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Studies on the Nature of Bacterial Hemolysin and Proteolysin.

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Many cultures of staphylococci and colon bacilli recently isolated from lesions are hemolytic when grown on blood containing media and usually such organisms are proteolytic when grown in nutrient gelatin. It has not been determined whether the hemolysin is identical with or produced parallel to the proteolytic substance. Both qualities are frequently transient and may exhibit a parallel decline. An attempt was made to study these substances with reference to individuality, interdependence or homologous nature.

Filtrates of broth cultures were tested for hemolytic activity as follows: measured quantities of filtrate were added to tubes containing 1 cc. each of a 0.5% suspension of washed red blood cells, incubated at 37°C., for 1 hour, placed in the refrigerator over night and the amount of lysis observed. Proteolytic activity was determined by streaking filtrate upon the surface of an agar-gelatin medium, incubating at 37°C., for 48 hours and testing for lytic action by flooding the plate with an acid bi-chloride of mercury solution according to the method of Frazier[1] and noting any change in the character of the gelatin as indicated by a clear zone at the site of "planting".

Filtrates of 4 to 8 day broth cultures of a proteolytic and hemolytic, Gram negative, motile organism, of intestinal origin and considered as belonging to the colon group were very active in lysing red blood cells and in digesting gelatin.

In filtrates heated to 55°C., for 5 minutes the hemolysin was de-

[1] Frazier, W. C., *J. Inf. Dis.*, 1926, xxxix, 302.

stroyed, while the proteolysin was destroyed only after 30 minutes at that temperature.

In absorption experiments it was found that by incubation of the active filtrate with red blood cells at low temperature it was possible to absorb and remove all of the hemolysin without marked decrease in proteolytic activity of the filtrate.

Experiments were also made with a proteolytic and hemolytic culture of *Staphylococcus albus,* isolated from a case of middle ear disease. Difficulty was encountered in preparing an enzymatic material containing both the hemolytic and proteolytic substances as filtrates of plain broth cultures were devoid of proteolysin and contained the hemolytic principle only irregularly. Broth cultures of various ages sterilized by addition of thymol were active in lysing red blood cells but were without effect upon gelatin. Living organisms as obtained from agar cultures, suspended in saline solution, manifested the hemolytic property. However, hemolysin production was probably coincident with bacterial activity during the hour of incubation at 37°C.

By sectioning cylinders of agar media below the surface growth of the organisms and extracting with salt solution the hemolytic substance was found to have diffused as far as 1.5 cm. below the surface culture. However, proteolysin could not be demonstrated in these sections.

The hemolysin in broth filtrates was most active in lysing red blood cells of the rabbit, less active toward those of the sheep and guinea pig and least active with those of man.

Dilution of the hemolytic filtrate with saline solution 1:10 did not interfere with activity. However, dilution with sterile broth 1:2 decreased lytic activity and inhibition was complete at a dilution of 1:10. Exclusion of air by covering the mixture of cells and filtrate with oil served to delay the appearance of lysis and quantitatively reduced lytic activity. Heating the filtrate to 50°C., for 40 minutes decreased lytic activity while at 56°C. for 10 minutes, hemolysin was entirely destroyed.

In later experiments, filtrates were made from 3 to 6 day cultures in proteose-peptone broth (pH 7.5) containing 0.5% of aminoids and sterilized by Seitz filtration. These filtrates contained hemolysin and proteolysin. It was possible to absorb and remove all of the hemolysin by incubating mixtures of filtrate and cells for 1 hour at 5°C. Cells sensitized in this manner and removed by centrifuging lysed upon re-suspending in salt solution. The supernatant fluid contained proteolysin but was devoid of hemolytic activity.

By forcing the dissociation of the original culture, 2 aberrant types were obtained upon subculture, one hemolytic and non-proteolytic, while the other was proteolytic and non-hemolytic.

The data indicate that hemolytic and proteolytic activities of filtrates prepared from cultures of both of these organisms are manifested by individual extracellular substances, and that hemolytic and proteolytic properties of viable staphylococci may be independently altered by means of bacterial dissociation.

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A Useful Modification in the Preparation of Therapeutic Sera.

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There are two recognized categories of individuals who are potentially subject to acute complications incident to serum therapy. One group is composed of those naturally hypersensitive to horse protein. The other is composed of individuals who are so constituted that they become hypersensitive to horse protein on the first exposure, and henceforth react like the individuals of the first group to the injection of the horse serum. (The latter category of individuals is likely to increase in the future due to toxin-antitoxin immunization.) While accidents of this sort can be avoided by the use of therapeutic sera derived from such animals to the protein of which the recipients show no hyper-susceptibility, in practice this procedure can not be carried out because therapeutic sera are prepared in horses almost exclusively. It is evident that if the protein of the therapeutic serum could be deprived of its species specificity the difficulty might be solved. The work of Obermeyer and Pick, of Landsteiner and of others has shown that by subjecting proteins to azotization, acetylation, halogenation, or by coupling with carbohydrates or lipoids, it is possible to destroy the species specificity of proteins while imparting to them new artificial specificities. In the past these experiments were made only with the view of eliciting the interdependence between the chemical structure and antigenic specificity. Accordingly chemical procedures selected were often so drastic that they would not be applicable to the problem at hand without some modification. By excluding such procedures which