

## Effect of Haemoglobin on Experimental Infections with *Pasteurella septica* and *Escherichia coli*

BOTH haematin hydrochloride and ferric ammonium citrate can abolish the protective effect of immune serum in experimental infections with *Pasteurella septica* in mice<sup>1</sup>. Ferric ammonium citrate has the same effect with *Clostridium welchii* Type A infections in guinea-pigs<sup>2</sup>. The importance of these observations depends on the fact that iron can abolish the bacteriostatic effect of sera against *Cl. welchii* *in vitro*<sup>3</sup>. Because iron compounds seem to have such similar effects both *in vitro* and *in vivo*, it seems quite likely that the bacteriostatic action of serum may play an important part in resistance to bacterial infections.

We find that homologous lysed red cells as well as heterologous crystalline haemoglobin are effective in abolishing passive immunity to *P. septica*. Homologous lysed red cells and crystalline haemoglobin also enhance the virulence of *Escherichia coli* (type 0111B4) in normal guinea-pigs.

Red cells were prepared from blood withdrawn from the tails of anaesthetized mice; they were separated by centrifugation, washed three times with normal saline and lysed by the addition of approximately 5 volumes of distilled water. The cell stroma was removed by centrifugation and the supernatant fluid sterilized by filtration through a Seitz EK pad. The haemoglobin in the filtered solution was measured colorimetrically by the carboxy-haemoglobin (Haldane) method. Lysed guinea-pig cells were prepared in the same way as lysed mouse cells except that the blood was removed by cardiac puncture from animals anaesthetized with ether. Chromatographically homogeneous haemoglobin was isolated from human blood by the method of Allen *et al.*<sup>4</sup> and the solution sterilized by filtration through a cellulose ester membrane (Oxo, Ltd., London). Crystalline human haemoglobin was prepared from the same sample of blood by the method of Drabkin<sup>5</sup>. Two samples of crystalline horse haemoglobin were used (twice recrystallized grade A, Calbiochem; and Koch-Light Laboratories). The haemoglobin solutions were sterilized by filtration through a Seitz EK pad. The lysed red cells and the haemoglobin solutions were mixed with the bacteria immediately before intraperitoneal injection.

The methods of passive immunization, and infection with *P. septica* (strain CN 3449, Wellcome Research Laboratories), were the same as those described previously<sup>1</sup>. For all the experiments the dose of bacteria injected intraperitoneally varied between  $1.5 \times 10^5$  and  $2.9 \times 10^6$ , but in any individual experiment the treated and control groups received identical numbers of organisms. The effects of lysed mouse red cells (equivalent to 7–28 mg haemoglobin) and the purified haemoglobins on passive immunity against *P. septica* are shown in Table 1. None of these compounds was toxic to normal mice.

*E. coli* strain 0111B4 was passaged in guinea-pigs by the same technique used for mice, but the lethal dose ( $> 3 \times 10^7$  organisms) for guinea-pigs weighing 250–300 g remained relatively high. For the experiments with lysed

Table 2. EFFECT OF LYSED GUINEA-PIG RED CELLS AND CRYSTALLINE HAEMOGLOBIN ON INFECTION BY *E. coli* (STRAIN 0111B4) IN NORMAL GUINEA-PIGS

Haemoglobin intraperitoneally with <i>E. coli</i>	Amount of haemoglobin (mg)	Number of guinea-pigs	Deaths (3 days)	Deaths (%)
Nil	Nil	25	0	0
Lysed guinea-pig red cells	166–236	30	29	97
Lysed guinea-pig red cells	50	15	8	53
Human haemoglobin: crystalline	135	10	9	90

red cells and haemoglobin shown in Table 2,  $2.5$  to  $6.6 \times 10^4$  *E. coli* were injected intraperitoneally. Neither the lysed cells nor the haemoglobin solution was toxic to normal guinea-pigs.

It is clear that homologous lysed red cells and a variety of purified haemoglobins can be effective in abolishing passive immunity to *P. septica* in mice, and enhance the virulence of *E. coli* in normal guinea-pigs. The mechanism of these reactions needs further investigation, but for the moment we have assumed, in the case of *P. septica* at least, that the reason for the effectiveness of these compounds is connected with the ability of the bacteria to acquire iron from the haemoglobin molecule. This is based on the observation that ferric ammonium citrate, a relatively simple iron compound, produces the same effect as haematin and haemoglobin. Whether the presence of free haemoglobin in wounds or infected tissue might play a part in encouraging bacterial infection is a matter for speculation, but it seems to be a possibility.

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- <sup>1</sup> Bullen, J. J., Rogers, H. J., and Cushnie, G. H., *Nature*, **214**, 515 (1967).  
<sup>2</sup> Bullen, J. J., Cushnie, G. H., and Rogers, H. J., *Immunology*, **12**, 308 (1967).  
<sup>3</sup> Rogers, H. J., *Immunology*, **12**, 285 (1967).  
<sup>4</sup> Allen, D. W., Schroeder, W. A., and Balog, J., *J. Amer. Chem. Soc.*, **80**, 1628 (1958).  
<sup>5</sup> Drabkin, D. L., *Arch. Biochem.*, **21**, 224 (1949).

## Mechanism of Closure of the Aortic Valve

THE human aortic valve consists of three cusps made of relatively inelastic, muscle-free material about 0.15 mm thick. It opens and shuts about once a second, and withstands a pressure difference of 100 mm of mercury when closed. It usually functions for 70 yr without failure, and works so efficiently that very little blood is regurgitated at each pulse. In order to support this large

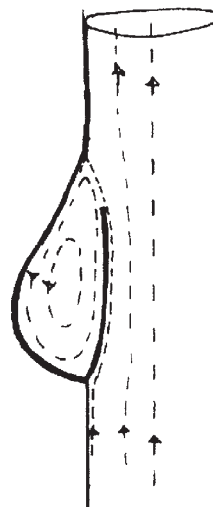


Fig. 1. Vortex formation in a sinus.

Table 1. EFFECT OF LYSED MOUSE RED CELLS AND PURIFIED HAEMOGLOBINS ON INFECTION BY *P. septica* (STRAIN CN 3449) IN PASSIVELY IMMUNIZED MICE

Haemoglobin intraperitoneally with <i>P. septica</i>	Amount of haemoglobin (mg)	Number of mice	Deaths (5 days)	Deaths (%)
Nil	Nil	327*	34	10
Lysed mouse red cells	7–28	60	58	97
Human haemoglobin: crystalline	7	10	10	100
Human haemoglobin: chromatographically pure	7	10	10	100
Horse haemoglobin: crystalline sample 1	28	10	10	100
Horse haemoglobin: crystalline sample 2	28	10	10	100

\* Including controls from other experiments.