

anaphylactic reactions have also been shown to be accompanied by low levels of 5-HT in the blood and a generalized sympathomimetic response, the above data further demonstrate the similarity between these two phenomena (10).

Summary. Elevated levels of epinephrine are found immediately after administration of a lethal dose of endotoxin. Following this, plasma catechol amine concentration is only slightly above control levels. Prior to death, toxic levels of epinephrine are attained. Serum serotonin concentration falls immediately after administration of endotoxin and remains low throughout the shock state.

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Variation with Age of Antistreptococcal Antibodies and Their Correlation With Antistreptolysin O Titers in Human Sera.* (25239)

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Halbert, Swick and Sonn(1) found that sera from non-rheumatic individuals gave from 0-4 bands, when tested by Ouchterlony's technic(2,3), against a crude ammonium sulfate concentrate of the supernate from a Group A streptococcus culture (C 203 S). Sera from active cases of rheumatic fever gave 2 to 7 bands when similarly tested. Harris, Harris and Ogburn(4) obtained similar results using Oudin's method(5) and the concentrated supernate of a type 4 culture. Halbert *et al.*(6) also observed a correlation between migration rate of the heaviest bands, antistreptolysin O (ASO) titers of the sera and intensity of the precipitate supposedly produced by the ASO system. This study is concerned with the immunogenic response to streptococcal antigens of normal individuals of different ages in a tropical environment. Possible correlations between precipitins, as evidenced by gel diffusion, and ASO titers have been investigated.

Materials and methods. Human sera comprised 9 taken from the umbilical cord of infants at birth, 74 from infants 1 to 12 months old, many of whom were suffering from diarrheal disease, and 331 from apparently normal adults aged 17 to 70 years, selected randomly at various factories, blood banks and public health dispensaries. The antigen used consisted of a type 3, group A, beta hemolytic streptococcus inoculated into 500 ml of trypticase soy broth and incubated at 37°C for 18 hours. Culture was divided into 50 ml portions, lyophilized and each dissolved in 5 ml of sterile saline just before use. Same batch of lyophilized material was used in all gel diffusion tests(1). Usually antigen was placed in the center and sera in the peripheral wells. Plates were placed in the refrigerator and precipitin bands read at intervals of 3, 7, 14 and 21 days. Antistreptolysin O assay was done with Bacto Streptolysin O reagents (Difco) and results expressed in Todd units.

Results. All sera from umbilical cord blood revealed the presence of 1 to 3 bands with an

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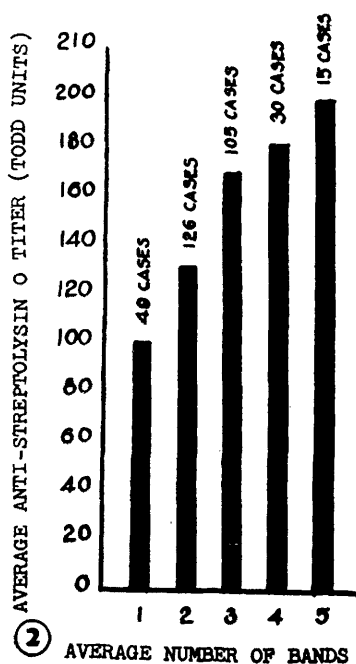
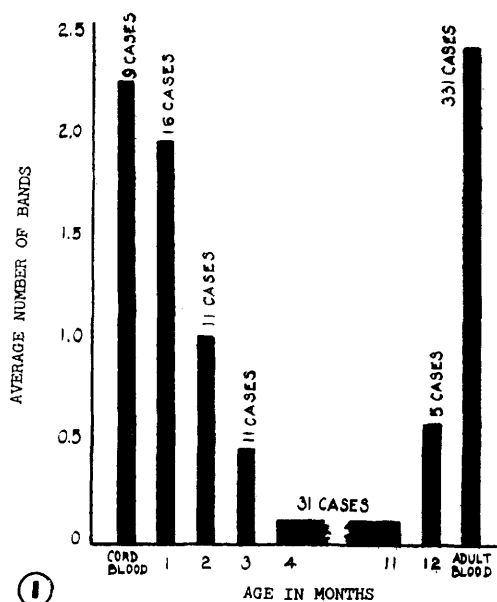


FIG. 1. Fluctuation with individual's age in the No. of bands (gel diffusion) in sera tested with a streptococcus whole culture concentrate.

FIG. 2. Correlation between ASO titers and No. of bands (gel diffusion) in adult sera.

average of 2.2 bands per individual. A sharp drop in the number of bands was observed in infants 1 to 5 months old (Fig. 1). Sera from 29 out of 31 infants 4 to 11 months old were

free from demonstrable anti-streptococcus antibodies. At 12 months the average number of bands per infant rose again to 0.6. The number of bands in 331 adults ranged from 0 to 5 with an average of 2.45 per individual which is about the same value obtained with cord sera. Forty-five adult sera gave 4-5 bands. This was not observed in any of the sera from infants in which the maximum number of bands was 3.

Findings in infant sera can probably be explained on the basis of transplacental transfer. These antibodies are short-lived and disappear from the infant's blood during the first 3 to 5 months of life. They reappear upon encounter with the streptococcus later in life. No significant fluctuation obtained in the average number of bands in individuals 17 to 70 years old.

Results suggest the desirability of a simultaneous study of the antibody pattern of mother and infant in an attempt to determine selective transplacental transfer of the different streptococcal maternal antibodies.

We did not perform ASO determinations in these infants' sera. There are reports in the literature of ASO determinations in infants which correlate with our observations(7). Three hundred thirty-one adult sera were assayed for ASO and by gel diffusion methods. Sera were classified into 5 groups according to number of bands obtained (from 0 to 5). Results are given in Fig. 2.

Good correlation obtains between number of bands and ASO titer which is not apparent when individual cases are considered. Average ASO titer for sera showing a single band was 100.6 units. ASO titers rose gradually in almost regular steps with each increase in number of bands. It is difficult to interpret the observed correlation between ASO titers and number of bands, representing a single antigen-antibody and a multiple antigen-antibody system respectively. The correlation found by Halbert, between migration rate of the ASO band and ASO titer is to be expected, as it has been established that rate of migration of a given band depends on respective concentrations of antibody and antigen(5). Our findings suggest that when ASO titer increases there is a concomitant stimulation of

antibody response to multiple streptococcal antigens elaborated *in vivo*. It seems that the more recent the date of the last streptococcal infection suffered by the patient, the stronger the antibody response observed. Apparently each antibody has a threshold level which has to be reached before antibody makes itself evident as a band by gel diffusion. Recent streptococcal infection could bring several of these antibodies to their threshold value resulting in an increase in number of bands and a concomitant increase in ASO titer. As time passes, concentration of antibody decreases with a corresponding diminution in number of bands and titer of the ASO system. The fact that a greater number of bands is obtained with the sera of rheumatic fever patients, in whom high ASO titers occur, seems to corroborate this interpretation of results.

Using a crude concentrated streptococcal supernate, Halbert found 0 to 4 bands in a series of 35 non-rheumatic individuals with an average of 1.51 bands per individual. We found an average of 2.45 bands. This may be due to our use of antigen which contained streptococci, from which cellular antigens could presumably be liberated by disintegration of the bacterial cell. Halbert(6) reports a surprising infrequency of bands with sonic vibrated cell extracts, as well as with individual intracellular constituents such as C carbohydrate and M substance.

It is interesting to note the almost complete absence of negative results (only one case) among adults, in contrast with the 31% of negative sera in Halbert's series. He believes that many of their apparently negative sera may have had bands masked by a non-specific turbidity of the serum-agar gel. In our experiments this interference was minimal. We observed that all adult sera, with the exception already mentioned, produced a band located approximately midway between the antigen and antibody wells, which gave reactions of identity with similar bands in adjacent sera, with the formation of a hexagonal pattern. Other bands were more variable in position and frequency.

An attempt was made to determine degree of contamination of our antigen with C carbohydrate and M substance. This was done by

comparing our preparation with antigenic preparations consisting of (a) hydrochloric acid extract of streptococci prepared by the method of Lancefield(8). (b) autoclaved extract of streptococci prepared by the method of Rantz and Randall(9). (c) C carbohydrate prepared by the method of Fuller(10). The 4 antigens were tested side by side against commercial Bacto anti-A serum. The last 3 antigens gave a prominent, well defined band, with reaction of identity with each other. Our antigen gave an extremely faint and diffuse band with reaction of identity with the other three. This indicates an extremely small contamination of our preparation with these intracellular antigens. In addition, we tested several normal sera against extracts a, b and c with completely negative results.

Summary. 1. Sera from newborn infants were examined by gel diffusion against whole culture concentrate of type 3, Group A beta hemolytic streptococcus. One to 3 bands of precipitation were obtained with average of 2.2 bands/individual. In the first 3 to 4 months of life the average number of bands/individual diminished sharply to almost complete absence. From 4 to 11 months of age the average number of bands/individual was 0.13. On twelfth month the average number/infant rose to 0.6, and in adults an average of 2.45 was obtained. These results are interpreted on the basis of transplacental transfer of antibodies which are short-lived and reappear with subsequent streptococcal infection later in life. 2. Sera from 331 apparently normal adults were studied by the gel diffusion method and simultaneously assayed for anti-streptolysin O. A close correlation was found between number of bands and ASO titers, not apparent when individual cases were compared. With each increase in number of bands there was a corresponding increase in ASO titer.

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Influence of Resting Tension on Contractility and Morphology of the Papillary Muscle.* (25240)

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The papillary muscle preparation has become a popular tool in the field of cardiac research. A wide variety of factors, *e.g.*, resting tension, manner of recording, composition of bathing media, have been studied with respect to their effects on contractility or morphology. A review of the literature discloses only 4 publications(1-4) in which resting tension was stated as an absolute value. Bennett(5) made a detailed study of the effect of tension on physiological response but did not examine the muscles for any associated morphologic changes. Tanz *et al.*(6,7) reported alterations in morphology of papillary muscles exposed to drugs, but failed to indicate the resting tension to which their preparations were subjected. In a preliminary report(8) experiments were described in which a study was made of the relationships between initial resting tension, stability of initial contractile amplitude or tension, and morphology of working papillary muscles. The present report includes studies on nonworking as well as working muscles.

Methods. Cats were anesthetized with ether, the heart removed, and placed in warm, oxygenated, modified Tyrode's solution of the following composition: (meq/l) Na, 145.00; K, 5.34; Ca, 4.75; Mg, 1.20; Cl, 129.55; PO₄, 1.79; SO₄, 1.20; HCO₃, 25.00; glucose, 5.0. A thin papillary muscle, 1.0 mm or less in diameter, was selected from the right ventricle and ligatures were placed around it at its

junction with the ventricular wall and the chorda tendineae. The muscle was then removed from the ventricle and placed in a muscle holder, care being taken not to apply tension to the muscle. The holder was immersed in a bath containing the previously described Tyrode's solution maintained at a temperature of 37.5°C and aerated by a rapid stream of 95% oxygen and 5% carbon dioxide entering through a sintered glass disk in the bottom of the bath. A supramaximal stimulus of 1 to 2 millisecon in duration was provided as a square wave from an American Electronic Laboratories, Inc., Model 751, stimulator at the rate of 1/sec. In isotonic preparations, the upper ligature was attached to a light heart lever adjusted to give the desired resting tension and record on a smoked drum. For isometric studies, the ligature was attached to the pin of a RCA 5734 transducer tube in a bridge circuit similar to that described by Ranney(9). This arrangement allowed the transducer to be calibrated with weights and the resting tension to be adjusted to that desired. A total of 30 muscles were studied, the tension ranging from 1.6 to 4.0 g. Some muscles were merely subjected to static tension and not stimulated. The muscles were removed from the bath after 6½ hours and fixed in 10% formalin. After fixation the muscles were dehydrated and embedded in paraffin. Sections approximately 5 micra in thickness were cut and stained with hematoxylin and eosin.

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Results. Muscles subjected to tensions of