BACTERIOLOGY

Immunological Activity of Precolostral Calf Serum

A failure of passage of immune and normal antibody across the placenta of the cow has been described by a number of authors[1,2]. Antibodies have been shown to be transferred to the calf, during the first 24 hr., in the colostrum, and this has been shown to be associated with a parallel rise in serum γ-globulin from around 2 per cent of the total serum proteins to 30 per cent, the level found in the adult cow[2].

Sera from 6 calves, taken before suckling could occur, were studied to determine the basic immunological activity of a mammalian serum before the acquisition or development of normal antibodies. The sera were titrated for their bactericidal activity, and estimates were made of the titre of properdin[3] and complement. An attempt was also made to determine whether there was any conglutinin activity in these sera. Titration of bactericidal activity of the fresh sera was performed by Nagington's method[4]. Properdin was titrated by the modified method of Howard, Rowley and Wardlaw[5] and the level of conglutinating complement by that of Hole and Coombs[6].

The serum bactericidal activity of calf *R*80 is shown in Table 1. Similar activity was found with the sera of the other 5 calves. There was little or no bactericidal activity against a wide range of smooth organisms tested, whereas the serum was highly bactericidal against the two rough strains tested, *Salm. typhi SW*540 and *Shig. shiga K*624. This activity was completely removed by absorption with 0·2 gm./ml. of zymosan for 2 hr. at 4° C.

Table 1. Mean Number of Bacteria killed by 0·02 ml. Serum of Calf *R*80

Smooth organisms		Rough organisms	
Salm. typhi 0901	< 1·1	*Salm. typhi SW*540	1·2 × 10⁵
Salm. paratyphi A	< 2·5	*Shig. shiga K*624	> 7·5 × 10⁵
Shig. shiga	1·3		
Shig. flexner 4	10		
E. coli	< 3·1		
K. pneumoniae	2·0		
Pr. vulgaris	1·4		
Ps. aeruginosa	< 4		
V. el tor	< 1·6		

The bactericidal activity was associated with a level of properdin which ranged within ± 50 per cent of the level found in a normal adult bovine serum, and with conglutinating complement titres of 16–32. No conglutinin was demonstrated by the standard method of Coombs and Coombs[7], but conglutination of starch grains (*Amaranthus cruentus*) and bacteria was observed under the phase-contrast microscope, associated with an inhibition of immune-adherence[8].

The bactericidal activity of precolostral calf serum is probably due to the interaction of properdin and complement. As antibody globulins cannot pass over the placental barrier, it is therefore probable that properdin and the components of complement necessary for the bactericidal activity of precolostral calf serum are developed by the fœtus *in utero* and are not related to the production of normal antibodies which appear to develop as a function of the age of the calf[2].

Precolostral calf serum is an example of the properdin system unaffected by normal antibody. In other systems where the bactericidal activity of properdin has been studied[9], there has always been normal antibody present which might have confused the picture. The experiment described suggests that the properdin system in the new-born calf is relatively inactive against smooth strains of bacteria, whereas it is highly bactericidal to rough strains. This is in keeping with the observation that calves deprived of colostrum are very susceptible to an *E. coli* septicæmia, despite having properdin and complement levels of the same order as those found in adult cattle.

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J. L. Turk

London School of Hygiene and Tropical Medicine,
Keppel Street,
London, W.C.1.
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A Source of Error in Microbiological Assays attributable to a Bacterial Inhibitor in Distilled Water

Most workers in microbiological assay will have had experience of the well-established assay procedure that will occasionally give sub-optimal growth in some of the tubes, and hence discordant replicates. In our experience, folic acid assays using *Streptococcus faecalis* R are particularly prone to troubles of this kind, and such behaviour can be a serious practical difficulty.

We have observed that replication is much better in some batches of 'clean' tubes than in others and that the difference is attributable to an unidentified contaminant in certain batches of distilled water used for the final rinsing of the tubes.

'Difco' folic acid assay medium (single strength) supplemented with 2·5 mμgm. folic acid/ml. was dispensed into 'Pyrex' tubes. After autoclaving and cooling, they were inoculated with one drop of a washed, undiluted suspension of *Streptococcus faecalis* R. cells from a culture 5–8 hr. old, and growth was estimated with a 'Spekker' absorptiometer after overnight incubation. The tubes were cleaned before use by boiling for 10 min. in 2 per cent washing soda, steeping overnight in 3·5 per cent hydrochloric acid, rinsing nine times in tap water and twice in distilled water, and drying in an oven. Such tubes are designated 'clean' tubes. For the work to be described below, 'clean' tubes were filled with distilled water, autoclaved for 15 min. at 15 lb./sq. in. pressure, emptied and dried in an oven immediately before use. Such tubes are designated 'rectified' tubes.

Excellent replication could invariably be obtained if the tubes were 'rectified'; 'clean' tubes, on the other hand, would often give lower and less regular responses. Often this difference would be slight or non-existent, but in extreme instances, some of the 'clean' tubes would show no detectable growth.