Hemolysis in Identifying Eaton's Pleuropneumonia-Like Organism

Abstract. *A method for readily demonstrating hemolysis of mammalian erythrocytes by Eaton's pleuropneumonia-like organism is described. This reaction may be useful in preliminary identification of this organism, since other strains from human sources are not known to have this property.*

Recent evidence that Eaton's agent is a pleuropneumonia-like organism (PPLO) (*1, 2*) which can be recovered easily from clinical specimens on artificial media (*3*) intensifies the need for a rapid means of identifying this micro-organism. The problem is compounded by the occurrence of nonpathogenic PPLO in the human respiratory tract from which Eaton's PPLO must be distinguished, and by the lack of generally accepted classifying schemata for PPLO.

It has been found recently that Eaton's PPLO grown on solid media hemolyzes erythrocytes of several animal species (*4*). In the present studies, hemolysis was evaluated by applying overlays of blood agar to established cultures. After brief incubation. Eaton's PPLO produced clear (beta) hemolysis of mammalian erythrocytes, whereas a number of other PPLO cultures (not Eaton) produced no hemolysis under similar conditions. This characteristic has proved useful for differentiating Eaton's PPLO from other mycoplasma strains.

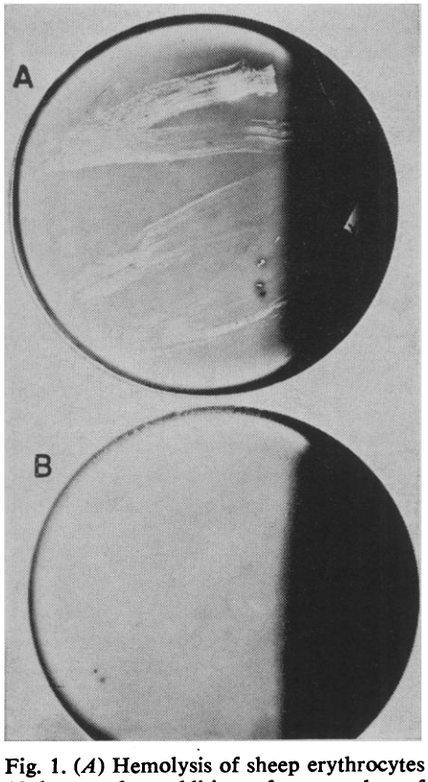

Fig. 1. (*A*) Hemolysis of sheep erythrocytes 18 hours after addition of an overlay of blood-agar to a culture of Eaton's PPLO on transparent medium. (*B*) Culture of a non-Eaton PPLO treated identically to the plate above, showing lack of hemolysis.

Eight strains of Eaton's PPLO were used in the experiments, as follows: four strains received from Dr. R. M. Chanock (Bethesda), isolated in tissue culture; the Mac strain from Dr. C. Liu (Kansas City), which was isolated in eggs, and passaged many times in eggs, tissue culture, and artificial media in our laboratory (*2*); an isolate made on agar from the original sputum of patient F. H. (Dr. Liu); and two strains isolated locally from sputum by the use of artificial media. The reactions of these strains were compared to each other and to 10 strains of PPLO recovered from the normal human oropharynx on agar, representing four distinct serotypes. In addition, a non-Eaton PPLO (DC-63) recovered from patients with atypical pneumonia and obtained from Dr. M. A. Mufson (Bethesda) was evaluated.

The technique employed was a modification of the method used by Freimer, *et al.*, for demonstration of hemolysis produced by streptococcal L forms (*5*). One percent Batco agar was prepared in normal saline, autoclaved, and cooled to 45°C. One part 50 percent sterile blood in Alsever's solution was added to three parts saline agar. The blood agar was poured onto PPLO colonies which were growing on a transparent medium (*2*) in Petri plates to produce a thin overlay. The plates were tipped slightly to permit excess blood agar to accumulate at one side, and were incubated at 37°C after the overlay had solidified.

All Eaton PPLO strains tested reacted similarly; they produced clear zones of hemolysis around individual colonies 18 to 48 hours after the application of the overlay of mammalian blood agar. Hemolysis was most striking when sheep or guinea pig bloods were used, and usually appeared within 18 hours. The gross appearance of a culture of Eaton's PPLO overlaid with sheep blood agar for 18 hours (Fig. 1*A*) is compared to the appearance of a non-Eaton PPLO (Fig. 1*B*) treated in like fashion. Figure 2 reveals the hemolytic zones around individual colonies. Hemolysis of human, horse, and rabbit erythrocytes was less marked and plates made with these bloods required longer incubation for development.

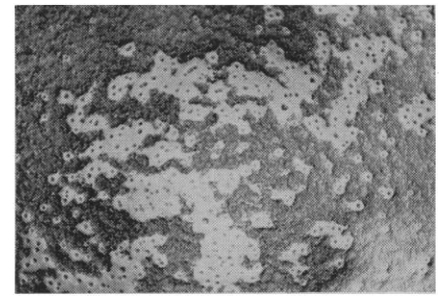

Fig. 2. Appearance of the plate shown in Fig. 1*A* at greater magnification. Hemolytic zones are evident around individual colonies of Eaton's PPLO, with confluence into larger areas of hemolysis where colonies are closely spaced (× 10).

The erythrocytes tested (chicken and the mammalian species listed above) were not lysed by any of 11 strains of non-Eaton PPLO studied. Hemolysis has not been reported as a property of the classified PPLO isolated from the human oral cavities (*6*).

These data suggest that the hemolytic reaction may serve as a useful basis for presumptive identification of Eaton's PPLO. The specificity of this reaction cannot be established until a more exhaustive search has been made for other strains of PPLO which may lyse erythrocytes under the conditions described. Confusion may also occur if L forms of hemolytic bacteria are induced by bacterial inhibitors that are frequently incorporated in PPLO media, as the hemolytic capacity of the parent organism can be retained by such variants (*5*). For these reasons, more definitive tests are advisable before making final identification of Eaton's PPLO by the means described (*7*).

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References and Notes

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7. Sponsored by the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and supported by the Office of the Surgeon General, Department of the Army.

10 October 1962