

hour period. Of 21 control suspensions on which the light transmission was determined 15 minutes after dilution and again in 1 to 3 hours, only 14 showed no change. Similar experiments using isotonic saline showed a change in about one-third, both before and after standing. The cells after standing in the modified Brinkman's solution had assumed a spherical shape with numerous small delicate projections, and no further change in shape was detected during the period between determinations. Presumably they had reached an approximate equilibrium with the surrounding fluid. This method is being utilized at present in studying the rate of hemolysis of the red blood corpuscles by various hemolytic agents.

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⁴ Brinkman, R., and Van Dam, E., *Biochem. Z.*, 1920, cviii, 52.

⁵ McGlone, B., *Am. J. Med. Sc.*, 1926, clxxii, 155.

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A Comparison of Indifferent Substances and Specific Antigen in Production of Local Streptococcus Immunity.

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Our recent communication¹ which described a high grade of localized antistreptococcus protection in the pleural cavity of the rabbit induced by the previous injection of indifferent, non-antigenic substances, left in question the relation of this protection to a specific immunity which might be produced by the streptococcus. A thickened granulating parietal pleural wall containing large numbers of phagocytic clasmatoocytes is produced by the inoculation three days previously of gum arabic broth or aleuronat-starch and such a cavity resists infection by many multiples of the normally fatal dose of our pathogenic streptococcus. The

duration of this protection is from ten days to two weeks and its disappearance is paralleled by a return of the pleural wall to approximately its original condition as we have learned from recent histological study.

Repeated injections of the original non-specific substance, or of considerable doses of the streptococcus in the second and subsequent inoculations, produced further thickening of the pleural wall, an increased number of cells, and an apparently still more marked resistance to the streptococcus. In other words, there was an indication that the degree of resistance varies directly with the number of clasmatocytes present in the protected area. We at once raised the question, however, of the significance of specific antibodies, whether localized or general, in conditions of this sort. In other words, will granulation tissue of a given thickness produced by gum arabic broth afford as much protection as an equal accumulation of cells produced by the streptococcus? A preliminary experiment which we published seemed to answer this question in the negative but was not regarded as conclusive, owing to certain technical difficulties involved.

Pleural cavities that have received aleuronat or gum arabic withstand repeated injections of virulent streptococci but, although the animal recovers perfectly and on examination the cavity is found sterile, it is frequently found obliterated by adhesions in whole or in part. In a similar way one of our control series consists of animals with granulating pleural walls produced by *Staphylococcus aureus*. The injection of this organism is usually not fatal but almost invariably leaves localized abscesses behind, from which pure cultures of the inoculated coccus may be obtained. These two conditions make it impossible to locate a test dose of streptococcus in the pleural cavity with any certainty. To obviate these difficulties we have proceeded as follows in our recent experiments.

Three series of rabbits are prepared by intrapleural injections of:

- A. Gum arabic broth.
- B. Gum arabic broth containing first dead and then living cultures of *Streptococcus* "H".
- C. Gum arabic broth containing living *Staphylococcus aureus*.

Three or four such injections were given at intervals of 4 or 5 days in equal total volumes for each series. Six or 7 days later rep-

representatives of each series were tested with increasing multiples of the minimal lethal intrapleural dose administered into the *left* and still unaffected pleural cavity. We are now well assured of the conditions under which crossed pleural immunity is possible as outlined in the preceding article.

The results of these latest experiments are uniform and unmistakable. Repeated injections of gum arabic broth alone protect as well or even better than the same injections including streptococcus or staphylococcus. Thus in one experiment representatives of each of the three series withstood 3,000 lethal doses, but, only the one with gum arabic alone withstood 15,000 doses. One hundred fifty thousand and 300,000 doses were fatal to representatives of each of the series. In another experiment gum arabic animals withstood 1,800 and 4,500 lethal doses, both of which were fatal to the streptococcus-gum arabic and staphylococcus-gum arabic animals.

An objection may still be raised that the conditions of our experiments do not include true specific immunity; we failed to demonstrate agglutinins in the blood serum of any of our animals before the test injection, including those whose treatment included the streptococcus. Agglutinins appeared in some of the animals of each series during their period of recovery from the test injection. At all events these experiments indicate that a high grade of local immunity may be produced by non-specific substances entirely dependent on increase of clasmatoocytes. The failure of the addition of the specific antigen to increase this protection seems somehow associated with the generally recognized inefficiency of streptococcus serum therapy.

¹ Gay, F. P., Clark, A. R., and Linton, R. W., *Arch. Path.*, 1926, i, 857.