PATHOLOGY

Döhle Bodies in *Bufo temporaria* afflicted with 'Red Leg Disease'

Döhle bodies, which occur as blue-staining inclusions in neutrophils on Romanowsky-stained preparations, have been described as an acquired anomaly in various conditions in man[1–5]. They have also been reported as a familial anomaly associated with platelet abnormalities in 4 families so far[6] and are then denoted by the eponym, May–Hegglin syndrome. They have not been reported in experimental animals.

During an investigation of the pigmented leucocytes in *Bufo temporaria* it was observed that 50–60 per cent of the neutrophils exhibited Döhle bodies (Fig. 1). These animals were captured in the Kommetjie area near Chapman's Peak in the Cape Peninsula and they developed 'red leg disease', caused by *B. hydrophilus fuscus*, in captivity. This disease bears a mortality rate of 90 per cent and is not uncommon in *Rana* and *Bufo* kept in captivity[7]. The animals were treated with sulphonamide therapy and responded favourably to therapy. (Prof. H. D. Brede diagnosed and instituted therapy for these animals.)

The ribonucleic acid nature of the inclusions could be confirmed by the red staining with methyl pyronin-green and the disappearance of these bodies when incubated with ribonuclease. They do not stain with sudan black, periodic acid–Schiff or Feulgen procedures.

The mode of origin of these bodies is obscure but it is usually held to represent areas of cytoplasm which failed to mature[4]. An alternative, or additional, mechanism appears to be possible in the case of *Bufo*. It was found that many lymphocytes had conspicuous cytoplasmic budding (Fig. 2), and occasionally globules apparently representing broken-off cytoplasmic buds were seen in the vicinity of such cells. Phagocytosis of such buds was occasionally observed (Fig. 3).

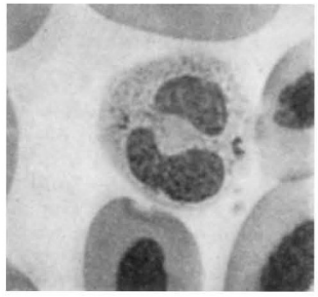

Fig. 1

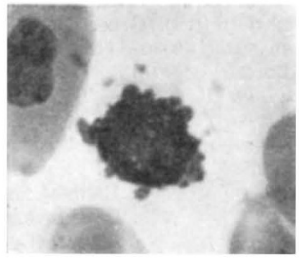

Fig. 2

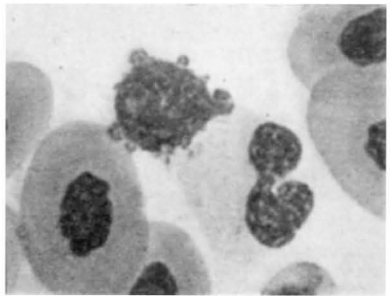

Fig. 3

A mean incidence of 15 per cent of lymphocytes with budding cytoplasm was found. There is no significant correlation between the number of cells with budding and the number of neutrophils with Döhle bodies. An average of 1·5 per cent of monocytes had similar inclusions.

The relationship of giant thrombocytes in May–Hegglin syndrome to these inclusions is also obscure, but it is of interest that amphibians have large thrombocytes ('spindle cells').

Although single inclusions per cell are the most frequent occurrence, up to six Döhle bodies in a single cell have been observed.

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In vivo and *in vitro* Activity of Tolerant Anti-leukæmia Serum

In previous work we have shown that after the induction of artificial immunological tolerance in the rabbit to normal mouse tissues, subsequent immunization with tumour produces tolerant anti-tumour sera. These show low murine toxicity and significantly greater toxicity for the tumour than do non-tolerant anti-tumour sera or anti-sera against normal tissues—tolerant or non-tolerant. So far we have prepared such tolerant specific antisera against the transplantable Ehrlich ascites tumour[1,2] and the spontaneous *AKR* lymphatic leukæmia[3,4]. The assays of these anti-sera have been done *in vivo*. Other workers have also reported tolerant specific anti-tumour sera using both homologous[5–8] and isologous[9,10] tumours.

Published works have used either *in vivo*[1–4,9,10] or *in vitro*[5–8] methods of analysis, with the exception of Trench *et al.*[10] where an attempt to correlate the *in vivo* activity of tolerant and non-tolerant anti-tumour serum with *in vitro* precipitin lines in Ouchterlony plates was made. The anti-sera they used failed to give precipitin lines with normal or tumour antigens. This finding agrees with our unpublished data[11] that in interfacial ring tests between tolerant anti-Ehrlich ascites tumour serum and supernatants from homogenates of this tumour, no precipitin lines were seen.

Until now the *in vivo* tolerance and anti-tumour activity of anti-sera could be shown only in the intact animal. About a third of all presumptively tolerant anti-sera which we have prepared have failed to meet criteria for tolerance. Thus an *in vitro* assay which would accurately predict tolerance toward normal tissue as well as tumour toxicity has been lacking and is needed for further investigations of tolerant anti-sera. The present investigation outlines such an *in vitro* method utilizing cytotoxic indices of anti-sera against leukæmic and normal cells and indicates the correlation of these findings with *in vivo* observations.

Artificial immunological tolerance was induced in neonatal Dutch rabbits to normal *AKR* mouse tissues by