The Use of Pronase for Dispersing Cells

In this laboratory difficulty has been experienced in dispersing 'clots' which formed when mouse spleen cells were incubated with sheep red blood cells in diffusion chambers of internal volume 0.38 ml in the peritoneal cavity of mice (S. G. ANDERSON, unpublished). The enzymes hyaluronidase (100 IU/ml), streptokinase (50,000 IU/ml) and/or streptodornase (streptococcal deoxyribonuclease, 5,000 IU/ml) did not disperse the clots when used at 37 °C or at room temperature. Crude collagenase (Koch-Light, 10 mg/ml in borate buffer with $CaCl_2$) had a slight effect but highly purified collagenase (kindly furnished by Dr. A. NORDWIG from the Max-Planck Institute, Munich, Germany: 1 mg/ml) had no effect. The effect of the crude enzyme was thought to be probably due to contaminants. This is in agreement with unpublished findings of P. L. WALTON of this Institute. In his hands, nitrogen analysis of dried chamber clots gave values (14%) which suggested that the bulk of the clot was protein. Quantitative estimation of hydroxyproline in hydrolyzed clots (6*N* HCl, 24 h) were negative. From the sensitivity of this test, it was possible to conclude that less than 2% of the clot could be collagen.

Fresh clots were incubated with different final concentrations of 'Pronase', Kalbiochem, Switzerland, (0.025 to 0.001%, W/V in balanced Hank's solution) in the presence or absence of normal mouse serum. Samples were stirred at room temperature and the number of cells released from each clot after 5, 10 and 20 min incubation was measured in a counting chamber. The results of the experiments are summarized in the Table.

Cells were liberated by 0.005% pronase but not by a 0.001% solution of the enzyme. The presence of 50% normal mouse serum did not significantly inhibit the action of pronase.

GWATKIN and THOMSON[1] used 0.25% pronase for dispersing the cells of mammalian tissues and WILSON and LAU[2] found 0.05 to 0.01% concentration of pronase suitable for dissociation of chick embryo cells. In agreement with KAHN et al.[3] we have found that 0.005% of pronase was still effective. KAHN et al.[3] described the inhibition of 0.005% pronase by 50% bovine serum; however normal mouse serum did not significantly inhibit even the diluted enzyme.

'Pronase' was found to be an effective enzyme for dispersing cells from 'clots' under the described conditions.

The number of cells liberated from diffusion chamber 'clots' by treatment with pronase

Concentration of pronase (%)	Time of incubation (min)		
without normal mouse serum	5	10	20
0.001	0	0.05[a]	0.35
0.005	1.27	1.63	2.18
0.010	1.40	2.40	4.92
0.025	2.42	3.30	4.80
0.050	2.28	3.50	5.04
with normal mouse serum			
0.005	1.03	1.40	1.95
0.010	1.41	2.28	4.80
0.025	2.46	3.20	4.88
Controls without pronase			
without serum	0	0	0.005
with serum	0	0	0.001

[a] Total No. of cells liberated from 1 clot · 10^6. Each figure is a mean of results from 2–8 clots treated separately.

Zusammenfassung. Zugabe von 0.005% Pronase bewirkt eine Freisetzung von Zellen aus Gerinnsel, welche sich bei der Inkubation von Mäusemilzzellen mit Schaferythrocyten bilden.

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[1] R. M. B. GWATKIN and J. L. THOMSON, Nature *201*, 1242 (1964).
[2] B. W. WILSON and T. L. LAU, Proc. Soc. exp. Biol. N.Y. *114*, 649. (1963).
[3] J. KAHN, M. J. ASHWOOD-SMITH and D. M. ROBINSON, Exp. Cell Res. *40*, 445 (1965).
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Ultrastructural Common Properties of Haemobartonellae and Mycoplasmatales

Bartonella bacilliformis, probably a true bacterium[1], presents few similarities to Haemobartonellae These organisms have revealed biochemical and morphological features, such as sensitivity to chemotherapics and antibiotics, that characterize pleuropneumonia-like organisms (PPLO, Mycoplasmataceae) and a classification of Haemobartonellae within Mycoplasmatales has been proposed[2]. Our recent work related to *H. muris* infection discovered an intra-erythrocyte phase of evolution. Morphological and developmental behaviour common to Haemobartonellae and Mycoplasmatales was shown[3].

Here we present some electron micrographs of metacrilate-embedded erythrocytes and plasma sediments

[1] TOPLEY and WILSON's *Principles of Bacteriology and Immunity* (Ed. Arnold Ltd., London 1964) vol. I, p. 1098.
[2] R. WIGAND, *Morphologische und serologische Eigenschaften der Bartonellen* (Georg Thieme Verlag, Stuttgart 1958).
[3] G. G. TEDESCHI, D. AMICI, O. MURRI and M. PAPARELLI, Annali Sclavo *8*, 197 (1966).

(30′–60,000 *g*) of splenectomized, hematuric, *Haemobartonella*-infected rats. DOMERMUTH et al.[4] have studied, with the aid of an electron microscope, many strains of *Mycoplasma* species: our results could be usefully compared to their observations.

Figure 1 shows the morphology which Haemobartonellae present when these organisms are externally located to erythrocytes, partially by peripheric protrusions of the red cells. In this condition, inside the scarcely visible membrane, one can observe a thin filamentous reticle and a particulated material variously distributed but mainly crowded in the vicinity of the membrane itself.

Figure 2 reports the contents of a vacuole inside the bulk of an erythrocyte: there are 4 Haemobartonellae that clearly show transformation between electron-dense corpuscles (in which granulated structures are still distinguished) and diaphanous shapes, like those of Figure 1. There is good evidence for a double-layered membrane in one of the conformations enclosed.

Figure 3 represents a section of the product of the plasma ultracentrifugation: also in this preparation the evolution of the optically dense elementary corpuscles into larger, widely vacuolated, shadow-like structures is clear. The latter contain a fine reticle with granulated material and present a peripheric limiting membrane which is composed of 2 opaque barriers separated by a more transparent layer.

The presence of elementary bodies with electron dense cytoplasm and about 100 nm transverse diameter, and the evolution of these structures into others of larger dimensions and well shaped by limiting membranes, containing filamentous material and assembled particles almost 20 nm in width, identifiable as ribosomes, are well settled points. These observations prove that also on an ultrastructural basis Haemobartonellae and microorganisms related to bacterial order *X* (Mycoplasmatales) are quite similar[5].

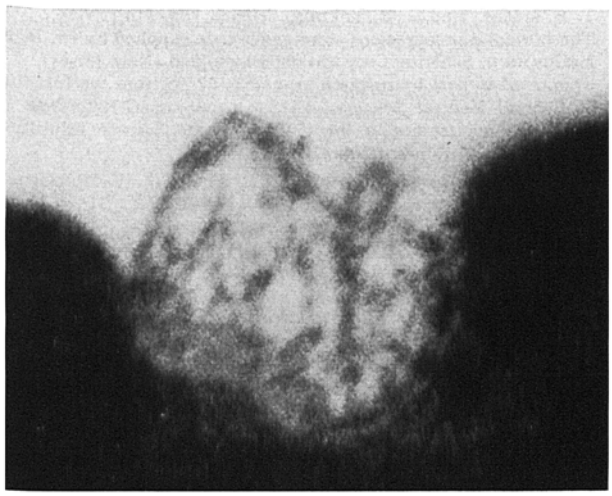

Fig. 1. *Haemobartonella muris* externally located into an erythrocyte. × 123,000.

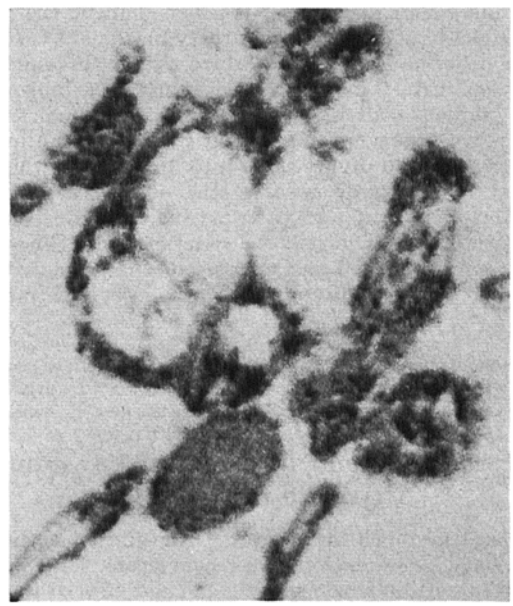

Fig. 3. Haemobartonellae at different stages of development, free in the plasma. × 149,000.

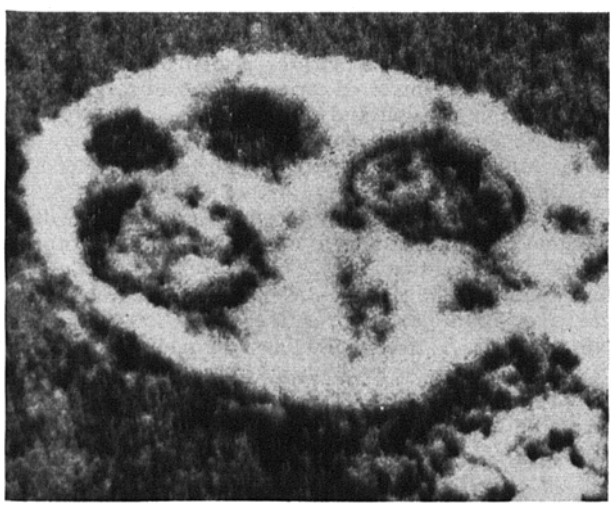

Fig. 2. Erythrocyte containing a vacuole with Haemobartonellae at different developmental stages. × 103,400.

Riassunto. È stata considerata la possibilità di classificare le Haemobartonellae nel gruppo delle Mycoplasmataceae; le presenti osservazioni dimostrano anche sul piano ultrastrutturale l'esistenza di strette analogie fra tali microorganismi.

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[4] C. H. DOMERMUTH, M. H. NIELSEN, E. A. FREUNDT and A. BIRCH-ANDERSEN, J. Bact. *88*, 727 (1964).

[5] This investigation was aided by a grant from the study group on morphology and function of ultramicroscopical cellular structures of the Consiglio Nazionale delle Ricerche.