tive) *S. aureus* strains, under study for a number of years in our laboratories. The quantitative immunofluorescence data, as shown in Table 1, are ratios between the degree of total fluorescence of bacteria after staining and that of their primary fluorescence, expressed in percentages. There are individual strain differences in the results obtained with both techniques, and also for the two types of fluorescent antibodies used. Differences between strains in each group are greater with quantitative immunofluorescence than with immunodiffusion if only the number of lines is considered. The numbers of precipitin lines in each group are not too far from their means. When comparing the means of each series of results for the two groups of strains, both immunological methods are able to differentiate pathogenic from nonpathogenic groups of strains. The two groups of strains are better differentiated, with quantitative immunofluorescence, by normal human gamma-globulin than by antitoxin; the reverse is true with immunodiffusion. All the observed differences between the two methods could be due to the fact that each of them detects partly different antigenic material, particularly diffusible antigens with immunodiffusion and structural cell antigens with quantitative immunofluorescence.

Quantitative immunofluorescence seems to add supplementary information to that obtained by other immunochemical methods. It could be used later even as an alternate method when technical improvements, like those suggested by Goldman and Carver (Exptl. Cell Res. **23**:265, 1961), would even permit its application to microscopic studies of isolated cells.

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# MEASUREMENT OF GROWTH-INHIBITING ANTIBODY FOR *MYCOPLASMA PNEUMONIAE*

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Antibodies to *Mycoplasma pneumoniae* (Chanock et al., Science **140**:662, 1963) have been measured by immunofluorescence (Liu, J. Exptl. Med. **106**:455, 1957) and complement-fixation techniques (Chanock et al., Proc. Soc. Exptl. Biol. Med. **110**:884, 1962). Measurement of specific antibodies inhibiting growth of the pleuropneumonia-like organisms (PPLO) should be a useful index of protective immunity similar to the measurement of neutralizing antibody in viral infections. We have found that growth of *M. pneumoniae* on agar can be suppressed by human or animal immune serum. A medium consisting of Difco PPLO Agar plus 20% horse serum, 2.5% yeast extract, 1% dextrose, 0.05% thalium acetate, and 1000 units of penicillin per ml was placed in the cups of transparent depression plates (Linbro Disposo-Trays, model 96CU). A broth culture or suspension of the PPLO of known concentration was mixed with dilutions of

TABLE 1. *Comparison of antibody titers by complement-fixation, immunofluoresence, and plaque-reduction tests*

| Serum | Complement fixation | Indirect fluorescence | Plaque reduction |
|---|---|---|---|
| *Guinea pig* | | | |
| Postinfection | | | |
| 2 weeks | <10 | 0 (1:10) | <10 |
| 4 weeks | 15 | 2+ (1:10) | 40 |
| 6 weeks | 15 | 3+ (1:10) | 20 |
| *Human* | | | |
| P.I.-843 | | | |
| Acute | <10 | 0 (1:10) | <5 |
| Convalescent | 40 | 1+ (1:160) | 10 |
| P.I.-1634 | | | |
| Acute | <10 | 0 (1:10) | <5 |
| Convalescent | 160 | 1+ (1:80) | 80 |
| Levine, convalescent | 100 | 1+ (1:160) | 160 |

test antiserum, and 0.1 ml of the mixture was pipetted onto the surface of replicate cups of agar. The plates were then sealed with transparent cellophane tape, and incubated at 37 C for 7 days. Because it was difficult to count individual colonies, we utilized lysis of guinea pig erythrocytes as an indicator of colonial growth (Somerson et al., Am. J. Hyg. **77**:112, 1963). This was accomplished by adding 0.3 ml of PPLO Agar base suspension of 4% red blood cells (prewarmed at 45 C before mixing) to each cup with a syringe and needle. The plates were then reincubated 40 hr at 37 C without resealing. Colonies were seen as zones of hemolysis or plaques which could be counted in control and serum-containing cups.

Best results were obtained when approximately 50 colonies developed per cup in the absence of immune serum. Because 100% growth suppression was irregular, we arbitrarily chose 90% reduction in the number of plaques forming as the titration end point. No advantage was gained by preincubation of the serum-organism mixtures.

Comparison of titers found in guinea pig and human convalescent sera by three methods shows that end points were in general agreement (Table 1). Although more laborious than complement-fixation or immunofluorescence techniques, growth-inhibiting antibody is probably a more significant measurement of resistance. Use of the multi-cup sealed plate should facilitate antibody surveys and further define the relationship of antibodies detected by these methods.

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# INHIBITION OF COAGULASE REACTION OF PATHOGENIC STAPHYLOCOCCI BY HEPARIN IN VITRO

## II. USE OF STERILE, CELL-FREE PREPARATIONS OF COAGULASE

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Prolongation of staphylococcal coagulase clotting time in the presence of heparin was reported recently (Sanders, Proc. Soc. Exptl. Biol. Med. **108**:185, 1962). This effect was observed when overnight broth cultures of pathogenic staphylococci were used as the source of coagulase in an in vitro experiment. Use of broth cultures containing viable cells as a coagulase source did not distinguish between two possible mechanisms of action of heparin: (i) inhibition of further cellular production of coagulase once added to the in vitro system, or (ii) direct inhibition of the coagulase enzyme. The present experiments permit this distinction.

Sterile, cell-free preparations containing crude coagulase were obtained by a method modified from Tager (Yale J. Biol. Med. **20**:487, 1948). Coagulase-positive staphylococci, of varying bacteriophage types, were obtained from 20 hospitalized patients. Each strain was inoculated into 30 ml of Trypticase Soy Broth (BBL), and allowed to incubate overnight. The next morning the cultures were centrifuged at 1,400 $\times$ $g$ in an International centrifuge for 30 min. The supernatant fluid to be used as a coagulase source was removed by suction, and sufficient Merthiolate was added to yield a final concentration of 1:10,000. These preparations were sterile after incubation of 10-ml samples with 25 ml of nutrient broth for 48 hr, and were free from cells and cellular debris on microscopic examination.

Coagulase Rabbit Plasma (Cappel Laboratory, West Chester, Pa.) was used to assay coagulase activity. The test system consisted of 0.1 ml of heparin (1.0 mg/ml), 0.5 or 1.0 ml of 1:10 rabbit plasma in isotonic saline, and 0.1 ml of the crude coagulase. Resultant heparin concentrations were 0.14 and 0.08 mg/ml. Isotonic saline (0.1 ml) was substituted for heparin in controls, which were otherwise comparable. Experiments were performed in duplicate at 37 C. Tubes were examined at 5-min intervals for coagulation.