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From the Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London

Generalized Tuberculosis in a South American Frog *Leptodactylus pentadactylus*

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Spontaneous tuberculosis is a well-recognised disease in amphibia. In view of the ubiquity of free-living acid fast bacilli in the environment of frogs and toads, it is surprising that the disease is not more widespread. However, amphibian pathology has not yet been studied at all fully and the paucity of reports in the literature is probably misleading. It is curious that no case has been recorded from North America, Africa or the East although amphibia exist in large numbers all over the world except at the poles. The fullest description of the naturally occurring disease in the wild comes from Northern Brazil where tuberculosis is endemic in *Leptodactylus pentadactylus* living in the neighbourhood of fresh-water ponds in the suburbs of Salvador (Bahia)[1]. We are reporting another example of this condition in such a frog because of the unusual nature of the eye lesion which arose as a complication of long-standing tuberculosis.

Case Report

A male South American "bullfrog", *Leptodactylus pentadactylus*, aged about 6 years and previously in good health, was first noticed to have partial opacity of the right eye in November 1963. The lesion was described by the owner as "a pale mistiness at the back of the eye". Subsequently, the whole eye became pale blue and apparently blind. At about this time a mass of soft, boggy, reddish tissue appeared and grew rapidly in the upper part of the eye, displacing the lid. This tissue, with part of the upper lid, was removed for microscopy on 16 January 1964 in the belief that it was a neoplasm. Penicillin powder was put in the cavity but the operation site continued to discharge sero-sanguinous material over the next few weeks. Redundant tissue reappeared in the wound and although the animal seemed well and had not lost weight, it was thought wise to destroy it. The photograph (Fig. 1) was taken immediately before ether euthanasia on 6 February 1964.

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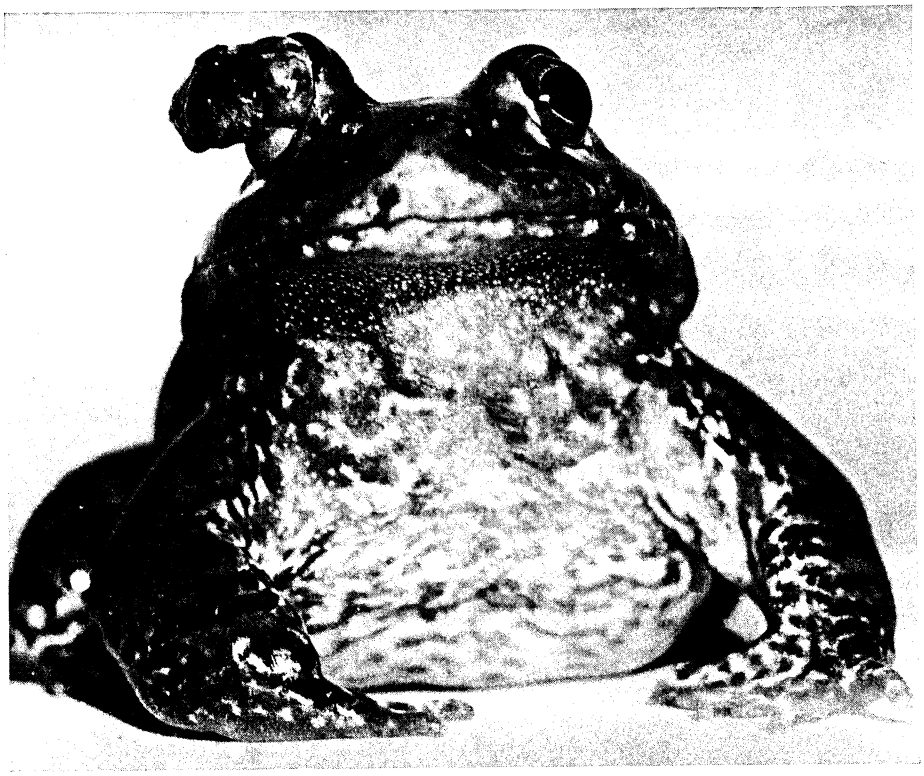

Fig. 1. Full-face view of frog just before death. Note the relation of the tumour to the upper eyelid and eyeball.

Post Mortem Findings

The animal measured 150 mm from snout to vent (range for this species 150–200 mm) and was well nourished. There was a soft, fleshy, haemorrhagic mass about 8 mm in diameter protruding from the right orbit and displacing the eyelid upwards and backwards. Most of the eyeball was overhung and apparently replaced by tumour. The lower part was smooth, pale blue and opaque.

There was a grey, translucent nodule $3 \times 3 \times 1$ mm in the visceral pericardium of the anterior wall of the ventricle. The lungs were congested but apart from a tiny yellow nodule on the pleura of the right lung, there were no focal lesions. The liver was dark brown, flecked with yellow and gritty. Several minute grey dots, less than the size of an average pinhead, were present on the posterior aspect beneath the capsule. Neither the liver nor the spleen, which was also flecked and gritty, were enlarged. The stomach was normal but the wall of the first 180 mm of the intestine contained numerous small round lesions, seen best with a $\times 8$ hand lens. In the remainder of the intestine the lesions were larger, measuring up to

1 mm, were clumped together and in some places extended into the adjacent mesentery. Serosal, pedunculated 1 mm nodules were scattered at intervals along the intestine.

Histology

The right eye was filled with abundant granulation tissue which occupied both anterior and posterior chambers and replaced the lens and retina (Fig. 2). This tissue was composed of many discrete nodules in a loose framework of connective tissue fibres (Fig. 3). The meshes of this framework contained inflammatory cells, deposits of melanin from the disorganised choroid coat and retina and pools of serous fluid. The focal lesions were composed of mononuclear cells surrounded by a reticulin capsule. There was central caseation in the larger lesions and some attempt at epithelioid cell formation. No giant cells of the Langhans type were present but foreign body giant cells were clustered around pieces of non-refractile material. The inflammatory process had expanded the eye, pushed the scleral cartilage downwards and forwards and entered the retro-orbital space. Small lesions were present in the substance of the upper lid but the skin was intact. There was considerable haemorrhage into the superficial part of the inflammatory mass. A few intracellular. long, narrow, acid-fast bacilli, beaded at each end, were seen in the superficial part of the lesion with Ziehl-Neelsen's stain but no other organisms were identified in Gram or periodic acid Schiff preparations.

The liver was studded with lesions of different ages. These lesions were scattered at random beneath the capsule and throughout the liver parenchyma. All gradations from purely cellular foci composed of mononuclear cells surrounded by a loose reticulin capsule to acellular lesions with central necrosis and peripheral concentric rings of dense collagen fibres were seen. Epithelioid but not giant cells were present in these foci. Eosinophils were seen in moderate numbers around the nodules but more extensive perinodular inflammation was absent and large areas of normal liver existed between the abnormal foci. The central necrotic zone of the older lesions were packed with small acid-fast particles. Melanin (normal for frogs) and iron pigment were present in macrophages in the stroma.

Numerous inflammatory nodules like those in the liver were present in the wall of the intestine and in the mesentery. The smaller lesions were restricted to the submucosa and were covered by a layer of intact mucosa. The larger, older lesions replaced all layers of the intestine and bulged into the lumen and general abdominal cavity. Granular, fragmented, acid-fast material was present in the necrotic lesions. Similar nodules of different ages were found in the spleen (Fig. 4) and kidney. The fat body contained a few such nodules but the most striking feature was a large, partly collapsed, multilocular cavity containing the remains of an inflammatory exudate. Very little normal fat body remained. The testes were normal.

The epicardial plaque was composed of loose granulation tissue containing nodular, cellular foci as in the orbit (Fig. 5). The stroma was more collagenous and the nodules were smaller than in the eye but in other respects were similar. No tubercle bacilli were seen in this lesion. There was a small inflammatory focus in the myocardium of the ventricle and a collection of inflammatory cells in the adventitia and outer media of the truncus arteriosus. The lung was moderately well

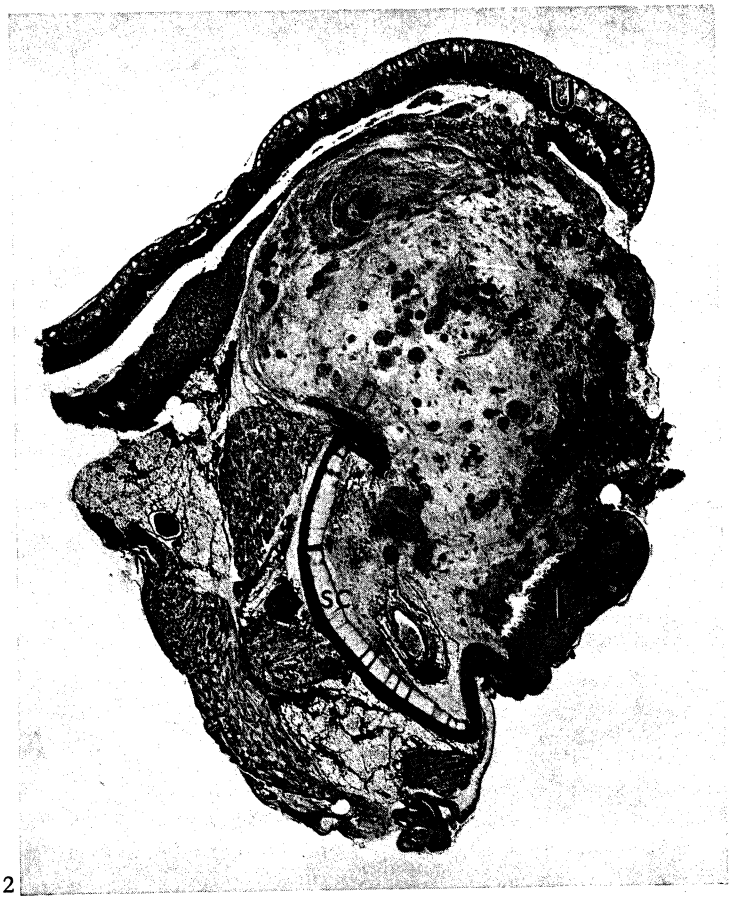

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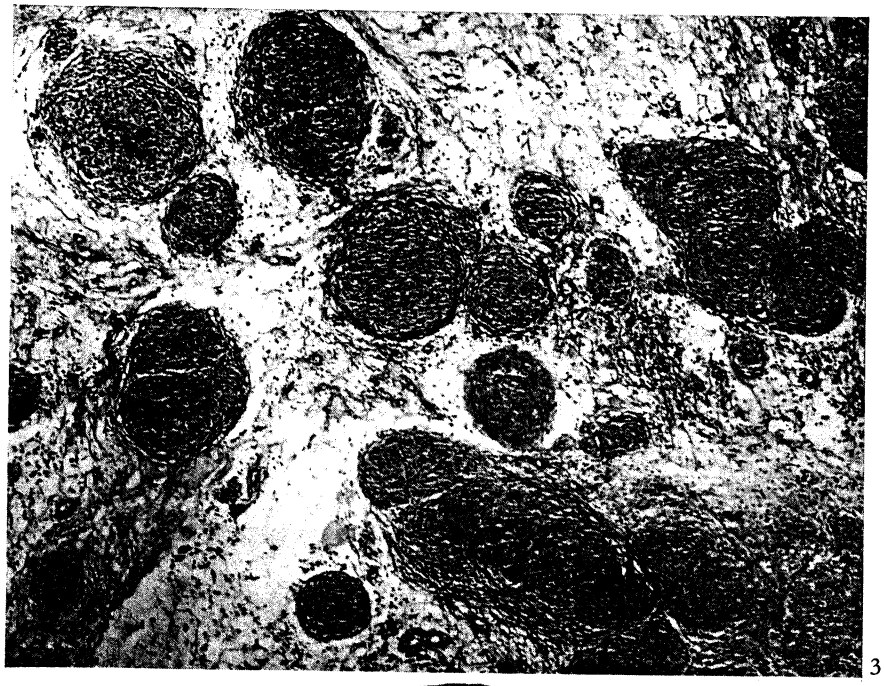
Fig. 2. Longitudinal section of eye to show extensive destruction of the globe. The scleral cartilage (SC) has become detached and displaced downwards. (U = upper lid; L = lower lid.) ×7.

Fig. 3. Higher magnification of area in center of Fig. 2, showing tuberculous granulation tissue. ×55.

Fig. 4. Low power view of spleen to show characteristic nodular lesions at different stages of maturity. ×20.



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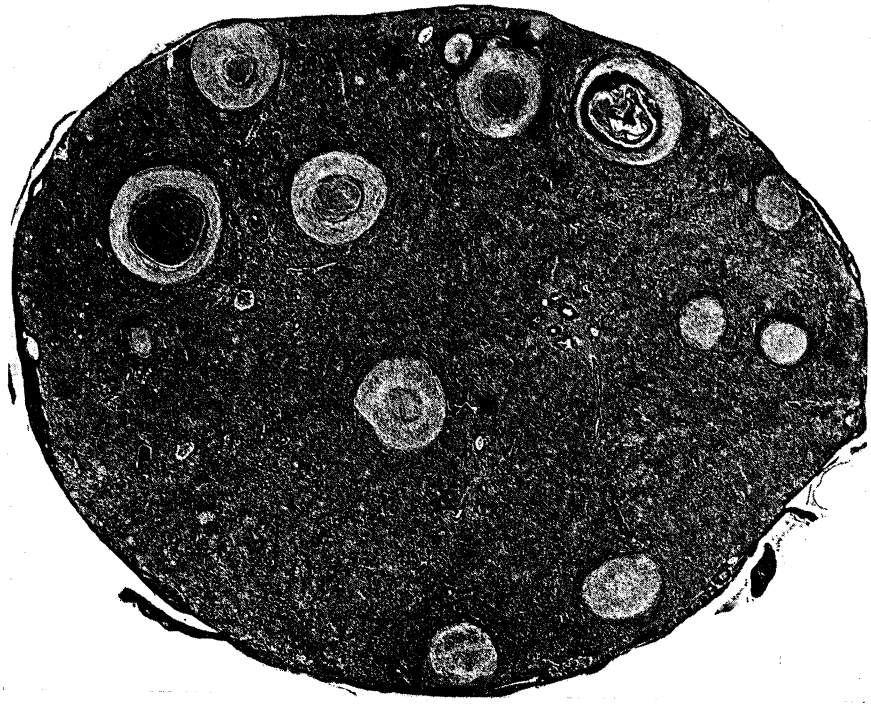

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aerated. There were a few small areas of intra-alveolar inflammatory cell exudate and an occasional small cluster of granulomatous foci in a connective tissue framework in the fibrous stroma of the lung.

Discussion

The nature of the chronic disease in this frog and its relation to the acute granulomatous process occurring in the eye cannot be proved beyond doubt in the absence of culture of the causative organism. However, we feel that there is strong presumptive evidence that this animal was suffering from chronic visceral tuberculosis with secondary, self-propagated infection of one eye.

Examination of sections stained by Gram and Ziehl-Neelsen's methods and by the periodic acid Schiff technique for parasites showed occasional slender, beaded, acid-fast bacilli in the acute inflammatory lesions and fragments of granular acid-fast material, possibly from degraded organisms, in the older lesions. In the light of this discovery, the most likely diagnosis was tuberculosis so we searched the literature to compare our findings with those of others in this disease.

The first case of spontaneous tuberculosis in a frog was reported by RUPPRECHT[13] in 1904. This animal was one of a group of 20 frogs examined in Freiburg by KÜSTER[8] who reported two further cases. In each, the liver was enlarged and studded with numerous prominent nodules. No other organs were affected. A similar lesion was described by LICHTENSTEIN[9] in a frog (species not stated) and by GRIFFITH[5] in a paradoxical frog, *Pseudis paradoxa*, which died in the London Zoo. Dermal tuberculosis was reported by SCHWABACHER[14] in an adult female *Xenopus laevis*, and renal tuberculosis, also in *Xenopus laevis*, by ELKAN[2] working in the same laboratory. Miliary tuberculosis has been described in several species: *Leptodactylus pentadactylus* (DARZINS[1]), *Hyla caerula* (HILL[6]), *Bufo bufo* (ELKAN[2]) and *Rana tigrina* and *Ceratophrys americana* (IPPEN[7]). The manifestations of the three main types of tuberculosis, dermal, intestinal and pulmonary, were described by REICHENBACH-KLINKE and ELKAN[12] with illustrative case histories from the latter author's vast collection of observations on captive species.

We were particularly interested in the endemic disease occurring in colonies of *Leptodactylus pentadactylus* living around fresh-water ponds in the outskirts of Salvador in Northern Brazil. The most severely affected animals were emaciated, feeble and underweight and at autopsy the liver, peritoneum, intestines and lung and sometimes the joints were more or less replaced by nodular lesions. No intraocular lesion was mentioned in this report. Lesser degrees of the disease could not be detected externally but DARZINS[1] found that all 60 animals examined from four different places were affected. There was no indication that our frog was diseased until the eye lesion appeared and at autopsy the visceral lesions were not nearly as advanced as in animals described by ELKAN[2]. Interference with sight led indirectly to debility and death in our animal and would probably have had the same result in the wild where binocular vision is needed for feeding, particularly in a nocturnal creature such as this.

We do not know if the intraocular granulomatous process was part of the systemic disease or a separate, independent infection. Metastatic spread may have occurred; the lesions elsewhere in the body were not of the same age and pre-

Fig. 5. Longitudinal section of heart to show epicardial tuberculous plaque. ×13.

sumably arose from blood stream infection from primary lesions in intestine and liver at different times. On the other hand, it is possible that the ocular lesion started as a dermal type of tuberculosis deep in the superior fornix beneath the upper lid. The eye of a frog moves continuously in the orbit. One can postulate that a foreign body contaminated with tubercle bacilli may have been forced under the upper lid and initiated the infection. The animal defaecated into his water bath as is common with amphibia. DARZINS[1] showed that water from ponds in which emaciated frogs lived contained free-living acid fast bacilli indistinguishable from those in the frog lesions. If reinfection did occur in this way, it is surprising that the resulting inflammatory process entered the eyeball as well as presenting in the palpebral fissure. However, once the scleral cartilage had become detached along its superior margin, extension into the eye would be as easy as along tissue planes in the lid. We regard this second explanation as the more likely.

The reasons for regarding this spontaneous chronic infection in frogs as tuberculosis are the constancy of the histological picture, the association with acid-fast bacilli which can reproduce similar lesions in susceptible animals and the likeness of the disease to human tuberculosis. Characteristically, the lesions are nodular when in solid organs and nodular in a loose granulation tissue when in soft tissues. Each nodule changes in time from a cluster of inflammatory and epithelioid cells surrounded by a light reticulin capsule to a fibrous body composed of a caseous necrotic core, an intermediate zone of flattened inflammatory cells and a peripheral layer of dense collagen fibres. This process resembles the conversion of a hard tubercle to a cold abscess in human tuberculosis. Eosinophils are plentiful around the nodule as it matures; giant cells of the Langhans type are found at no stage. Extracellular organisms which may be intact or fragmented and which are intensely acid-fast with Ziehl-Neelsen's stain, are common in the centre of older nodules. It is very much easier to examine the morphology of these organisms in a fresh smear preparation than in histological sections[3]. The nodules are always quite distinct because of absence of inflammation in the adjacent host tissue.

The supposition is that allergy provoked by tuberculoprotein is as important in producing the characteristic lesions of tuberculosis in frogs as in man. In warm-blooded animals, the presence of tubercles correlates well with the degree of allergy developed and, in general, the more pronounced the allergy, the smaller the number of organisms in the lesions. The degree of caseation depends on a balance between the host response and the number of bacilli in the tissues[4]. Because of an inadequate number of observations, it is not known if amphibia are highly susceptible to tuberculosis (the present view is that they are not) but it is certain that they are highly resistant to the systemic effects of infection. One of the striking dissimilarities of tuberculosis in different species is the varying degree of acquired resistance (RATCLIFFE and PALLADINO[11]). LONG and VORWALD[10] showed in the guinea pig that the initial response to testicular inoculation with various strains of Mycobacteria was much more uniform than the late effects. The mechanism of immunity to tuberculosis in amphibia is unknown. Nor is it understood why giant cells are absent in frogs although epithelioid cells are always present. Experimental tuberculosis in cold-blooded vertebrates has been studied by IPPEN[7] who also reviewed the literature.

The taxonomic position of the causative organism of frog tuberculosis is still under debate. The subject has been reviewed by SCHWABACHER[14] who has com-

pared the growth characteristics, pathogenicity and sensitivity to anti-tuberculous drugs of saprophytic, frog *(Mycobacterium xenopei)*, bovine, avian and typical and atypical human strains. She concluded that a separate group of organisms exists midway between saprophytic and pathogenic forms of which *Mycobacterium xenopei* is a member. DARZINS[1] has shown that *Mycobacterium giae*, the name that he has given to the acid-fast bacillus in his neighbourhood can be distinguished from *Mycobacterium ranae*, the causative organism of European frog tuberculosis. Neither is pathogenic for man.

Summary

A frog is described which had chronic visceral tuberculosis for some time before an acute, granulomatous lesion, in which acid-fast bacilli were identified, appeared in the right eye. In the absence of culture of the causative organism, the diagnosis was inferred from the presence of characteristic morphological changes. The disease process is essentially similar to that in man but the systemic effects are much less marked.

Zusammenfassung

Es wird ein Frosch beschrieben, der eine Zeitlang an chronischer Organtuberkulose litt, ehe eine akute, granulomatöse Veränderung im rechten Auge auftrat, bei der säurefeste Bazillen identifiziert wurden. In Ermangelung einer Erregerkultur wurde die Diagnose nach den charakteristischen morphologischen Veränderungen gestellt. Der Krankheitsprozess gleicht grundsätzlich dem des Menschen, jedoch sind die Allgemeinwirkungen erheblich geringer.

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