# Effect of Hemoglobin on the Development of *Pseudomonas aeruginosa* Infection

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The disease caused by injection of *P. aeruginosa* suspension in a hemoglobin solution was more severe (greater weight loss and larger abscesses in primary foci) than that induced by the same dose of the microbe in Hanks' solution (control). It was supposed that in the control the viability of the microorganisms was suppressed by iron deficiency in surrounding tissues, which was particularly pronounced upon accumulation in a limited space of great numbers of bacterial cells competing with each other for iron. Iron released from hemoglobin provides better conditions for the development of *P. aeruginosa*.

**Key Words:** *bacterial infection; Pseudomonas aeruginosa; iron*

Infection caused by immobile microorganisms is less severe if the infecting dose is injected as a concentrated suspension [1]. The same dose injected as diluted suspension induces severe infection with greater weight loss and formation of larger abscesses in primary foci. The severity of infection caused by the mobile microbe *Pseudomonas aeruginosa* strain 453 (L. A. Tarasevich Institute for Standardization and Control of Medical and Biological Preparations) was practically the same after injection of concentrated and diluted suspension. It was hypothesized that in the organism the bacteria lack some vital substance, particularly when they are accumulated in great numbers in a limited space. Consequently, accumulation of immobile microorganisms is unfavorable for them and favorable for the macroorganism. The significance of this factor for mobile microbes is much lower. The present study is an attempt to check up this hypothesis employing iron as a vital substance.

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## MATERIALS AND METHODS

Two groups of rats weighing 180-200 g were infected by injection of $5\times10^8$ cells/ml *P. aeruginosa 453* (0.3 ml of suspension washed from 24-h agar culture) in the right gastrocnemius muscle. Control animals ($n$=9) were given the injection in Hanks' solution. Experimental rats ($n$=9) were infected with bacterial suspension in Hanks' solution with hemoglobin in the same concentration as in the blood. Hemoglobin was prepared by hemolysis of washed erythrocytes from intact rat in distilled water. Erythrocyte ghosts were removed by centrifugation, and isotonicity of suspension was adjusted with a 10-fold Hanks' solution.

The severity of infection was assessed by weight loss 3 and 7 days after injection of *P. aeruginosa* and by the weight of purulent-necrotic exudate in primary foci after 7 days. The significance of differences was evaluated by Wilcoxon's test.

## RESULTS

The intergroup difference in the examined parameters were statistically significant (Table 1). The disease was more severe in experimental rats, as

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**TABLE 1.** Body Weight of Rats and Weight of Exudate in Sites of Infection

| Vehicle | Number of infecting bacteria | Body weight change, g | | Exudate weight, mg |
|---|---|---|---|---|
| | | 3 days of infection | 7 days of infection | |
| Hanks' solution (control) | $1.5\times10^8$ | +0.5 | -2 | 61 |
| Hanks' solution+hemoglobin | $1.5\times10^8$ | -7 | -16 | 320 |

**Note.** For all parameters $p<0.05$ compared with the control.

evidenced by greater body weight loss and larger abscesses in primary foci. Since hemoglobin is degraded in the intercellular space with the release of iron, it can be concluded that the increase in the concentration of iron in primary focus was favorable for the microorganism and unfavorable for the host. All living organisms require iron because it is a component of respiratory enzymes [2-6]. In mammals all iron is in the intracellular bound form. It was shown that *P. aeruginosa* produces proteins (siderophores) that compete with transferrin for free iron [3]. Our results indicate that this mode of adaptation to life in a macroorganism is not ideal, since bacteria lack iron. Therefore, artificial enrichment of primary focus by iron improves their viability. Presumably, in addition to iron, the bacteria lack other vital elements. Irrespective of the nature of one or several substances, their deficiency will be most pronounced in the sites where microbial cells competing with each other for these substances are accumulated in a limited space. In immune organism, agglutinating antibodies provide accumulation of bacteria in a limited space, thus suppressing their viability.

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