DETECTION OF TOXIC SUBSTANCES IN KILLED CULTURES OF PNEUMOCOCCI USING ADRENALECTOMIZED MICE (SHORT COMMUNICATION)

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The nature of the factors causing death of the host from infection has not been identified in the case of many species of pathogenic agent, including the microorganisms of the cocci group.

The object of this investigation was to investigate one such factor in the pneumococcus.

EXPERIMENTAL METHOD AND RESULTS

Adrenalectomy increases the sensitivity of animals to the action of bacterial toxins and, in the endotoxins of the Gram-negative microorganisms. The author has used this method to detect the toxic substances produced by pneumococci.

Virulent (smooth) and avirulent (rough) forms of pneumococci of types I and II were tested. Cultures were grown on serum broth for 24 h and killed by heating to 58° for 30 min. The test preparations, in a volume of 1 ml, were injected intraperitoneally into mice adrenalectomized 2 days beforehand. Death of the animals during the next 48 h was noted.

The results of preliminary experiments showed that cultures of type I pneumococci, killed by heat or by incubation for 24 h with sodium merthiolate, caused death of the adrenalectomized animals in approximately the same number of cases (from 64 to 70%). Heated cultures of type II pneumococci caused death of 57% of adrenalectomized mice. Death of intact animals was rare (under 6% of cases). If the cultures were heated to higher temperatures (70° or higher) they lost their toxic properties.

Storage of the pneumococcal cultrures at constant temperature for 48 h or more caused a marked decrease in their toxicity, after subsequent heating, toward adrenalectomized mice. Cultures kept for a long time (11-20 days) at 4° also lost their activity, and if heated before injection, caused death of only 20% of the experimental animals. Injection of cultures stored at 37°, but heated before being stored, caused death of 80% of the animals. Preliminary heating thus stabilized the lethal factor (as the toxic component is conventionally called).

To determine the localization of the lethal factor, the whole heated culture and the decantate and suspension of thrice washed bacterial cells prepared from it were injected intraperitoneally into adrenalectomized mice. These preparations caused death of 59, 25, and 50% of animals respectively, from which it may be concluded that most of the lethal factor is contained in the bacterial cells.

One of the most important questions is the content of lethal factor in cultures of different degrees of virulence. If, in fact, the toxic component is related in any way to the virulence of the bacterial culture, the virulent strains must contain more of it than the avirulent. A series of experiments was performed to determine the toxicity of the heated virulent (smooth) and avirulent (rough) cultures of type I. Injections of the virulent cultures led to death of twice as many adrenalectomized mice as injection of avirulent cultures (120 of 150 mice in the first case, 57 of 135 in the second). Evidently the lethal factor contained in the cells of the virulent pneumococci in an increased amount may play a definite role in the death of the animals from pneumococcal infection. This raises the issue of the presence of pneumococcal lethal factor in the tissues and organs of infected animals.

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A group of mice was inoculated intraperitoneally with a large dose (44 000 cells) of virulent type I pneumococci. Next day blood was taken from the animals (some of which were in an agonal state) and smears were obtained from the peritoneal cavity, spleen, and liver. Suspensions of the organs, ground with physiological saline, were centrifuged. The preparations were heated from 30 min at 55°, after which the tissue extracts were again centrifuged. The suspensions were sterile. Preparations obtained from animals infected with pneumococci and from control animals were injected into adrenalectomized mice. In the first group most of the mice died within 24 h. The tissues of the uninfected animals were nontoxic toward the adrenalectomized mice. The results of this experiment confirmed the hypothesis that the lethal factor is present in the body of mice with a lethal pneumococcal infection.