

found no difference between the serum and plasma pH, electrometrically determined. The correction in Cullen's method, however, is slightly greater for serum than for plasma as stated by Cullen.

2854

The skin response of rabbits to non-hemolytic streptococci.

C. L. DERICK and C. H. ANDREWES (Introduced by H. F. Swift).

[From the Hospital of the Rockefeller Institute for Medical Research, New York City.]

A study of the skin reactions in rabbits which were induced by intra-dermal injection of non-hemolytic streptococci demonstrated an unusual phenomenon. Following the injection of these microorganisms a local reaction took place which reached its maximum within 24 to 36 hours. The lesion then diminished until the eighth day, when an exacerbation occurred which at times approached the original lesion in intensity. This persisted for about 2 days and disappeared slowly. The late lesion is referred to as the "secondary reaction."

This reaction was first encountered while studying a green streptococcus isolated from a rheumatic subcutaneous fibroid nodule; with it definite secondary reactions were induced in 50 per cent of normal adult rabbits and doubtful reactions in an additional 13 per cent. On further extending the strains of green streptococci studied, the reaction was found to be incited by 5 out of 10 strains from nodules, blood or heart of rheumatic fever patients; by 1 out of 4 strains from the blood of subacute bacterial endocarditis patients; by 1 strain from the urine of a nephritic patient and by 1 anomalous strain isolated from a rheumatic nodule. It never followed the injection of green streptococci from normal throats, nor of hemolytic streptococci, staphylococci, *micrococcus catarrhalis*, *B. coli*, and *B. influenza*.

The material used for intra-cutaneous injection consisted of the centrifuged sediment from 5 cc. of an 18-hour growth of the

bacteria in blood broth. It was subsequently found that the reaction could be obtained with heat killed organisms.

Lesions were excised daily and studied bacteriologically and histologically. In the early stages an abundant growth of streptococci was obtained, but the number of viable organisms progressively diminished until the 7th day, when none was present, although many could still be seen in the smears. At the time of the secondary reaction only occasional contaminants were found. Histologically the primary reaction always consisted of a well circumscribed collection of cells, mostly polymorphonuclears, and bacteria, with edema of the surrounding tissue. Aside from more marked congestion and edema, there was nothing of importance to distinguish a secondary lesion from a primary one with the exception of the presence of a few macrophages in the outer cellular zone, which made their appearance about the 6th day. This secondary lesion rarely broke down and discharged pus.

By means of previous inoculation with any type of streptococcus within a period of nine weeks it was possible to prevent the secondary reaction following inoculation with a suitable strain. This protection did not exist after the ninth week. Protection was afforded not only by streptococci, but occasionally by pneumococci. Additional experiments with the latter organism are now under way. The secondary reaction was neither caused by nor prevented by nucleoprotein, soluble precipitable substance, or filtrates from an active strain.

In attempting to explain this phenomenon the possibility of renewed bacterial growth at the time of the secondary reaction was eliminated by using killed cultures and also by the inability to recover the original organism at this time. It would appear then to be caused by one of the following substances: (1) a product of the streptococci; (2) a product of the animal host; (3) a product of the interaction of streptococci and animal host.

The first is unlikely unless it takes about 9 days for the organisms to be broken down so that a toxic product is liberated. The second is hardly plausible, since such a hypothetical substance is called into action by only a limited number of bacterial strains. The third possibility has more claim for consideration.

The secondary reaction has certain analogies with serum sickness in man and the Arthus phenomenon in rabbits. To accept such an hypothesis it must be assumed that the injected strepto-

cocci or some antigenic product of them exists at the site of inoculation until the appearance of antibodies about the 8th or 9th day when there is a union of antibody and antigen; this product then acts as an irritant and excites the secondary reaction. In attempting to prove this many of the experiments of Opie¹ were repeated.

- (1) Injection of streptococci into an immune animal.
- (2) Injection of antibodies (immune serum) into an animal in whose tissues streptococcal antigen was known to exist.
- (3) Injection of *in vitro* mixtures of antigen and antibodies, *i. e.*, homologous immune serum.
- (4) Attempt to find some correspondence between the occurrence of the secondary reaction and the presence of antibodies in the body fluids at that period, *i. e.*, agglutinins, precipitins.

None of the experiments place the secondary reaction in a class with the Arthus phenomenon. As yet the causative factor or factors for the secondary reaction have not been elucidated, but further experiments are in progress toward this end.

¹ Opie, E. L., *J. Immunol.*, 1924, ix, 231.