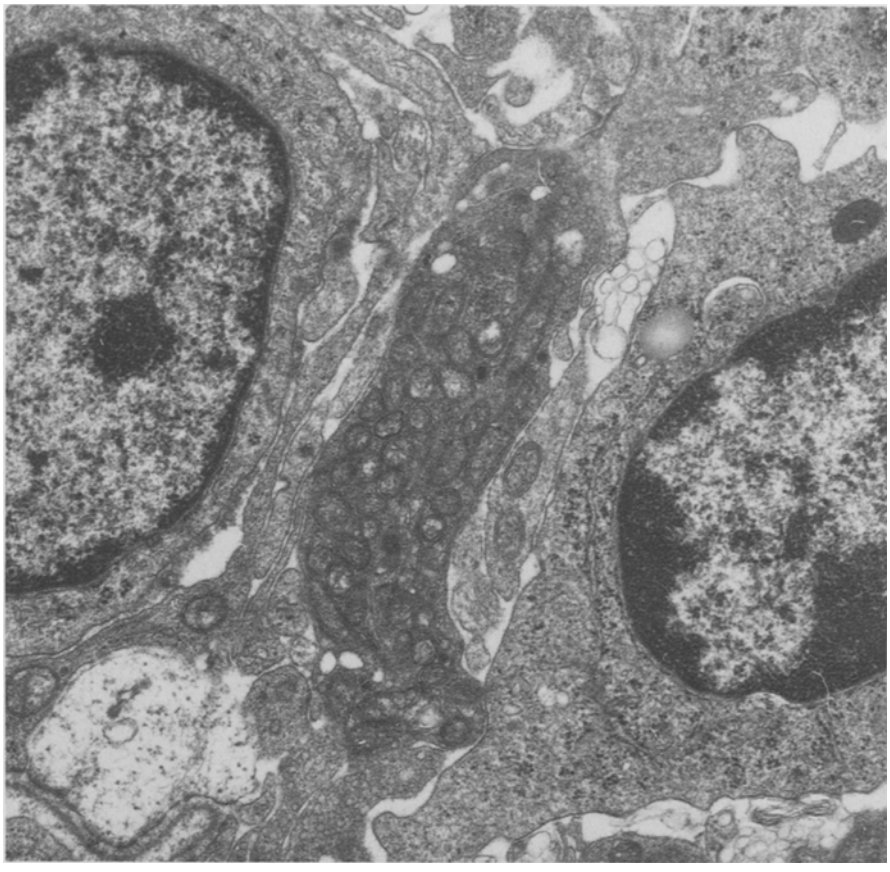

Figure 6. Transmission micrograph reveals scattered cell processes packed with mitochondria occurring at the periphery of the follicular cells. The size and location of this inner segment-like structure correlates with that of the I-A positive dots. 15600 ×.

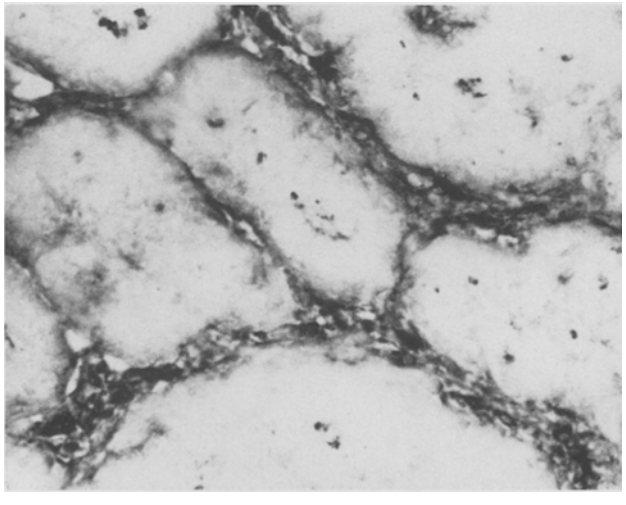

Figure 7. Anti-Lambda light chain stains intensively the connective tissue septae where plasma cells are located. A few follicles stain weakly. Lambda positive dots rarely appear in areas identified by I-A. 90 ×.

substance was present approximately 20–25 μM from the lumen in scattered locations among the parafollicular cells (fig. 4). The I-A positive dots were sharply outlined which might exclude their intercellular location. Transmission microscopy did not support the presence of extracellular substances in this area, but it revealed scattered cell processes with mitochondria (fig. 6). Since these cell processes are reminiscent of the inner segments of photoreceptor cells, the I-A positive dots may represent inner segments of the modified photoreceptor cells. The IgA positive substance in the pineal was not observed until 7 days of age. By 53 days of age the I-A positive substance disappeared from the luminal surface but it was still present with less frequency at other topographical sites. Observations that after 60 days of age pinealocytes formed dense groupings[9,11] suggest that structural and physiological changes continue to occur in the pineal as late as approximately 8 weeks of age. While the I-A monoclonal stained the luminal portion of the pinealocytes, the anti-light chain reacted strongly with connective tissue, identified plasma cells, and did not produce a pattern similar to the I-A mAb (fig. 7).

Failure of the anti-light chain to stain the luminal portion of the pinealocyte suggests that the I-A positive substance in the luminal pinealocyte may not be a complete Ig. Thus, the I-A mAb may be identifying either free α-heavy chains or a polypeptide possessing α-chain homology.

Since the pineal is capable of antibody production[6] the Ig-like substance associated with the B type pinealocytes may be a product of the immune cells of the pineal. On

the other hand, it might be produced by non-lymphoid cells of the pineal. It is conceivable that the substance associated with the modified photoreceptor cell may have an immuno-endocrine role.

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Asiatic cobras: Systematics and snakebite

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Summary. The population affinities of the Asiatic cobras of the genus *Naja* are investigated, using multivariate analysis of a range of morphological characters. This complex, which was formerly thought to be monospecific, consists of at least eight full species. In some cases, species whose bites require different antivenoms occur sympatrically. The new understanding of the systematics of the Asiatic cobra complex calls for a reappraisal of cobra antivenom use in Asia, and for more research into venom composition.
Key words. Naja; cobra; Serpentes; systematics; snakebite; antivenom.

The Asiatic cobras of the genus *Naja* are of considerable medical, social and economic importance in many parts of Asia, killing thousands of people every year[1]. The complex is usually thought to consist of a single species, *Naja naja*, with ten generally recognized subspecies[2-4] (fig. 1). Many of these are poorly defined and heterogeneous, and the population systematics of the group have been a source of confusion for many years. Our study has already shown that some of the conventional subspecies are in fact full species[5-7].

The poor understanding of the population systematics of these snakes is regrettable in view of their medical importance: as has been demonstrated in the carpet viper (*Echis carinatus*) species complex, closely related and morphologically similar species can have venoms with different antigenic qualities. Consequently, the antivenom against one species may not neutralize the venom of another[8], which can lead to greatly increased fatality rates[9,10]. The aim of this paper is to summarize the population affinities of the Asiatic cobra complex, and to relate these findings to the literature on the treatment of bite victims and to venom research.

This study is based on the multivariate analysis of morphological characters recorded from approximately 700 preserved specimens loaned from 29 museums in Europe, the United States and India[11]. 66 morphological characters, listed in table 1, relating to scalation, colour pattern, dentition, internal anatomy and body proportions were recorded from each specimen. Specimens were grouped into operational taxonomic units (OTUs) on the basis of collecting gaps and potential physiographic barriers. The homogeneity of the OTUs was confirmed by principal components analysis. Canonical variate analysis (CVA), one of the most widely used techniques in the analysis of population affinities[12-15], was used for the investigation of the population systematics of the group.

The four CVAs, presented in figure 2, show that the Asiatic cobra populations comprise eight distinct taxa, which we regard as separate species:

- the Indian spectacled cobra, *Naja naja*, from India and neighbouring areas;
- the Central Asian cobra, *Naja oxiana*, ranging from the Caspian Sea to northern India;
- the monocellate cobra, *Naja kaouthia*, which occurs from northern India to Malaysia, Vietnam and the Andaman Islands;
- the Chinese/Indochinese spitting cobra, *Naja atra*, which occurs from eastern China to Thailand. The affinities of the Indochinese populations here included in this species are still unclear, and under further investigation. More than one species may be involved;
- the equatorial spitting cobra, *Naja sumatrana*, from the Malayan Peninsula, equatorial Indonesia and Palawan;