

superambient air of broth cultures of the Type I pneumococcus greatly reduced or prevented bacteriostasis by sulfanilamide.

2. When the oxygen concentration was reduced to 0.04% an actual stimulus of growth by sulfanilamide was found.

3. When the oxygen was further reduced by the presence of pyrogallate or hydrosulfite the inhibition by sulfanilamide reappeared.

4. Hydrogen peroxide was detected only in cultures in equilibrium with atmospheres containing 10% oxygen or more. These concentrations correspond to those permitting effective bacteriostasis and are comparable to those obtainable in the plasma.

5. The lack of inhibition at intermediate concentrations shows that oxygen plays a vital rôle in the action of sulfanilamide. The failure to form peroxide at these same concentrations is taken as evidence that oxygen exerts its influence through the agency of hydrogen peroxide. The stimulus and recurring inhibition at the lowest values is interpreted as evidence of the formation of a toxic reduction compound, possibly a sulfide. The latter type of inhibition may play a rôle in any bacteriostatic effect against anaërobes.

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Advances in the Serological Typing of *Streptococcus hemolyticus*.

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Griffith's¹ technic for typing Group A hemolytic streptococcus has been applied in this laboratory to the classification of more than one thousand strains recovered from a variety of infections. It was possible to type 70% of the organisms examined in 1935 and 1936 by the procedure described previously.² At that time several difficulties were pointed out: (1) the elimination of anti-C cross-reactions; (2) the granular character of matt organisms; and (3) the failure of certain strains to be agglutinated by any of the 28 available type-specific sera.

As shown previously² interfering cross-reactions due to anti-C substance in the sera could be eliminated by absorbing the sera with

¹ Griffith, F., *J. Hyg.*, 1935, **34**, 542.

² Pauli, R. H., and Coburn, A. F., *J. Exp. Med.*, 1937, **65**, 595.

purified C-substance, but the purification of C-substance according to Heidelberger's method was a matter of considerable difficulty. However, C-substance prepared by another method (Fuller³) has has proven quite satisfactory for this purpose⁴ so that the preparation of type-specific sera has been greatly simplified.

The granular nature of the suspensions produced by many matt organisms interfered seriously with the typing of these organisms up to 1937. Subsequently two methods have been found to obviate this difficulty. Kodama⁵ has made granular suspensions homogeneous by subjecting them to supersonic vibrations. In collaboration with Dr. L. Emmet Holt, Jr., we have confirmed this observation, but found the method impractical. A simpler alternative consists in subculturing granular organisms in broth containing trypsin. The trypsin (Difco-Bacto Trypsin supplied in solution in 10 cc ampoules) is stored at 5°C until used. One cc of the solution is added to 9 cc of phosphate-buffered broth *just prior to seeding*. A heavy inoculum of culture (about 0.1 cc) added to the mixture and incubated at 37°C usually gives satisfactory growth in 6 to 18 hours. Suspensions are then made in the customary way. Out of 188 matt strains which we had been unable to type with available methods, only 28 were still too granular for typing after one subculture in broth to which trypsin was added. Only 8 of these remained refractory on subsequent subculturing in this mixture.

The third difficulty, failure of agglutination with any of the 28 type-specific sera has been largely overcome by Griffith's addition of 2 new sera (type 29 "Coggins"; type 30 "Quinn"). Most of the strains which we previously had to classify as "no type" have fallen into one of these 2 types.

These modifications have made it possible for us to assign types to 90% of the Group A cultures examined since our previous report, about 900 in all. There are still 3 minor difficulties: (1) the sera of types 6 and 18 are weak; (2) types 15 and 17 are frequently difficult to distinguish and (3) a few strains are still not agglutinated by any of our 30 sera. Most of these "no type" strains were recovered during the early fall months from throat cultures of healthy subjects. It may be significant that the time of their isolation corresponds with the season of minimal activity of hemolytic streptococcus in New York City.

Type 30 was the predominant type of hemolytic streptococcus

³ Fuller, A. T., *Brit. J. Exp. Med. and Path.*, 1938, **19**, 130.

⁴ Little, Paul A., personal communication.

⁵ Kodama, T., *Kitasato Arch. Exp. Med.*, 1937, **16**, 245.

recovered by us in 1937 and 1938, accounting for 35% of our typed organisms. Types 13, 15, 25, 27 and 28, together accounted for 25%. Types 4 and 22, which had been predominant in 1935-36, were recovered only occasionally. Type 10 (N.Y.5) was the only type not recovered in New York City during these 4 years. In contrast, types 10, 8, and 1 were most prevalent in the throat flora of Tokyo during this period of study.⁶

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Concentration of Prothrombin in Blood of Babies (3 to 7 Days Old).

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The ease with which nutritional deficiency states develop in young infants suggests the advisability of studying their prothrombin level, since it is now known that vitamin K is required for the synthesis of this clotting factor.

Blood was obtained by venipuncture from normal healthy infants varying in age from 3 to 7 days. The prothrombin was quantitatively determined by the senior author's method¹ on both the undiluted plasma and on plasma diluted with an equal volume of saline solution. Typical cases of this series are presented in Table I.

The results plainly show that the prothrombin concentration in the blood of the babies studied was essentially the same as that of normal adult blood. It was found, however, that the prothrombin diminished in infants' blood more rapidly than from that of adults, and curiously in a few bloods, such as Case 3, the decrease of prothrombin was strikingly rapid when the plasma was diluted.

These findings are completely at variance with those reported by Brinkhous, Smith and Warner.² In their series the prothrombin concentration of the bloods of the 3 babies who were less than 11 days old were 27, 36, and 44% of normal. Furthermore, even in infants 2 months old, they found that the prothrombin was less than

⁶ Kodama, T., personal communication.

¹ Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

² Brinkhous, K. M., Smith, H. P., and Warner, E. D., *Am. J. Med. Sci.*, 1937, **193**, 475.