

# DIFFERENTIATION BETWEEN SOME TOXIC SUBSTANCES IN ANAEROBICALLY PRODUCED AUTOLYSATES OF PNEUMOCOCCI (TYPES I AND II).

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(Received for publication, January 22, 1929.)

In preceding papers (1, 2), it was shown that anaerobically produced autolysates of pneumococci cause necrosis when injected intradermally, and marked lung lesions and death when injected intratracheally, into small guinea pigs. In the present study, I wish to describe experiments made to determine whether or not the necrotizing and the lung-toxic effects are due to the same or to different substances present in these autolysates.

## EXPERIMENTAL.

### *Preparation of the Poisonous Autolysates.*

The method of preparation of the toxic autolysates has been described in detail in previous papers (1, 2). Briefly, the method is to autolyze in fresh broth at room temperature under vaseline seal, the centrifuged pneumococci obtained after 18 hours growth on double strength veal infusion broth which contains 4 per cent Witte peptone. The Berkefeld filtrates of these autolysates are toxic for both the skin and the lungs of young guinea pigs and both these toxic effects can be preserved without deterioration when such filtrates are kept in the ice box under vaseline seals.

### *Heat Lability of the Toxic Autolysates for the Lungs of Guinea Pigs.*

In a previous article it was shown that the necrotizing principle was completely destroyed when heated to 60° for 5 minutes under vaseline seal. It was found that under these same conditions the lung-toxic principle was also destroyed. Guinea pigs injected intratracheally with 0.2 cc. of the heated autolysate did not appear sick at any time and did not die spontaneously. Several of these pigs were

killed with ether 1 to 3 days after the injection of heated poison, and all showed at autopsy a small red patch of consolidation in one or more lobes of the lungs near the hilum, but never the diffuse lesion caused by the unheated autolysates.

*Sensitiveness to Oxidation of the Toxic Autolysates for the Lungs of Guinea Pigs.*

It was noted in a previous paper that the skin-necrotizing poison in the autolysates was extremely sensitive to oxidation and deteriorated rapidly when exposed to the air. We found that the lung-toxic substance also weakened quickly after removal of the vaseline seal, even when the tubes containing the preparations were kept in ice water. A few experiments were made with aerobically prepared autolysates,—autolysates prepared exactly like the anaerobic autolysates, except that the pneumococci were autolyzed without the vaseline seal and the filtrates preserved in the ice box were also without a vaseline seal. These aerobically prepared autolysates produced no skin reaction when injected intradermally and no symptoms or death when injected intratracheally into young pigs. In some pigs killed after the injection of aerobic autolysates, small areas of consolidation near the hilum were noted, similar to those found after the injection of heated autolysates.

*Is the Lung-Toxic Product Neutralizable by the Anti-Autolysate Serums Which Neutralize the Necrotizing Principle?*

It was shown previously that there was an apparent neutralization of the necrotizing principle when necrotizing autolysates from either *Pneumococcus* I or II were mixed *in vitro* with the serum of rabbits immunized against *Pneumococcus* I autolysates. It was important to determine whether the substance or substances which were responsible for the death of guinea pigs injected intratracheally with the toxic filtrates could also be neutralized by the antisera which neutralized the necrotizing principle.

The tests for neutralization of the lung-toxic product were set up in a way similar to our former tests for neutralization of necrotizing substances. These tests were carried out as follows: 0.9 cc. of a well chilled necrotizing filtrate was placed in

each of three narrow test-tubes. To the first tube was added 0.1 cc. of the anti-serum; to the second tube 0.1 cc. of normal rabbit serum; and to the third the same amount of broth. The contents of the tubes were well mixed, and a heavy vaseline seal was then added to each tube. After standing at room temperature for 1 hour, the tubes were again chilled, the vaseline seals removed, and the preparations in amounts of 0.25 cc. were injected intratracheally into small guinea pigs of approximately equal weights. The results of these experiments were clear-cut. The pigs which received the toxic autolysate and immune serum showed no symptoms and survived. The other two pigs died with the usual symptoms and the autopsy findings were typical. This experiment has been repeated several times and always with the same results.

ABLE I.

|                              | Toxicity for<br>guinea pigs'<br>lungs. 0.2 cc.<br>injected intra-<br>tracheally | Toxicity for<br>guinea pigs'<br>skin. 0.1 cc.<br>injected intra-<br>cutaneously |
|------------------------------|---|---|
| Necrotizing filtrate.....    | +++   | +++   |
| Adsorbed with red cells..... | 0   | +++   |

These experiments have been repeated with two potent anti-autolysate horse serums. These serums in amounts of 0.0002 cc. neutralized at least one lethal dose of the lung-toxic autolysates, *viz.*—0.1 cc. of these serums diluted 1–100 neutralized 0.9 cc. or more than five lethal doses of the toxin. (These last experiments will be given in detail in a later publication.)<sup>1</sup>

From the fact that the anti-autolysate serum prepared with Type I neutralized the lung-toxic action of the autolysates of both *Pneumococcus* Type I and Type II, it is evident that the lung-toxic poison, like the pneumococcus necrotizing poison, is antigenically similar for both Types I and II and is therefore not type-specific.

These experiments seem to show that the necrotizing and lung-toxic poisons in the anaerobic autolysates are similar in their sensitiveness to heat and to oxidation and in their ability to be neutralized by the same anti-autolysate serums.

<sup>1</sup> These horses were immunized to the autolysate filtrates at Eli Lilly Co., in Indianapolis, under the supervision of Mr. W. A. Jamieson.

*Toxicity of Red Cell-Adsorbed Autolysates for the Lungs of Guinea Pigs.*

In a preceding paper, it was shown that necrotizing filtrates from which the hemotoxin had been removed by adsorption with red cells, were just as toxic for the skin of guinea pigs as untreated autolysates. It seemed of interest to determine whether or not such treatment had any effect on the toxicity of these autolysates for guinea pig lungs.

Accordingly guinea pigs of approximately equal weight were injected intratracheally with 0.2 cc. of necrotizing filtrates or the same filtrates which had just previously been adsorbed with red cells. Six different autolysates were tested in this manner. All the guinea pigs which received the untreated autolysates died with characteristic symptoms and autopsy findings, while none of the pigs which were inoculated with adsorbed autolysates showed any symptoms or died. Five of the latter pigs were killed with ether from 1 to 3 days after the injection, and at autopsy showed very much the same picture grossly as the pigs which had been injected intratracheally with the heated toxin described before. None showed the diffuse pneumonia regularly found in control animals. The consolidation about the hilum was, however, more extensive than in the pigs given heated toxin. All autolysates, both treated and untreated, were tested each time for necrotizing activity by inoculation into guinea pigs' skin and always proved to be equally toxic for the skin. These last experiments are summarized in Table I.

It is evident from these experiments that the substance or substances in the necrotizing autolysates, which when injected intratracheally bring about marked symptoms and death of guinea pigs, had been removed or inactivated by treatment with the red cells. An important question to be answered was whether or not the toxic substance for the lungs which had been removed was the pneumococcus hemotoxin, which is known to be adsorbed by red cells.

*Toxicity of Pneumococcus Hemotoxin for the Lungs of Guinea Pigs.*

To investigate this point, we produced pneumococcus hemotoxin by the freezing and thawing method of Avery and Neill (3) and inoculated the filtrates of these extracts, which were strongly hemolytic, intratracheally into guinea pigs. As a general rule, these hemotoxin preparations caused no symptoms and only slight lung involvement when injected intratracheally. However, we have obtained two preparations which were toxic for the lungs of guinea pigs, causing a diffuse lesion similar both macroscopically and microscopically, to that

caused by a weak lung-toxic autolysate. It is probable, therefore, that the pneumococcus extracts do occasionally contain the lung-toxic poison in considerable amounts. That the lung-toxic action of our autolysates is not due to the hemotoxic properties of the hemotoxin itself seems probable from the fact that no evidence of hemolysis was seen in the sections of the lungs of the pigs injected with the toxic autolysates and that the lung-toxic action of an autolysate may be neutralized by quantities of anti-autolysate serums which have no effect on the hemotoxin. (This last point will be brought out in greater detail in a later paper.) We have also found no definite parallelism in our autolysates between the toxic action for lungs of

TABLE II.

| Toxic products in anaerobic autolysates | Present in aerobic autolysates | Present in anaerobic autolysates | Present in frozen and thawed pneumococcus extracts | Present in anaerobic autolysates adsorbed with red cells |
|---|--------------------------------|----------------------------------|--|--|
| Hemotoxin.....                          | 0                              | +                                | +  | 0  |
| Skin-toxic.....                         | 0                              | +                                | 0  | +  |
| Lung-toxic.....                         | 0                              | +                                | ±  | 0  |

guinea pigs and the amount of hemotoxin such autolysates contain. We think it probable, therefore, that the pneumococcus hemotoxin present in the autolysates is not responsible for either the dyspneic symptoms, or the death with marked lung lesions of guinea pigs injected intratracheally with the toxic autolysate, but that preparations of hemotoxin, produced by the freezing and thawing method, may contain definite amounts of the lung-toxic product.

It seems clear, therefore, that although the necrotizing and lung-toxic principles in the autolysates are similar in several respects, they differ in the important point that the necrotizing poison is not inactivated or adsorbed by red cells as is the lung-toxic principle. This fact appears to prove that the necrotizing and lung-toxic principles are separate entities. These facts are summarized in Table II.

## CONCLUSIONS.

The necrotizing and lung-toxic principles present in certain anaerobically prepared autolysates of *Pneumococcus* Types I and II are similar in respect to extreme sensitiveness to heat and to oxidation, and to their ability to be neutralized by the same anti-autolysate serums.

These two poisons differ, however, in their ability to be adsorbed or inactivated by red cells; the lung-toxic principle being adsorbed or inactivated by such procedure while the necrotizing principle is not.

Since pneumococcus hemotoxin is present in the anaerobic autolysates and is also adsorbed by red cells, it seemed possible that it was this substance in the autolysates which caused the diffuse lung lesions and death of guinea pigs. However, it was found that the intratracheal injection of pneumococcus hemotoxin prepared by the method of Avery and Neill only occasionally produced the characteristic reaction caused by the intratracheal injection of the anaerobic autolysates. From these experiments we believe, therefore, that the necrotizing and lung-toxic principles, and probably the pneumococcus hemotoxin also, are all separate entities in the anaerobically produced autolysates described.

## BIBLIOGRAPHY.

1. Parker, J. T., *J. Exp. Med.*, 1928, xlvii, 531.
2. Parker, J. T., and Pappenheimer, A. M., *J. Exp. Med.*, 1928, xlviii, 695.
3. Avery, O. T., and Neill, J. M., *J. Exp. Med.*, 1924, xxxix, 745.