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red blood cells in diffusion chambers of internal volume 0.38 ml in the peritoneal cavity of mice (S. G. ANDERSON, unpublished).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 This is in agreement with unpublished findings of P. L. WALTON of this Institute.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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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

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[2] R. WIGAND, *Morphologische und serologische Eigenschaften der Bartonellen* (Georg Thieme Verlag, Stuttgart 1958).
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