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Comparative Pathology of Inflammation in the Higher Vertebrates (Reptiles, Birds and Mammals)

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Introduction

Metchnikoff's original treatise and subsequent comparative studies of inflammation in animals established that the complexity of the inflammatory process increased with phylogeny (Dobberstein, 1960; Messow, 1960; Metchnikoff, 1891; Zwart, 1963). Inflammation in lower invertebrates is a localized response; in sponges and jellyfish, primitive mesodermal cells (amoebocytes) capable of phagocytosis are mobilized to the point of injury; healing is accomplished with collagen fibril proliferation and ectodermal hyperplasia. In earthworms, pathogens too large to be phagocytosed (e.g. metazoan parasites) are surrounded by coelomocytes and encapsulated in the coelomic cavity. Higher invertebrates, such as molluscs, react to noxious and infectious agents with granuloma-like lesions to which some of the participating leucocytes migrate from closed circulatory systems (Cooper, 1986; Sparks, 1985). This tendency for universal granuloma formation carries over to the vertebrates. Fish respond to most types of micro-organisms with granulomas (Wolke and Stroud, 1978); reptiles and birds appear to react to a wider spectrum of infectious agents with granulomas than do mammals (Frye, 1981b; Jortner and Adams, 1971).

This article will deal with morphological comparisons of inflammation in the higher vertebrates (reptiles, birds, and mammals). Leucocyte morphology and function will be reviewed and the gross and microscopic appearances and relationships of inflammatory cells will be examined in reptiles and birds with emphasis on granuloma formation.

Morphology of circulating leucocytes

Circulating "mononuclear" cells, such as lymphocytes and monocytes, are similar among the higher vertebrates (Andrew, 1965; Bradley, 1937; Hawkey, 1975; Lucas and Jamroz, 1961; Mateo, Roberts and Enright, 1984b), but there is considerable heterogeneity within the granulocytic series. The reptilian and avian granulocyte (heterophil) corresponding to the mammalian neutrophil has a high isoelectric point and stains intensely eosinophilic (Kelenyi and Nemeth, 1969). Heterophil nuclei are usually monomorphic or bilobed but,

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like mammalian neutrophils, can be polymorphic. The term acidophil can be applied to heterophils and eosinophils collectively.

Reptilian acidophils show wide species variation in their size, and granule and nuclear morphology. Chelonia (turtles, tortoises and terrapins) and Crocodilia (alligators, crocodiles, caymans and gavials) have two types of acidophils that were originally designated as morphological variants of a single cell line (Girons, 1970) and were referred to as type 1 and type 2 eosinophils by Pienaar (1962). The stronger evidence, however, supports the view that these acidophils are each derived from separate heterophil and eosinophil cell lines (Mateo *et al.*, 1984b). In Squamata (snakes and lizards), a rare second type of acidophil is described (Pienaar, 1962) and there is some ultrastructural evidence for more than one acidophil precursor in lizards (Kelenyi and Nemeth, 1969; Zapata, Leceta and Villena, 1981), but most squamates have only one type of discernible acidophil in their peripheral blood. In this article, "heterophil" and "eosinophil" will be used for the two types of acidophils in Chelonia and Crocodilia and "heterophil" for the one in Squamata (Ryerson, 1943, 1949; Will, 1979). Morphological details of these acidophils are as follows in Wright-stained smears of peripheral blood from Chelonia and Crocodilia, the heterophil has a single, basophilic peripheral nucleus with eosinophilic fusiform cytoplasmic granules (Fig. 1). The eosinophil is of comparable size with a peripheral, singular or bilobed nucleus and eosinophilic spherical cytoplasmic granules (Fig. 2). In Squamata, the heterophil has a round or multilobed nucleus with eosinophilic, angular or pleomorphic cytoplasmic granules. Snake heterophils usually have monomorphic nuclei and contain poorly formed granules (Fig. 3); lizard heterophils are polymorphic with spindle, rod-shaped or pleomorphic granules (Fig. 4). Most reptiles have basophils with large, round, deeply basophilic granules that often obscure the nucleus (Fig. 4).

Squamata also have a unique leucocyte, the azurophil, a round cell with a single nucleus and fine azurophilic cytoplasmic granules, and have morphological features of the monocyte and granulocyte in one cell (Fig. 3). In lizards, the azurophil frequently gives rise to intermediate stages that appear similar to neutrophils or other granulocyte types (Will, 1978).

Birds have two well-defined acidophils, the heterophil and the eosinophil (Campbell and Dein, 1984; Dein, 1984; Hodges, 1977; Lucas and Jamroz, 1961). In Wright-stained blood smears of most avian species, the heterophil has a basophilic nucleus with one or more lobes and prominent eosinophilic spindle-shaped cytoplasmic granules; the eosinophil usually has a bilobed nucleus (Maxwell, 1985b) and brightly eosinophilic, oval to round, cytoplasmic granules. Variations of granule shape and tinctorial qualities occur in some of the more exotic birds. The Indian spot-bill (*Anas p. poecilorhyncha*), Stanley crane (*Anthropoides paradisea*) and blue-crowned pigeon (*Goura cristata*) have rod-shaped rather than round cytoplasmic granules in their eosinophils; the heterophil granules of hoopoes (*Upupa epops*) tend to stain blue. Despite these differences, acidophils from these uncommon species can be categorized as either heterophils or eosinophils with careful study.

In contrast with heterophils, chelonian, crocodilian and avian eosinophils are peroxidase-positive with the benzidine or p-phenylenediamine methods

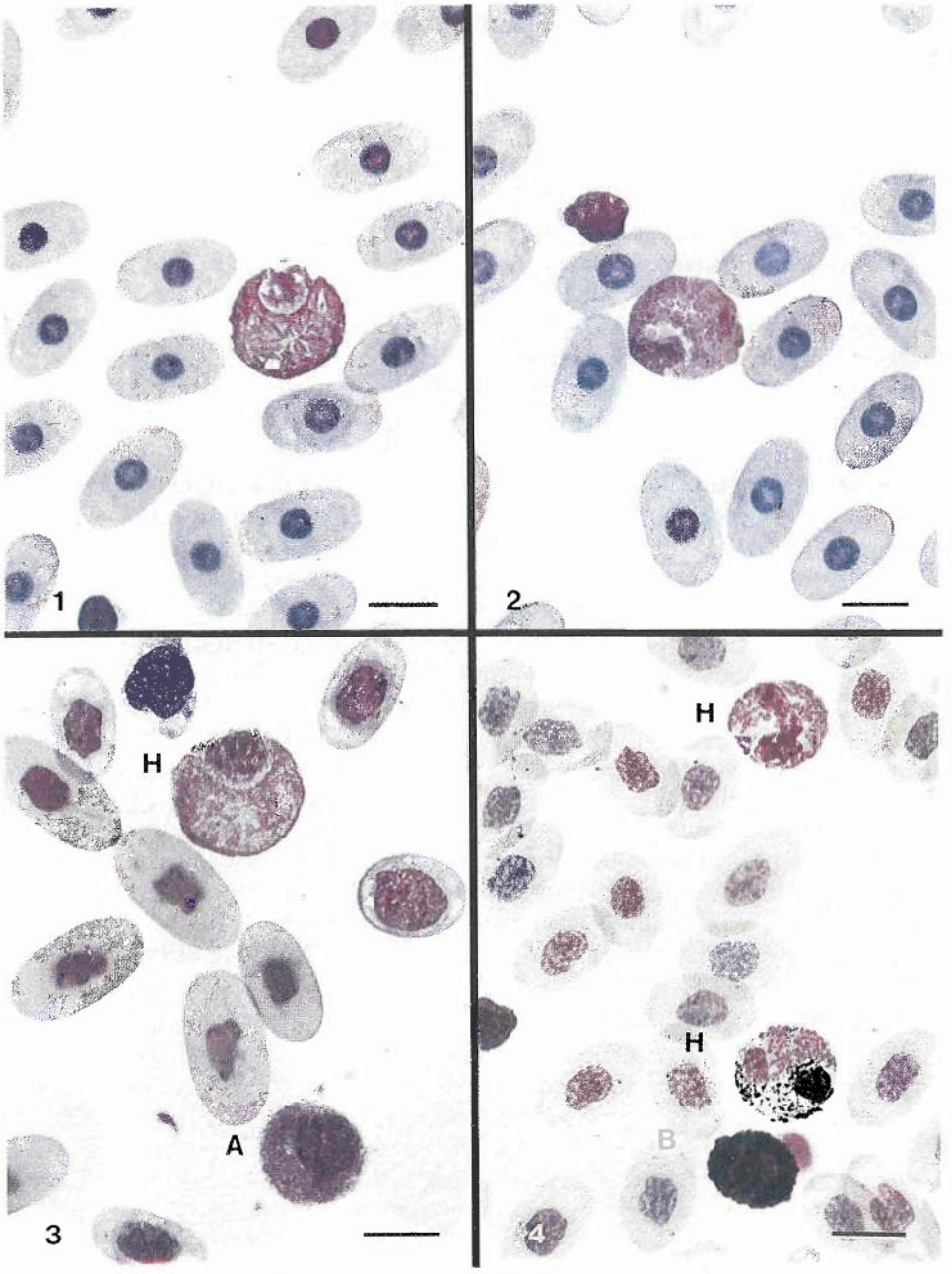

Fig. 1. Peripheral blood smear from an Aldabra tortoise (*Geochelone gigantica*) showing a heterophil. Note angular cytoplasmic granules and monomorphic nucleus. Wright's stain. Bar = 10 μm.

Fig. 2. Peripheral blood smear from Aldabra tortoise showing an eosinophil. Compare granules with those in the heterophil in Fig. 1. Wright's stain. Bar = 10 μm.

Fig. 3. Peripheral blood smear from an emerald tree boa (*Corallus canina*) showing a heterophil (H) and an azurophil (A). Wright's stain. Bar = 10 μm.

Fig. 4. Peripheral blood smear from a basilisk lizard (*Basiliscus plumifrons*) showing two heterophils (H), and a basophil (B). Wright's stain. Bar = 10 μm.

and can be readily differentiated from heterophils by simple cytochemical procedures (Kelenyi and Nemeth, 1969; Mateo *et al.*, 1984b; Maxwell, 1984a). Heterophils and azurophils from Squamata examined in our laboratory, however, show a moderate peroxidase reaction. Reptilian and avian acidophils range from 8 to 20 μm and are largest in snakes, intermediate in chelonians and crocodilians and smallest in lizards and birds (Fig. 5). Table 1 is a comparison of the specific dimensions of leucocytes in a reptile species with intermediate sized leucocytes.

The ultrastructure of acidophilic granulocytes varies widely among reptiles (Desser and Weller, 1979; Kelenyi and Nemeth, 1969; Taylor, Kaplan and Hirano, 1963; Zapata *et al.*, 1981), and they remain mostly unclassified. In birds, two populations of granules are consistently evident by electron microscopy; a large electron-dense type (specific granule) corresponding to the spindle-shaped granule seen by light microscopy and a smaller oval or short, electron-dense rod-shaped granule (Campbell, 1968; Fujimori, Masa-oki and Katsuyuki, 1979; Mateo *et al.*, 1984b; Maxwell, 1973; Maxwell and Trejo, 1970). A third small, round electron-lucent granule has been variably reported in avian heterophils (Brune and Spitznagel, 1973; Daimon and Caxton-Martins, 1977; Dinghra, Parrish and Venzke, 1969). In mammals, crystalloid structures within eosinophil granules differentiate them from neutrophils; similar crystalline arrays have been identified in the eosinophils of some aquatic birds (e.g. duck, geese, shag, and cranes) but crystalloids do not occur in the eosinophils of gallinaceous birds or reptiles (Maxwell, 1978, 1979; Maxwell and Siller, 1972).

Paradoxically, the granulocytes of the tuatara (a primitive reptile), amphibians and fish appear more mammalian-like than those of species that are phylogenetically closer. Amphibian neutrophils have segmented nuclei with fine, almost indiscernible cytoplasmic granules; eosinophils are large, with round eosinophilic granules (Desser and Weller, 1969; Kelenyi and Nemeth,

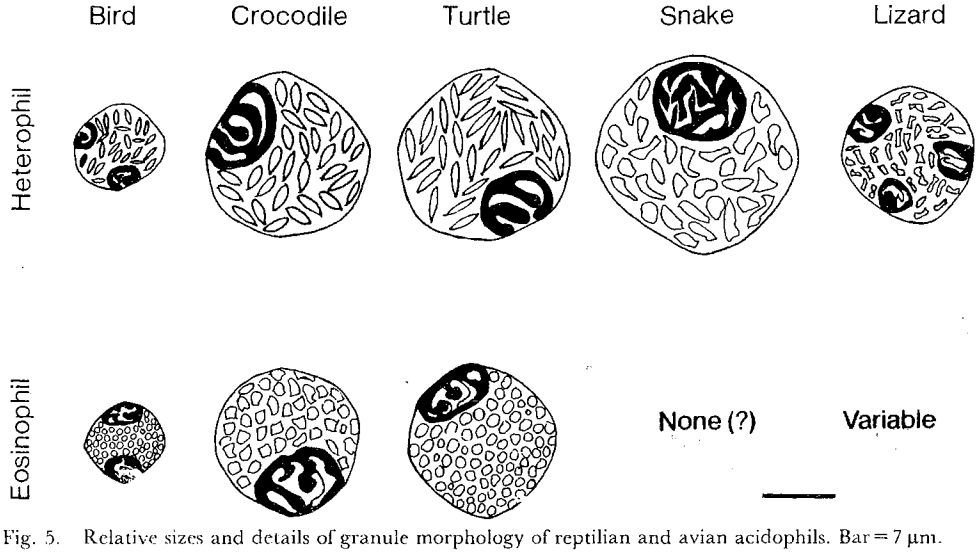

Fig. 5. Relative sizes and details of granule morphology of reptilian and avian acidophils. Bar = 7 μm.

Table 1
Alligator peripheral blood cell dimensions

Cell	*Cell diameter (μm)*	*Nucleus diameter (μm)*
Heterophil	17·3 ± 1·2	7·0 ± 0·02
Eosinophil	14·9 ± 1·9	7·3 ± 0·9
Basophil	12·8 ± 1·3	7·7 ± 0·6 (n = 12)
Lymphocyte	10·7 ± 1·6	9·6 ± 5·7
Monocyte	14·3 ± 1·9	7·1 ± 0·03
Erythrocyte	20·3 ± 1·6	4·9 ± 1·0
Thrombocyte	14·3 ± 3·0	8·0 ± 0·8

Data are expressed as mean ± S.D. n = 35, except where otherwise indicated. Reprinted with permission, Mateo *et al.* (1984b).

1969; Ryerson, 1943). Considerable variation occurs in fish granulocytes; most teleosts (bony fish) have neutrophils with fine to coarse, pale-staining granules and eosinophils with indistinct to large granules. Piscine basophils are almost never observed (Anderson and Mitchum, 1974; Andrew, 1965; Grizzle and Rogers, 1976; Weinreb, 1963).

The terms heterophil or pseudo-eosinophil are often applied to rabbit, guinea-pig, and hystricomorph rodent neutrophils (e.g. porcupine and capybara, *Hydrochoerus hydrochaeris*) because of their prominent eosinophilic cytoplasmic granules (Hawkey, 1975; Kozma, Macklin, Cummins and Mauer, 1974; Schermer, 1967). Rabbit heterophils have been shown to be functionally and biochemically equivalent to the neutrophil of other mammals; in fact, their granules were the first of the mammalian leucocytes to be designated as lysosomes (Cohn and Hirsch, 1960).

Acidophil components and their role in the inflammatory response

Cytochemically, lipids, glycogen, peroxidase and acid and alkaline phosphatases have been identified in the leucocytes of several species of reptiles (Efrati, Nir and Yaari, 1970; Horii, Hayashi and Kumashiro, 1951; Pienaar, 1962). Beta-glucuronidase and naphthol AS-D chloroacetate esterase were identified in West African lizards and in crocodiles, but the specific types of granulocytes or enzyme locations were not defined in those studies (Caxton-Martins, 1977; Caxton-Martins and Nganwuchu, 1978). Table 2 illustrates the cytochemistry of blood cells in *Alligator mississippiensis*. Moderate in vitro phagocytic and microbicidal activity of heterophils to live *Staphylococcus aureus* has been shown in the alligator (Mateo *et al.*, 1984b), and the heterophils of certain lizards are capable of phagocytosing inert substances (Efrati *et al.*, 1970).

Experimentally induced inflammatory responses with irritants are described in several species of reptile. Turpentine injected subcutaneously in turtles will elicit a heterophilic response (Ryerson, 1943). In alligators kept at 25°C and injected subcutaneously with turpentine, heterophils begin to accumulate 4 to 8 h after injection, and monocytes are evident in 24 h. A granulomatous type of change begins to develop after 1 week associated with an inner necrotic layer of heterophils and an outer layer of vacuolated macrophages with multinucleated

Table 2
Cytochemical reactions of alligator blood cells

Test	*Heterophil*	*Eosinophil*	*Basophil*	*Lymphocyte*	*Monocyte*
Naphthol AS-D chloroacetate esterase	—	—	—	+	+
Non-specific esterase	+	—	—	—	+
Acid phosphatase	+	—	+	—	+
Alkaline phosphatase	+	+	—	—	+
Peroxidase (3-amino-9-ethyl carbazole)	+	+	—	—	—
Peroxidase (benzidine)	—	+	—	—	—
Periodic acid-Schiff (no diastase)	+	+	+	+	+
Periodic acid-Schiff (diastase)	—	—	—	—	—
Toluidine blue	—	—	+	—	—
Luxol fast blue	—	+	—	—	—
Alcian blue (pH 1·0 and 2·5)	—	—	—	—	—

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giant cells occasionally attendant. After a month, these lesions develop into mature granulomas surrounded by connective tissue capsules (Mateo, Roberts and Enright 1984a).

Although biochemical differences exist, reptilian heterophils appear to be functionally homologous to mammalian neutrophils. Heterophils are the predominant cell type elicited in experimental irritant-induced inflammation and they appear to play an important role in suppressing microbial invasion. Heterophilia with leukaemoid reactions commonly occur in response to a variety of bacterial and fungal pathogens (Ryerson, 1943). Experimental infections with *Aeromonas hydrophila*, a common Gram-negative bacterial pathogen of reptiles, elicits heterophilia in alligators (Glassman and Bennett, 1978b).

The single acidophil of Squamata probably combines the actions of the heterophil and eosinophil into one cell. This is supported by our findings of a peroxidase reaction in squamate heterophils, since eosinophils are the only strongly peroxidase-positive acidophils otherwise found in non-squamate reptiles (Kelenyi and Nemeth, 1969). Other multi-functional cells occur in snakes, e.g. the gastric granular cell consolidates into one cell the functions carried out by the chief and parietal cells in mammals (Andrew and Hickman, 1974; Leake, 1975).

The function of reptilian eosinophils is currently unknown. Eosinophilia of up to 60 per cent occurred in alligators with experimental leech infections (Glassman, Holbrook and Bennett, 1979), but in natural infections and despite

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Non-specific esterase	+	—	—	—	+
Acid phosphatase	+	—	+	—	+
Alkaline phosphatase	+	+	—	—	+
Peroxidase (3-amino-9-ethyl carbazole)	+	+	—	—	—
Peroxidase (benzidine)	—	+	—	—	—
Periodic acid-Schiff (no diastase)	+	+	+	+	+
Periodic acid-Schiff (diastase)	—	—	—	—	—
Toluidine blue	—	—	+	—	—
Luxol fast blue	—	+	—	—	—
Alcian blue (pH 1·0 and 2·5)	—	—	—	—	—

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the prevalence of parasitism in reptiles, truly eosinophilic responses (in the reptilian species with discernible eosinophils) are not recognized. The specific function of the azurophil of Squamata is also not known; it has been designated by some as the reptilian neutrophil (Frye, 1981a; Girons, 1970; Jordon, 1938; MacMahon and Homer, 1975), but it clearly does not function as the neutrophil does in mammals. The azurophil appears to be derived from a monocyte and contains peroxidase-positive granules similar to mammalian monocytes. Transition from the granulocyte-like azurophil to a mononuclear type under certain conditions may reflect the phylogenetic link between monocytes and myelogenous cells (Will, 1978). In mammals, features that distinguish neutrophils from monocytes become less clear when the neutrophils are altered by infections and there are leukaemias classified as myelomonocytic in which the leukaemia cells appear to share monocytic and granulocytic phenotypes.

Despite some conflicting studies in the 1960s, considerably more is known about the biochemistry and function of avian granulocytes. The earlier studies reported the presence of little or no hydrolytic enzyme activity in mature heterophils of chickens and Japanese quail and raised some doubt as to whether heterophil granules were actually the lysosomal counterparts of mammalian neutrophil granules (Atwall and McFarland, 1966; Merkal and Mora, 1962). This alleged enzyme inactivity was considered to be the reason why suppurative exudates were not liquefied in birds (Carlson and Allen, 1969) as they are in mammals—a process attributed to the proteolytic enzymes of the neutrophil granules (Florey, 1970). Subsequent work showed that the specific (spindle-shaped) granules of bird heterophils are lysosomes, based on a number of hydrolytic enzymes demonstrated cytochemically or by electron microscopy (Table 3). Most of these studies have been in chickens and turkeys (Ericsson and Nair, 1973; Maxwell, 1984a; Osculati, 1970; Topp and Carlson, 1972a). Some of the avian lysosomal enzymes and their activities are contrasted with those of mammals in which considerable differences occur even amongst mammal species (Table 4). Avian heterophil granules also contain trimetaphosphatase, a lysosomal marker that occurs in rabbit granulocytes (Maxwell, 1984b).

The major difference between avian and mammalian granulocytes is that avian heterophils lack peroxidase (myeloperoxidase, MPO) and alkaline phosphatase (Brune, Leffell and Spitznagel, 1972; Brune and Spitznagel, 1973). Both of these enzymes are considered as markers for neutrophil lysosomes and MPO is believed to be the primary mediator of the cell's bacteriocidal properties. Despite not having MPO, chicken heterophils achieve an in vitro killing power against *Serratia marcescens*, *E. coli*, and *C. albicans* that is equivalent to mammalian neutrophils. Phagocytosis by heterophils was originally reported to be weaker than for neutrophils (Atwall and McFarland, 1966), but it has since been shown that chicken and turkey heterophils are capable of active phagocytosis and rapid degradation of staphylococci and other organisms (Carlson and Allen, 1969; Nair, 1973; Topp and Carlson, 1972b). The microbicidal activities of avian heterophils are associated with two electrophoretically-distinct cationic proteins (lysozymes) located in the

Table 3
Comparison of lysosomal enzymes and proteins of avian heterophilic and human neutrophilic granules

Lysosomal enzyme or protein	*Human neutrophil* (primary granules)*	*Avian heterophil (specific granules)*
Acid hydrolases		
acid phosphatase	+	+(fowl†,‡,§,¶, turkey‖)
β-glucuronidase	+	+(fowl[1], goose[1], turkey‖)
n-acetyl-β-glucosaminidase	+	ND[4]
α-glucosidase	+	+(fowl[2])
α-mannosidase	+	ND
arylsulfatase	+	+(fowl ¶)
β-galactosidase	+	ND
5-nucleotidase	+	ND
α-fucosidase	+	ND
acid protease (cathepsin)	+	ND
Neutral proteases	+	ND
Cationic proteins	+	+(fowl[2,3])
Myeloperoxidase	+	NP[5]
Lysozyme	+	+(fowl[1,3] goose[1])

* Klebanoff and Clark (1978).
† Daimon and Caxton-Martins (1977).
‡ Ericsson and Nair (1973).
§ Maxwell (1984b).
¶ Osculati (1970).
‖ Topp and Carlson (1972a).
[1] Rausch and Moore (1975).
[2] Fujimori *et al.* (1979).
[3] Brune and Spitznagel (1973).
[4] Not determined.
[5] Not present.

Table 4
Enzyme activities of neutrophils and heterophils in mammals and birds

Species	*Number tested*	*β-Glucuronidase (nmoles per min)**	*Myeloperoxidase (o.d. per min × 10^{-13})**	*Lysozyme (μg)*	*Alkaline phosphatase (nmole per min)**
Man	7	7·7 ± 2·4	109 ± 44	86 ± 11	11 ± 4
Rhesus monkey	7	3·0 ± 1·3	38 ± 7	< 1	< 1
Cow	4	0·4 ± 0·2	27 ± 2	< 1	38 ± 8
Horse	3	1·0 ± 0·3	39 ± 10	16 ± 1	471 ± 76
Cat	3	2·9 ± 0·7	9 ± 1	< 1	< 1
Dog	6	0·6 ± 0·2	95 ± 26	9 ± 2	8 ± 4
Rabbit	5	0·7 ± 0·2	13 ± 6	15 ± 3	99 ± 46
Rat	10†	5·1 ± 0·6	46 ± 4	10 ± 2	95 ± 34
Guinea pig	6	2·1 ± 0·2	11 ± 3	15 ± 3	471 ± 91
Mouse	2†	0·7	18	10	< 1
Hamster	3†	0·7 ± 0·1	16 ± 3	< 1	273 ± 23
Fowl	3	1·1 ± 0·5	0	84 ± 12	< 1
Goose	4	0·9 ± 0·1	0	24 ± 4	< 1

* Units per 5×10^6 cells ± S.E.M.
† Number of pools tested.
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specific granules as determined by sucrose density gradient (Brune and Spitznagel, 1973) and by ammoniacal silver reaction studies (MacCrae and Powell, 1979). The peroxidase-free chicken heterophil has been a useful model with which to study antibacterial mechanisms which are not dependent upon MPO activity (Bretton-Gorius, Coquin and Guichard, 1978). Chicken granulocytes were also observed in one study to be the main effector cells of natural cytotoxicity against two virus-transformed chicken cell lines (Mandi, Seprenyi, Pusztai and Beladi, 1986).

Mammalian eosinophils are generally accepted as modulators of immediate hypersensitivity and suppressors of helminth infections (David, Vadas, Butterworth, De Brito, Carlvalho, David, Bina and Andrade, 1980; Weller and Goetzle, 1980), but the exact function of avian eosinophils is still unclear. As with mammalian eosinophils, the granules of fowl, duck, and quail eosinophils are lysosomal and contain peroxidase (Maxwell, 1986a, 1986b). It is quite difficult to induce experimental eosinophilia in birds with parasite antigens (Maxwell, 1980), but multiple intraperitoneal injections of bovine serum albumin with aluminium hydroxide into chickens will induce a systemic eosinophilic response; if horse serum is used, a high concentration of reaginic antibody will develop (Maxwell and Burns, 1986). In one study, dinitrochlorobenzene (DNCB) induced a delayed type hypersensitivity and stimulated local eosinophilia of chicken skin (Awadhiya, Vegad and Kolte, 1982) suggesting that avian eosinophils may play a role in delayed hypersensitivity responses (Maxwell and Burns, 1986). In a similar study with DNCB in chickens, heterophils predominated instead of eosinophils and the accumulation of lymphocytes (usual for avian tuberculin delayed responses) did not occur (Huynh and Chubb, 1987). Unexplained peripheral eosinophilias and eosinophilic dermatitis have been reported in fowl (Maxwell, Siller and Mackenzie, 1979) but eosinophils have not been related to avian anaphylaxis or other acute hypersensitivity reactions. There have been some limited associations between eosinophils and nematode infections in grouse and other fowl; in one instance, adult bantams with natural *Trichostrongylus tenuis* infections developed eosinophilia but had no eosinophils in the tissue reactions to the worms nor elevated serum IgE (Maxwell and Burns, 1985).

The acute inflammatory response induced by turpentine in the wing web and mesentery of birds begins with an influx of heterophils and monocytes within hours (Awadhiya, Vegad and Kolte, 1981b, 1982; Carlson and Allen, 1969; Jortner and Adams, 1971; Smith, Blythe and Patterson, 1975); chemical mediators have not been identified yet (Ito and Böhm, 1986). The cellular events in birds differ from mammals by the numerous basophils elicited with the heterophils and by the syncytial giant cells that form from 12 to 72 h after the phlogistic stimulation. Vasoactive amines from degranulated basophils and mast cells initiate early vascular changes at the inflamed site (Carlson and Hacking, 1972; Wight and Mackenzie, 1970a, 1970b). Leucocyte migration is independent of vascular permeability in birds (Awadhiya, Vegad and Kolte, 1980) but the latter gives rise to oedema formation indirectly (Ito and Böhm, 1986).

Exudates: gross and microscopic appearance

Liquefied, creamy pus is not the typical suppurative response in reptiles and birds that it is in mammals. The exudate derived from an accumulation of heterophils usually forms a yellow-white caseous mass which can be "shelled" readily from an abscess (Figs 6 and 7) or peeled from a mucous membrane. The inspissated material can be confused with caseous granulomas, tissue or organ necrosis, hyperkeratosis, sebum or organizing fibrin. Suppurative material accumulated in hollow spaces (e.g. pulmonary airways), forms semi-solid clumps (Fig. 8). Wright-stained smears reveal these exudates to be predominantly mixtures of degranulated and degenerated heterophils. The dynamics of pus formation have not been defined in vertebrates below mammals, but the differences probably relate to hydrolytic enzyme activity variances (Table 4), or the lack of proteases (e.g. cathepsin), which have not been identified thus far in heterophils (Maxwell, M. H., pers. comm.).

In tissue sections stained with haematoxylin and eosin (HE), reptilian heterophils usually appear heavily granulated (Fig. 9). The granules stain deeply eosinophilic, but occasionally appear yellow-brown or copper-coloured like haemosiderin (Fig. 10). In sections prepared from well-preserved chelonian or crocodilian tissues in buffered neutral 10 per cent formalin, the fusiform shape of the heterophil granules is sometimes retained, but more often than not, the granules lose their definition and "round up". Avian heterophils in tissue sections appear as brightly eosinophilic cells with bilobed or round nuclei (Fig. 11), but their granules usually become round or confluent. For these reasons, it is difficult to distinguish heterophils from eosinophils in routine sections; acidophils in tissue sections usually are called heterophils until proven otherwise with spccial techniques. Bird eosinophils can be distinguished from heterophils in tissue sections with Sudan Black B as this stains them for phospholipids. An indirect peroxidase stain with p-phenylenediamine dihydrochloride will differentiate bird eosinophils from heterophils in paraffin wax sections. The eosinophils stain deeply brown while the heterophils show only the counterstain (Maxwell, 1984a, 1985a).

Once heterophils accumulate in tissues, they degranulate and clusters of these cells may be mistaken for degenerated mononuclear cells, particularly when found on mucosal or serosal surfaces (Fig. 12). Azure-eosin or tissue Giemsa stains will highlight granules within partially degranulated cells or dispersed into extracellular locations (Fig. 13).

Progression and resolution of inflammation in reptiles and birds with naturally occurring infections

Lesions in birds with early pulmonary aspergillosis are characterized by heavy accumulations of heterophils in the bronchi and capillary air spaces (Fig. 14). In tissue sections, these appear as eosinophilic masses of round cells with round nuclei. The most centrally located cells are necrotic and difficult to identify as heterophils. Cells in the mid and outer zones are better preserved, but are usually without granules. More intact heterophils can be discerned in the margins of the lesions (Fig. 15). In more fully developed lesions, the heterophilic

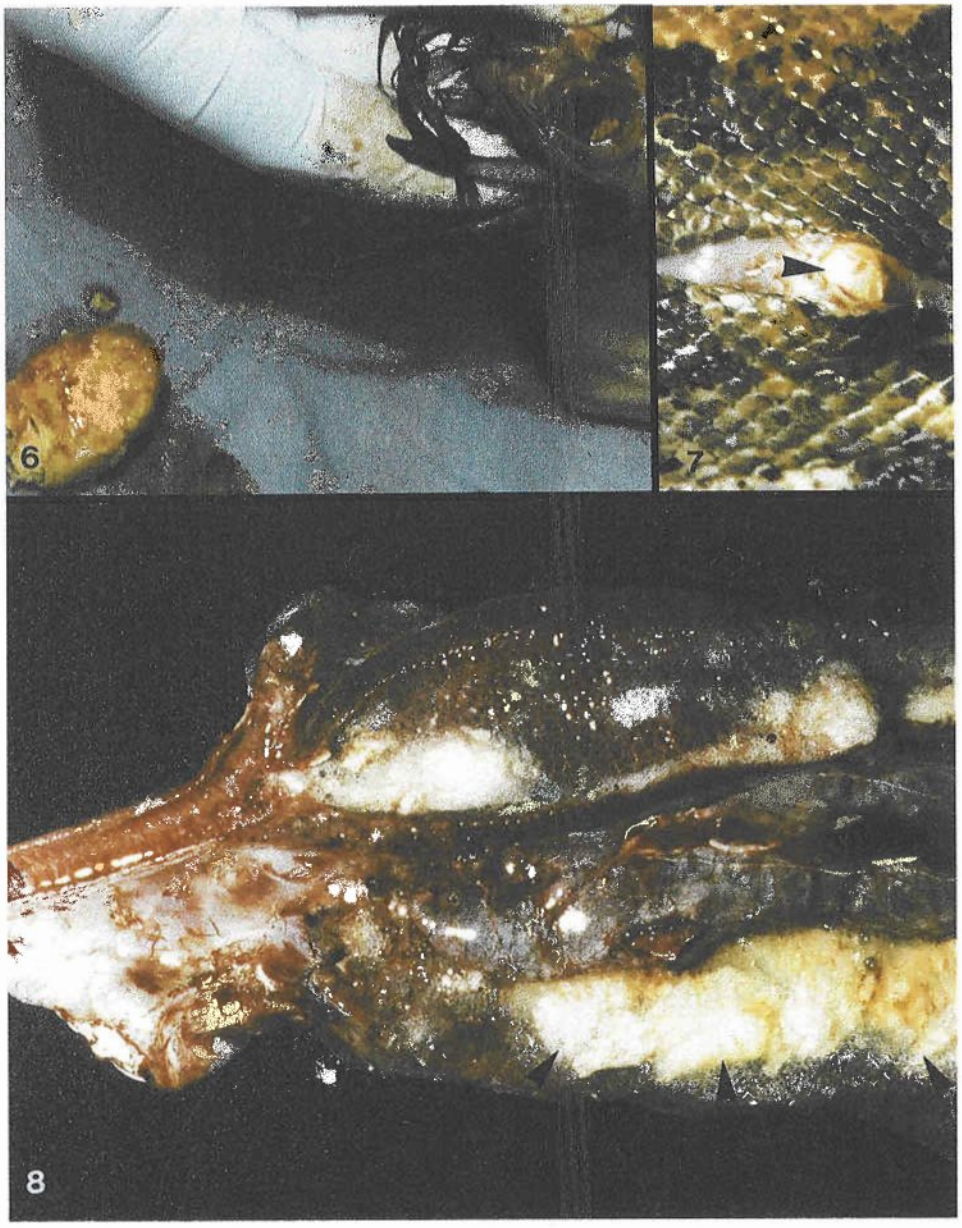

Fig. 6. Gross appearance of the exudate (left) "shelled" from an abscess on the wing tip (right) of a rufous hornbill (*Buceros hydrocorax*).

Fig. 7. Skin abscess in a boa (*Boa constrictor*) with exudate still intact (arrow).

Fig. 8. Gross appearance of lungs from a Burmese python (*Python m. bivittatus*) with bacterial pneumonia. Mesobronchus distended with semi-solid masses of exudate (arrows) mixed with serous fluid and mucus.

masses undergo necrosis and become surrounded by macrophages (Figs. 16 and 17) and eventually by giant cells (Fig. 18). Lesions that began as focal accumulations of heterophils (heterophilic abscesses) progress to granulomas (Fig. 18) and this can happen within a week or less; heterophilic abscesses and the developing granulomas can frequently be observed in the same section. These "acute" types of granulomas (heterophilic granulomas) have no lymphocytes or plasma cells associated with them and do not undergo calcification. Giant cells in the lesions are predominantly the foreign body type containing cytoplasmic vacuoles and many nuclei.

In birds surviving the acute stages of aspergillosis, heterophilic granulomas appear to develop into "chronic" granulomas with epithelioid cells, lymphocytes, plasma cells and connective tissue elements (Fig. 19). Fungal hyphae persist in these lesions, often within giant cells. These lesions commonly spread to the liver, kidneys, brain and other organs by vascular routes.

Granulomas also develop in birds in response to many different kinds of Gram-positive and Gram-negative bacteria including staphylococci, salmonella, and arizona. Coligranuloma (Hjarre's Disease) of domestic fowl is a classic example of a Gram-negative enteric organism (*Escherichia coli*) producing granulomas in the digestive tract, mesentery and liver (Gross, 1984).

Reptiles respond similarly to fungi with heterophilic granulomas and also to bacterial infections, particularly by pseudomonas, aeromonas, citrobacter, salmonella, arizona and proteus (Jacobson, 1984). Subcutaneous bacterial abscesses, common in reptiles, develop into heterophilic granulomas. With some systemic types of infections, such as bacterial pneumonia, there is first an accumulation of heterophils into the air spaces (Fig. 20) followed by degranulation and necrosis of the heterophils (Fig. 21) and a progression to heterophilic granulomas (Fig. 22) as described in birds. If the reptile survives, "chronic" granulomas develop.

Granulomas induced by intracellular pathogens such as mycobacteria (histiocytic granulomas) at certain stages resemble heterophilic granulomas, but those induced by tissue-invasive metazoal parasites infecting reptiles and

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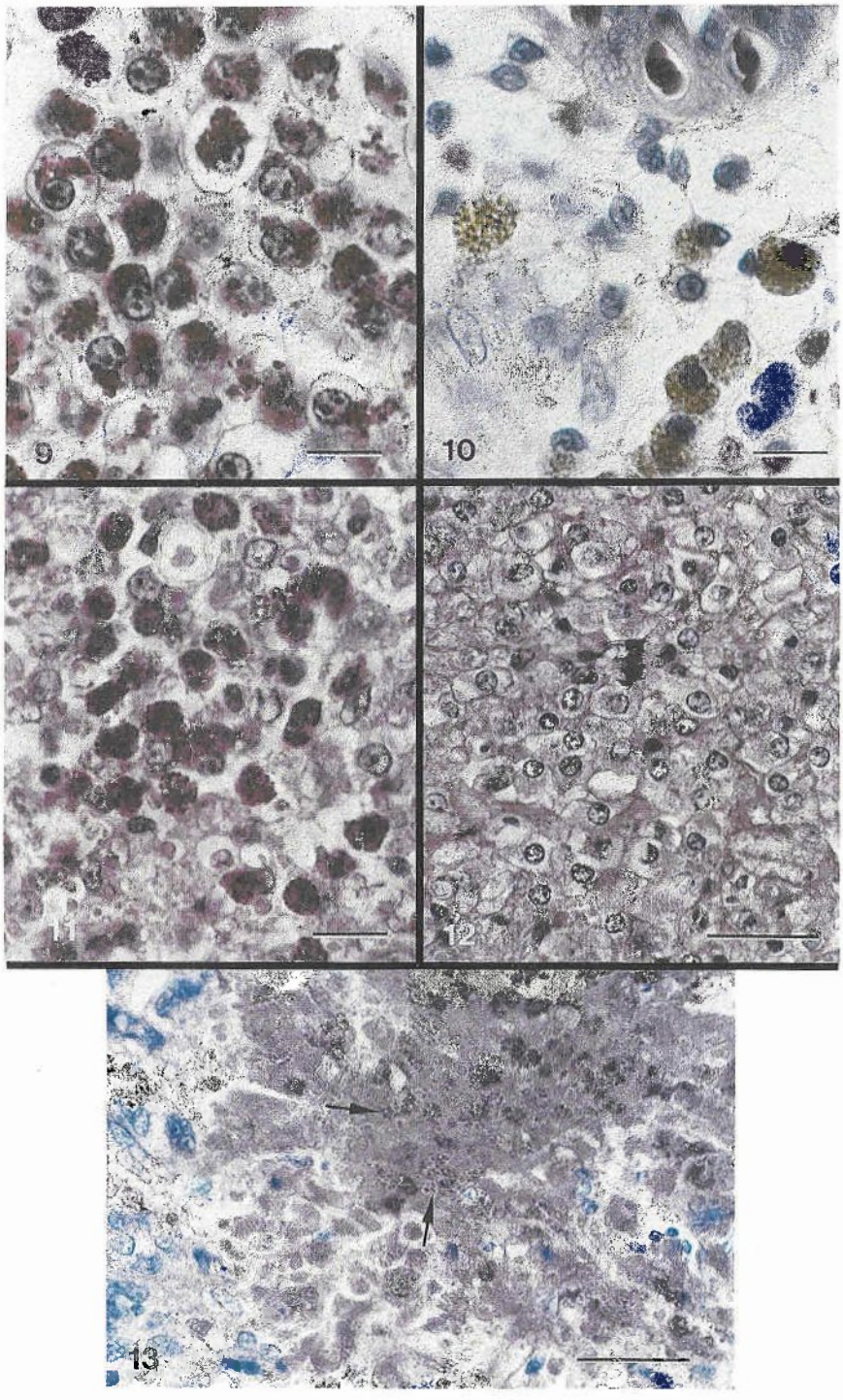
Fig. 9. Heterophil accumulation in the colonic crypt of a leopard tortoise (*Geochelone pardalus*) with *Aeromonas hydrophila* infection. Note the prominent eosinophilic cytoplasmic granules and the predominantly monomorphic nuclei. HE. Bar = 10 μm.

Fig. 10. Heterophils, with brown cytoplasmic granules, which have infiltrated the lung of an emerald tree boa. HE. Bar = 10 μm.

Fig. 11. Heterophil infiltration in the brain of a hybrid macaw with cerebrospinal nematodiasis. Most cytoplasmic granules are swollen or have become confluent. Compare their size with those of tortoise heterophils in Fig. 9. HE. Bar = 10 μm.

Fig. 12. Aggregates of degranulated heterophils from the colonic mucosa of a red-footed tortoise (*Geochelone carbonaria*) with *Edwardsiella tarda* infection. Cells are mostly monomorphic. HE. Bar = 25 μm.

Fig. 13. Giemsa stain of the necrotic centre of a subcutaneous heterophilic granuloma from an iguana lizard (*Iguana iguana*) highlights the extracellular granules (arrows) released from degenerated heterophils. Bar = 25 μm.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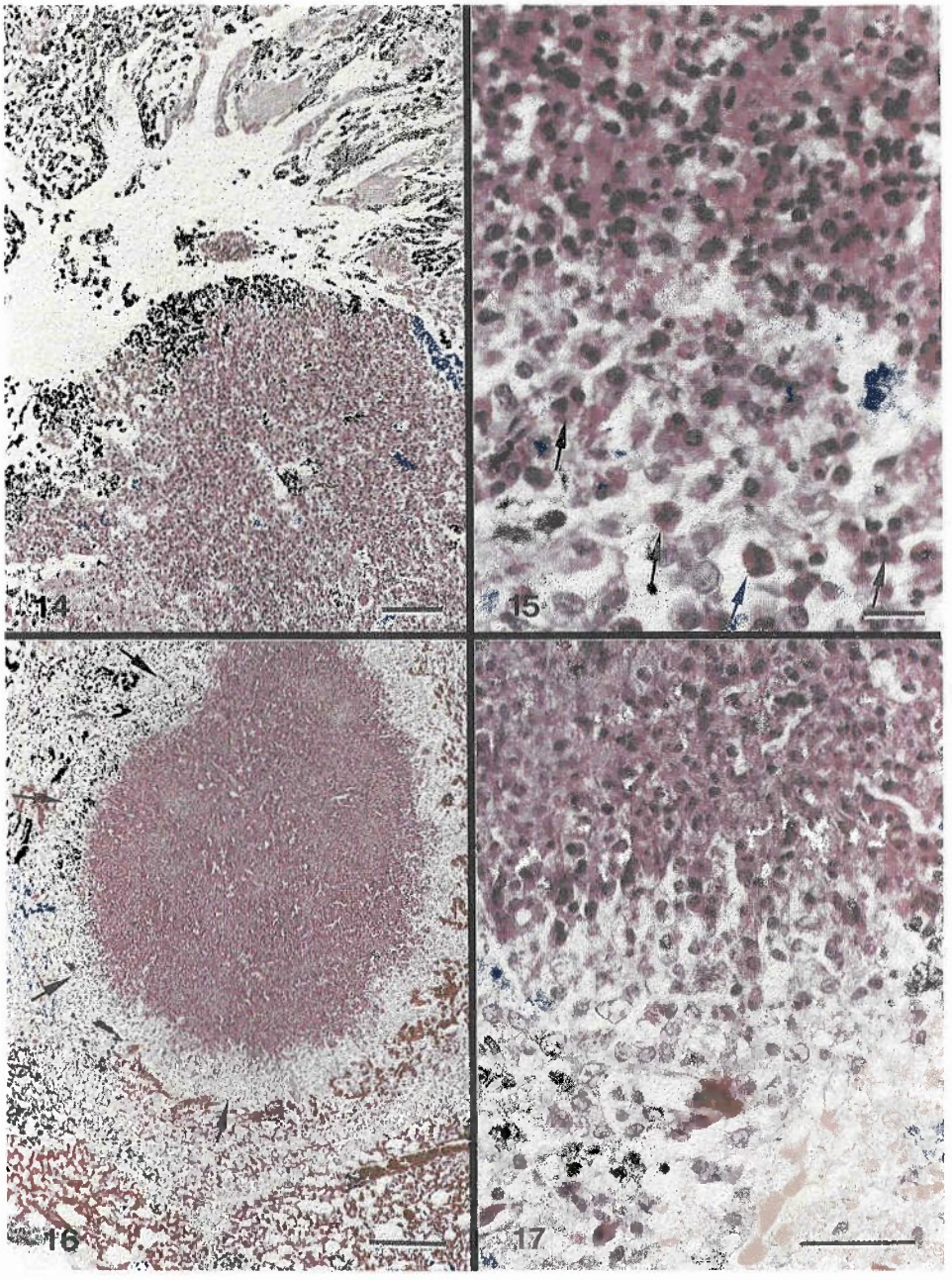

Fig. 14. Tertiary bronchus partially filled with heterophils (lower half) from a red-crested pochard duck (*Netta rufina*) in the early stages of aspergillosis. HE. Bar = 50 µm.

Fig. 15. Magnified area of bronchial heterophils from Fig. 14. Inner zone of cells are degranulated and degenerated (upper half); more intact heterophils are in the periphery (arrows). HE. Bar = 10 µm.

Fig. 16. A bronchus from an American eider duck (*Somateria m. dresseri*) with aspergillosis showing a mass of degenerated heterophils encircled by macrophages (arrows). HE. Bar = 100 µm.

Fig. 17. Magnified edge of lesion from Fig. 16. Macrophages accumulated (lower half) at the periphery of the degenerated mass of heterophils in the bronchus. HE. Bar = 25 µm.

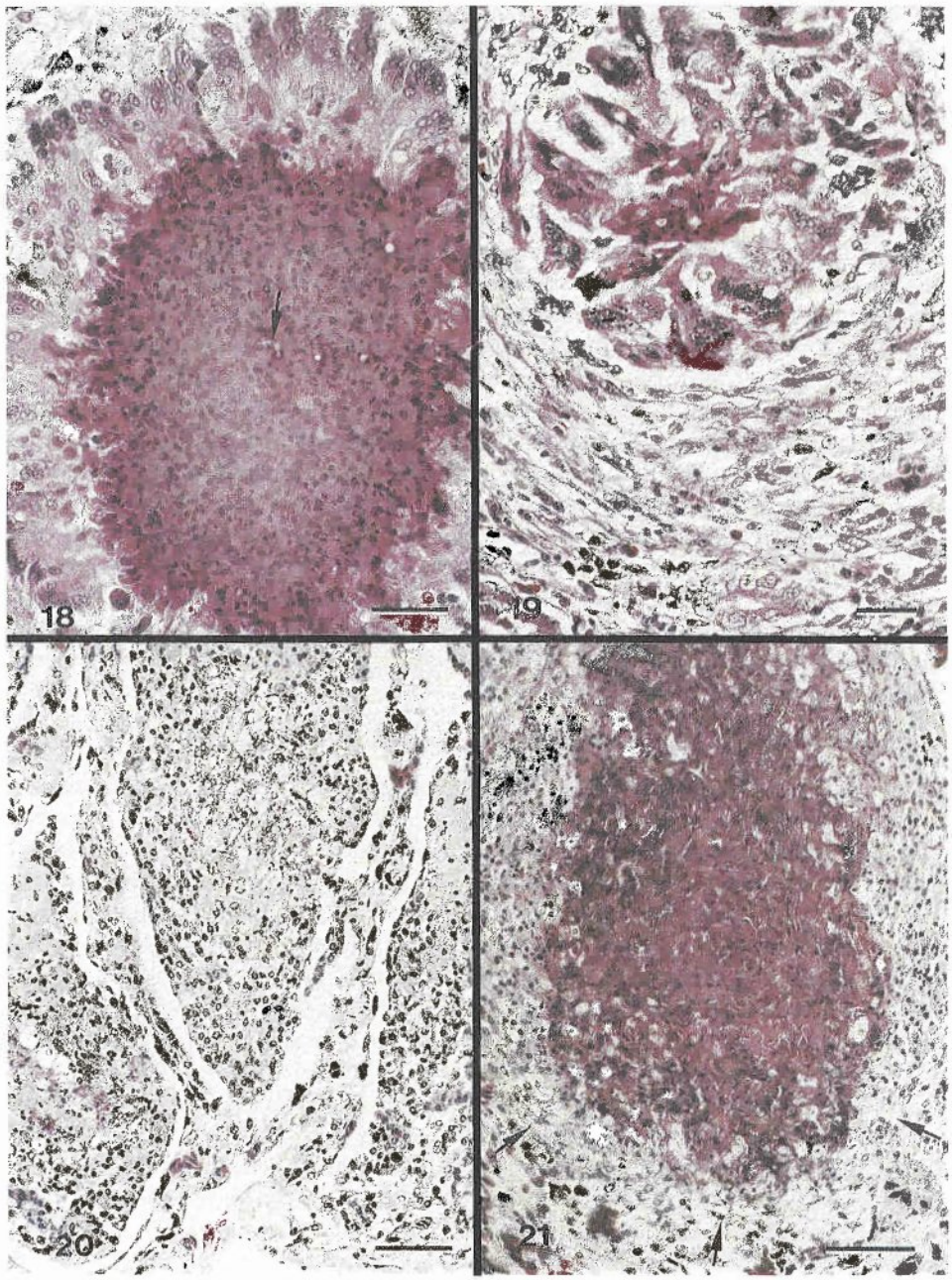

Fig. 18. The later stage of acute pulmonary aspergillosis in an American eider duck shows a condensed mass of necrotic heterophils surrounded by giant cells (heterophilic granuloma). Remnants of fungal elements are evident centrally (arrow). HE. Bar = 25 μm.

Fig. 19. Granuloma in the liver of a Patagonian crested duck (*Anas s. specularioides*) with chronic aspergillosis. Epithelioid cells infiltrated with lymphocytes and plasma cells surround a cluster of giant cells. HE. Bar = 25 μm.

Fig. 20. Lung from tegu lizard (*Tupinambis teguixin*) with a Gram-negative bacterial pneumonia. Air spaces are filled with heterophils many of which are degranulated. HE. Bar = 100 μm.

Fig. 21. Magnified field from Fig. 20 shows aggregates of degenerated heterophils with macrophages accumulated around the periphery (arrows). HE. Bar = 50 μm.

birds tend to be chronic, fibrosing and usually undergo calcification. Other types of classic responses such as phlegmonous (cellulitis), muco- or fibrinopurulent and catarrhal inflammations are also observed in these species. These inflammatory reactions may resolve completely or persist as subacute or chronic forms with fibrosis or granulation tissue. "Sulphur granule" formation in actinomycotic and fungal infections as described in mammals (Splendore–Hoeppli reaction), appears to be uncommon in reptiles and birds but recently has been reported in iguana lizards associated with neisseria infections (Plowman, Montali, Phillips, Schlater and Lowenstine, 1987).

Proliferative types of inflammation are also seen in which adjacent epithelial cells or the inflammatory cells themselves proliferate. Cryptosporidiosis, for example, induces a lymphocytic response in the gastric mucosa of snakes accompanied by extensive hyperplasia and hypertrophy of the gastric mucous-neck cells (Brownstein, Strandberg, Montali, Bush and Fortner, 1977). An unusual proliferative lesion occurs in pheasants particularly the golden pheasant (*Chrysolophus pictus*), which are susceptible to the caecal nematode, *Heterakis isolonche* (Griner, Migaki, Penner and McKee, 1977). The parasite causes prominent caecal nodules which are composed of elongated fusiform epithelioid cells. These were reported earlier as parasite-induced sarcomatous lesions (Helmboldt and Wyand, 1972).

Reptilian and avian granuloma formation: possible mechanisms

In heterophilic granulomas, the central masses of necrotic heterophils appear to act as "foreign bodies" and stimulate a strong macrophage response. Possible necrotoxic substances (Ryan and Magno, 1977) from freed heterophil granules or other cellular components in the necrotic mass could mediate this type of reaction. The organisms, inciting and potentially altered by the original acute response, would be further degraded by the ensuing granuloma. Giant cells can be initiated in birds in a matter of hours (both in vivo and in vitro), and do not necessarily indicate chronicity as they tend to do in mammalian lesions (Awadhiya, Vegad and Kolte, 1981a; Jortner and Adams, 1971; Olah, McCorkle and Glick, 1980).

Granuloma induction by necrotic tissue has been observed in experimental studies of inflammation in the chicken (Jortner and Adams, 1971), and in ducks inoculated subcutaneously with killed salmonella and lymphocyte antigens (Bell, 1974). In the latter case, heterophils accumulated, degranulated and underwent necrosis and then were acted upon by macrophages and multinucleated giant cells, all within a week of inoculation. In turkeys with natural *Salmonella arizona* brain infections, granuloma formation was attributed to the necrosis resulting from the inflammatory process rather than to the inciting organisms themselves (Jortner and Larson, 1984). Since, as previously discussed, liquefaction of exudates derived from heterophils does not occur readily, heterophilic granuloma formation may represent a less specialized mechanism for the dispersal of such exudates than the apparently more evolved proteolytic enzyme systems operant in mammals.

Heterophilic granulomas are qualitatively similar in reptiles and birds;

however, the temporal staging of their formation in reptiles may depend on the ambient temperature since reptiles are ectothermic and their metabolic regulation is reliant upon environmental conditions (Glassman and Bennett, 1978b). Induction of inflammation in alligators described earlier was considerably slower than that of birds, but this may have been because the experiments were carried out at suboptimal temperatures (Mateo *et al.*, 1984a). Variations in the inflammatory response rate may have clinical relevance; cutaneous "abscesses" often persist in Squamata probably because of their relatively cool skin temperature. The lesions are therefore best surgically ablated as they are usually refractory to antibiotics and become indolent (Cooper and Jackson, 1981).

Histiocytic granulomas induced by intracellular bacteria appear to have a different pathogenesis from heterophilic granulomas. As with experimental mycobacterial infections in reptiles (Marcus, Stottmeier and Morrow, 1975) and birds (Feldman, 1938), the early lesions are formed from organized collections of large foamy macrophages. In birds, the macrophages in these granulomas are frequently laden with mycobacteria. Eventually, necrosis of the most centrally located macrophages develops (Figs. 23 and 24) often accompanied by a few heterophils. In birds, extensive proliferation of giant cells occurs, apparently in response to the necrosis (Fig. 25); in reptiles, the degree of giant cell formation is more variable. Histiocytic granulomas progress to either large caseo-necrotic masses harbouring many extracellular bacilli, or to more typical tubercles of the type associated with delayed hypersensitivity reactions in mammals (Dannenberg, 1978). In reptilian and avian tuberculosis, caseous material often accumulates and persists, probably because lesions do not undergo liquefaction or cavitation and at this stage, macrophages might be less phagocytic (Arecchi, Saliba and Mariano, 1980); also calcification, as seen in mammalian tuberculosis, seldom occurs. Heterophilic granulomas may be confused for mycobacterial lesions and must be differentiated by careful histological examination and by isolation and identification of causative organisms (Brownstein, 1980; Montali, Bush, Thoen and Smith, 1976).

In short, the major morphological difference between heterophilic and histiocytic granulomas lies in their necrotic centres. The necrotic centres of heterophilic granulomas represent remnants of heterophil aggregates which accumulated in response to the pathogens initiating the reaction (Fig. 26); the necrotic centres of histiocytic granulomas represent remnants of macrophage aggregates which were stimulated by intracellular pathogens, such as mycobacteria (Fig. 27). Each of the cell types that gave rise to the necrotic centres is discernible histologically during the genesis of the lesions but not usually at the final stage.

Inflammation and immunity

Anatomically, birds do not have an elaborate lymphatic system and lymph nodes like mammals, but lymphoid aggregates occur throughout their respiratory and digestive tracts (Hodges, 1974) and sometimes within their

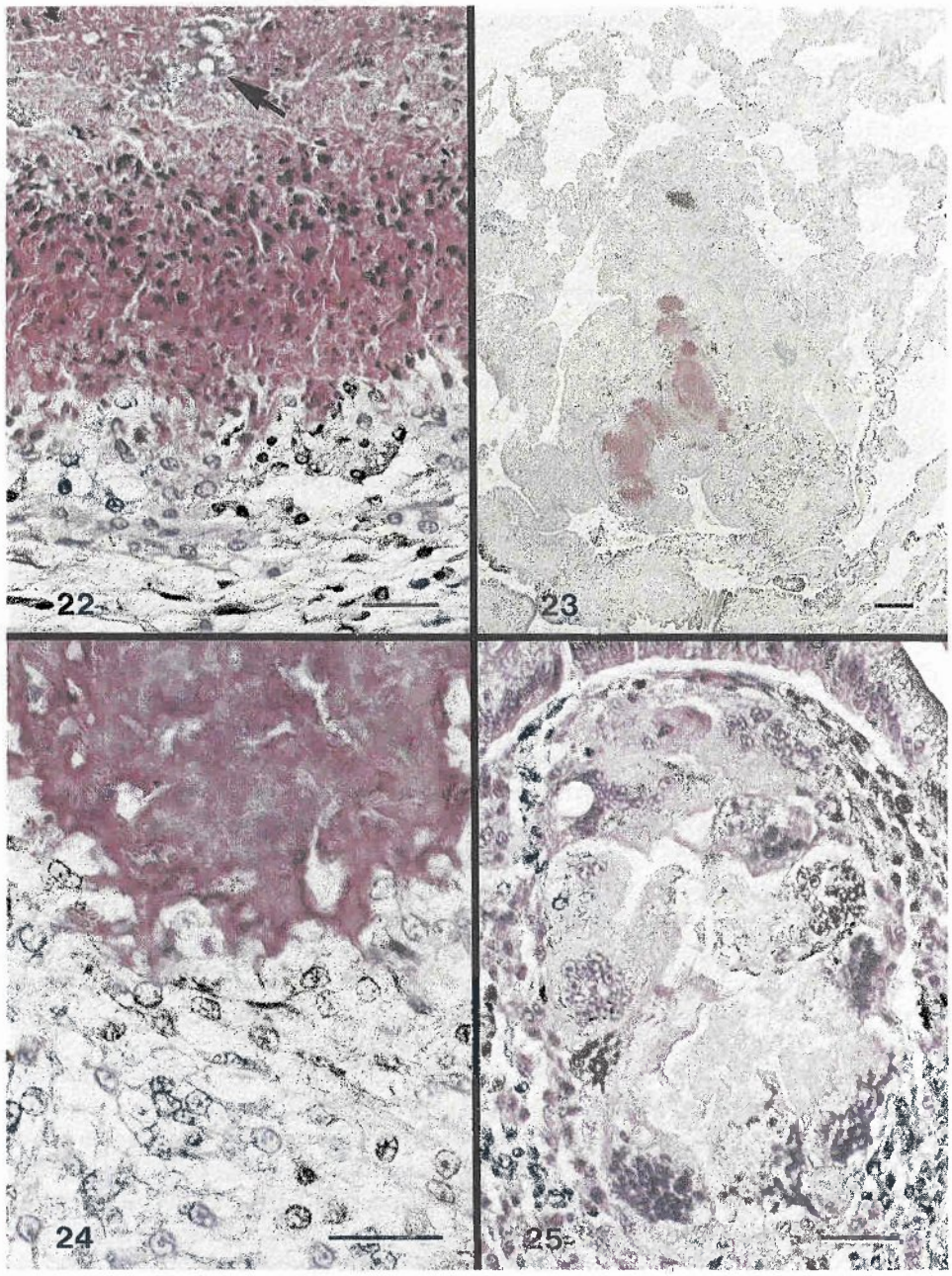

Fig. 22. Edge of heterophilic granuloma from tegu lizard with pneumonia. Aggregates of necrotic heterophils with bacterial colonies (arrow) surrounded by organized masses of macrophages (lower half). HE. Bar = 25 μm.

Fig. 23. Lung from emerald tree boa with early tuberculosis. HE. Bar = 200 μm.

Fig. 24. Magnified field from Fig. 23 shows details of aggregate of necrotic macrophages (top). HE. Bar = 20 μm.

Fig. 25. Tubercle from the small intestine of a rufous motmot bird (*Baryphthengus ruficapillus*) shows a cluster of multinucleated giant cells within the necrotic centre. HE. Bar = 25 μm.

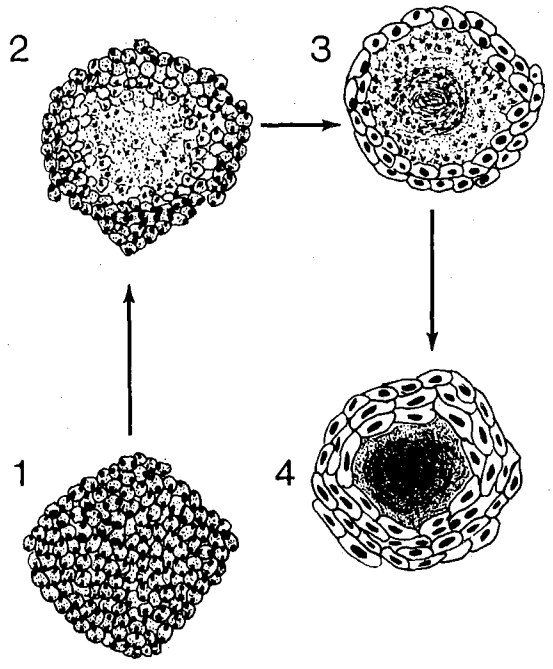

Fig. 26. Heterophilic granuloma formation in reptiles and birds. 1. Heterophils accumulate in response to extracellular pathogen. 2. Stage of heterophil degranulation and degeneration. 3. Macrophages surround necrotic aggregates of heterophils. 4. Formed granuloma.

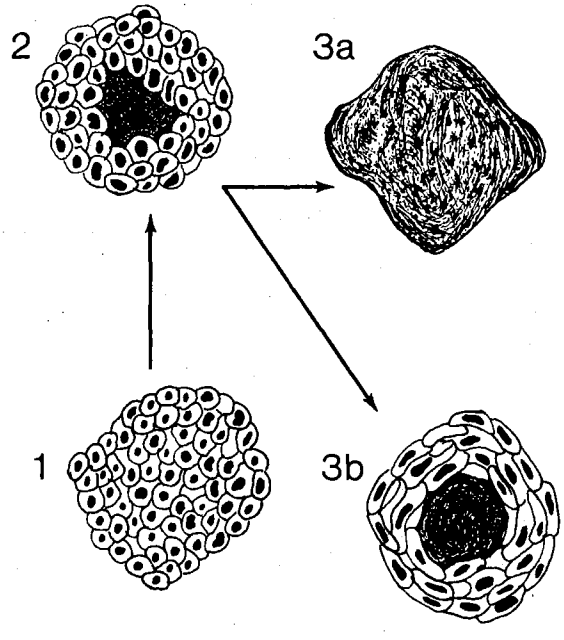

Fig. 27. Histiocytic granuloma formation in reptiles and birds. 1. Macrophages accumulate in response to intracellular pathogen. 2. Aggregates of macrophages undergo necrosis centrally. 3a. Progression to large areas of caseation or, 3b, formed granuloma.

parenchymal organs. Lymphoid aggregates commonly noted in the hepatic portal triads in birds are probably the result of previous antigenic stimulation, since perivascular lymphoid aggregates readily occur at the end stages of experimentally-induced inflammation in fowl (Awadhiya *et al.* 1980). They persist in ducks inoculated with cellular and soluble antigens after resolution of the acute responses (Bell, 1974). Most reptiles do not have lymph nodes or even a bursa of Fabricius, but like birds they do have a thymus, spleen, and lymphoid aggregates throughout body organs (Borysenko, 1978).

As with all vertebrate classes, reptiles and birds are capable of mounting both cell-mediated and humoral responses (Marchalonis, Rosenshein and Schluter, 1985). With the exception of the anatomic dichotomy of the immune system, the immunology of the fowl is similar to that of mammals; fowls synthesize both 7S (IgY, equivalent to IgG) and 17s (IgM) immunoglobulins; their cellular immune functions include interaction of helper and suppressor T cells, delayed hypersensitivity, graft-versus-host reactivity, macrophage activation and cytotoxic responses (Chi, Galton and Thorbecke, 1980; Glick, 1985). Reptiles are capable of eliciting similar immunoglobulins as birds, but antibody production is temperature-dependent and macroglobulin responses predominate; allograft rejection also occurs (Cohen, 1971; Du Pasquier, 1973). Although much is known about the ontogeny of the immune system in reptiles, there is a deficiency of clinically applicable immunological data (Kollias, 1984).

In fowl, the role of cellular immunity as an important defence against infectious disease is best exemplified by tuberculin skin response, lymphocyte transformation and ELISA tests. These all correlate well in tuberculous chickens (Thoen and Karlson, 1984). Reactions to a host of intradermally injected antigens in turkey and quail also fulfil the criteria for delayed hypersensitivity mechanisms (Rose and Bradley, 1977). In exotic birds, however, reliance on intradermal tuberculin testing to diagnose tuberculosis has been unfavourable (Bush, Montali, Smith and Peratino, 1980). This, along with the great variability in lesion types seen in tuberculous exotic birds (Montali, Bush and Smith, 1980) suggests that considerable heterogeneity exists amongst the majority of avian orders in their immunological sensitivity and reactivity to the same organisms. Susceptibilities of different groups of zoo birds to opportunistic pathogens such as *Aspergillus fumigatus* are also known to vary widely. Most pelagic birds (for example, sea ducks) develop fatal acute aspergillosis with heterophilic abscesses and granulomas, while other bird groups respond with more chronic forms of the disease and develop epithelioid granulomas. This might indicate inherently suppressed cellular immune reactions in the pelagic birds since it is unlikely that they would ever encounter such opportunistic fungal pathogens in their natural environment far from land; in the birds more resistant to aspergillosis, delayed hypersensitivity evidently plays a role as with fungal infections in mammals.

The use of the term "granuloma" to characterize lesions composed of orderly arrangements of macrophages as described in this paper, is in keeping with the general definition of granuloma that has been applied to mammals (Adams, 1976). Attempts to further classify the heterophilic granuloma according to existing schemes for mammals, however, is difficult (Adams, 1976;

Ridley, 1983), but it is a unique lesion that explains the prevalence of granulomatous responses in reptiles and birds.

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