then, contained transcribed sequences that also occurred within the short repeats subfraction. The frequency of these transcribed sequences was significantly greater in the long repeats. The chances that hybridization of long repeats to hnRNA was due entirely to a few single-copy sequences that might be present in the DNA subfraction were, accordingly, diminished.

The findings in this study introduce the possibility that long reiterated sequences are transcribed into hnRNA. The value of the hypothesis is that it may account for some of the observed size differences between hnRNA and mRNA. If long reiterated sequences are transcribed at all, they must be transcribed in entirety, since introns and exons within a single transcription unit are physically continuous[2]. In this connection, it may be noted that the sizes of the longest repeats were only slightly less than that of the longest primary transcripts found in human cells, about 20,000 nucleotides long[23].

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Babesial antigens in a saline eluate of sucrose washed bovine erythrocytes infected with *Babesia bovis*

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Summary. A saline eluate from sucrose washed bovine erythrocytes infected with *Babesia bovis* contains at least 4 babesial antigens. The antigens are located not only in the parasite but also in the internal matrix of the infected erythrocyte.

Bovine erythrophylic proteins constitute a group of partly characterized proteins obtained by washing freshly collected bovine erythrocytes several times with an isotonic sucrose solution and then eluting bound proteins from the erythrocytes with physiological saline[1]. Both quantitative and qualitative changes occur to these proteins during acute infection of cattle with the intra-erythrocytic protozoan parasite *Babesia bovis*. Such changes contribute markedly to the pathophysiology of infection, namely the hypotensive shock syndrome and the sludging of infected erythrocytes within the microcirculation[2,3]. Although it seemed reasonable to assume that a saline eluate from infected erythrocytes might also contain babesial antigens, we were unable to detect any antigens in initial studies using established immunological assays such as hemagglutination and immunodiffusion. The present studies, however, demonstrate that the eluate does contain babesial antigens which may be detected by electrophoresis and nitrocellulose transfer and are located in situ by immunofluorescent staining.

Blood (9 vols) was collected into sterile 3.13% trisodium citrate (1 vol.) from cattle experimentally infected with *B. bovis* by blood passage from carrier animals infected with the Samford strain[4]. The blood, containing ~ 10% infected erythrocytes, was centrifuged at 250 × g for 10 min and the supernatant platelet-rich plasma removed. The blood was washed 3 times in 10 vols of 0.27 M sucrose in 0.0004 M phosphate buffer pH 7.2 at 1500 × g for 10 min at 4 °C. After each wash the top layers of residual leucocytes and platelets were removed. The sediment of erythrocytes was mixed with 10 vols of saline (0.85% aqueous NaCl) and centrifuged at 10,000 × g for 30 min. The supernatant saline eluate was concentrated by ultrafiltration, using a membrane of 10×10^3 exclusion, to the original volume of blood and stored at −20 °C until required. For control purposes, a similar preparation was obtained from pooled blood of five normal cattle.

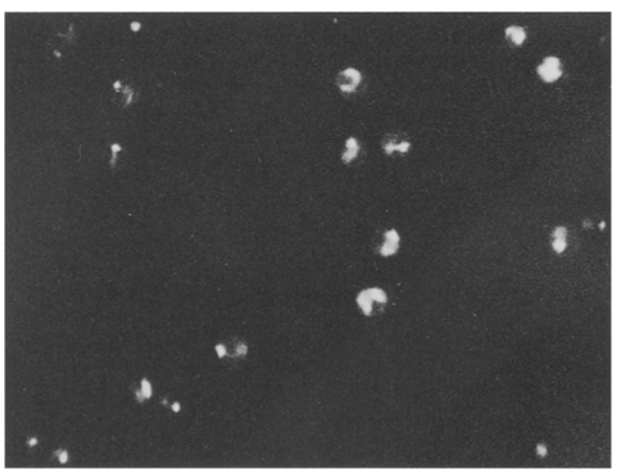
A rabbit antiserum to the saline eluate was prepared by a series of i.m. and i.v. injections as described by Coombs and Gell[5]. The rabbit antiserum and a commercially prepared goat antirabbit IgG coupled to fluorescein isothiocyanate (FITC) were used to stain thin films of acetone-fixed *B. bovis* blood as described by Goodger[6]. Both infected and non-infected erythrocytes stained avidly. However absorption of the rabbit antiserum with a suspension of normal bovine lysate and stroma abolished the staining of the non-infected erythrocytes. Infected erythrocytes stained moderately while the parasite stained avidly (fig.). No staining occurred in films fixed in either methanol or

ethanol which render the erythrocytic membrane impermeable to marker antibodies in contrast to acetone which causes membrane permeability.

Electrophoresis of eluate proteins was performed on 2.5–27% gradient acrylamide slabs according to the method of Margolis and Kenrick[7]. The proteins were then electrophoretically transferred to a sheet of nitrocellulose[8]. Antigens in the sheet were detected by sequential applications of rabbit antiserum to saline eluate (absorbed with normal bovine plasma, lysate and stroma), goat antiserum to rabbit IgG coupled with horseradish peroxidase, and a solution of 4-chloro-1-naphthol and hydrogen peroxide using concentrations, buffers, and incubation times as suggested by Hawkes et al.[19]. Six eluates were tested, each giving the same result. Three strongly reacting antigens were detected, the major one having a size of 190×10^3 with the others being 280 and 380×10^3. Sizes were obtained assuming pore limit retention and from comparison with the relative electrophoretic mobilities of a series of native protein standards[10] of sizes ranging from 67 to 669×10^3. A very weakly reacting antigen of size 120×10^3 was also detected. No equivalent bands were detected when either bovine plasma or the eluate from control erythrocytes was tested in a similar manner or when normal rabbit serum was substituted for rabbit antiserum. Similarly, bovine antiserum to *B. bovis* (Samford) and goat anti-bovine IgG coupled to horseradish peroxidase developed the same antigens. Protein staining of acryamide slabs with Amido Black 10B confirmed a previous analysis[1] that the main proteins were of plasma origin. Bands with identical mobilities to the antigens were not detected indicating the latter were in low concentration.

Thus the saline eluate of sucrose washed *B. bovis* infected erythrocytes contained proteins from membranes of non-infected erythrocytes and in addition contained babesial antigens located in both the parasite and the infected erythrocyte. As these antigens were released from sucrose washed infected erythrocytes by simple washing with saline, it seems reasonable to assume that in vivo they might be released into the circulation during parasite development or by degradation of sludged erythrocytes. As such they would be readily available to the immune system and might be important in the protective response. Nevertheless, their release mechanism in vitro is not easily explained. The change from sucrose to saline did cause some lysis, but this was minimal as the hemoglobin concentration in the eluates never exceeded 3% of the total hemoglobin of the washed erythrocytes[11]. Moreso the erythrocytes appeared intact when examined in suspension by either normal or dark ground microscopy. If the antigens are passively expelled due to some type of selective osmotic shock, one might expect antigens to be similar in size to hemoglobin or alternatively to mirror the spectrum of antigens found in distilled water lysates of infected erythrocytes[12]. Neither expectation holds. Firstly, the antigen sizes are vastly larger than hemoglobin. Secondly, whereas the eluate antigens are part of the lysate antigen spectrum, the latter contains at least 17 other babesial antigens[12]. Hence it seems reasonable to assume that some selective release mechanism must occur. One could argue that a small number of infected erythrocytes may have been completely lysed but a counter to this is that infected erythrocytes are far more resistant to lysis than uninfected erythrocytes[13]. One could also argue that the antigens were located on the external membrane but infected erythrocytes with intact membranes did not stain with antiserum whereas the parasite and the infected erythrocyte stained after acetone fixation. Hence the antigenic determinants must be masked by membrane proteins in intact erythrocytes or the antigens are located within the infected erythrocyte. As the parasite stained, the latter suggestion may be more credible. Alternatively, one or more of the antigens could be located externally but be at a concentration too low to be detected by fluorescent antibodies.

Previous work has shown that the rabbit, but not the bovine, readily produces antibodies to modified erythrocytic host protein[14]. Hence the similar staining of nitrocellulose blots by rabbit and bovine antisera suggests the eluate antigens must be totally of babesial origin. The antigens in vivo may be the source of exoantigens or soluble serum antigens, a protective group whose precise identification has proved difficult[15]. Alternatively, if partially exposed during membrane distortion by the developing parasite, they may be related in some manner to variant specific antigens, an esoteric and elusive group of high specificity[16]. In either case, their potential as protective antigens should be assessed.



Indirect fluorescent antibody staining of a thin smear of blood infected with *B. bovis*. Stained firstly with rabbit antiserum to the saline eluate of sucrose washed infected blood and secondly with goat anti-rabbit IgG coupled to FITC.

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