transfer was measured by a heat flow disk (Sensable B-1, Hy-Cal Engineering). Thermal insulation in clo units was calculated from the following equation (*1*):

$$\text{clo} = \left(\frac{T_1 - T_2}{H}\right)\left(\frac{1}{0.18}\right)$$

where T_1 is the temperature of the surface of the heat source (in degrees Celsius), T_2 is the temperature of the heat sink's lower surface, and H is heat flow [the product of heat energy (calories) and area (square meters) divided by time (hours)].

Temperatures and heat flow were observed until readings were constant over a period of 30 minutes. The experiment was repeated two more times, each preceded by dismantling and reassembling of the apparatus. The data were averaged, tabulated (Table 1), and plotted (Fig. 1).

Loft of the natural and synthetic insulating materials ranged from 0.6 to 3.6 cm. During the heat transfer measurements, lofts were reduced by compression to 0.4 to 2.6 cm. Density ranged from 170 to 424 g/m^2 per centimeter. Insulation ranged from 0.93 to 1.34 clo/cm (mean, 1.12 clo/cm), values which correspond closely with published values (0.7 to 1.33 clo/cm; mean, 1.08 clo/cm) (*2*).

Insulating capabilities varied little among materials. Down provided somewhat higher value (mean, 1.22 clo/cm) than polyester (mean, 1.08 clo/cm). The polyolefin fibers and wool filler provided intermediate degrees of insulation (1.13 and 1.17 clo/cm, respectively). These variations are unlikely to be detectable by the consumer. If one considers weight an important factor, down (233 g/m^2 per clo) had a decided advantage over wool (339 g/m^2 per clo), polyester (328 g/m^2 per clo), and polyolefin (464 g/m^2 per clo).

Many physical scientists have maintained that no particular fabric provides superior insulation; air is the insulating material, and how it is immobilized makes little, if any, measurable difference. The importance of this study is in the application of a single heat transfer measurement technique to a variety of natural and synthetic filler materials, especially the newly developed polyolefin fillers.

Questions might be raised regarding a technique that measures heat transfer of compressible materials subjected to a compression force of 1.2 g/cm^2. Filler materials exhibit different qualities of compliance. If compression of a swatch were to increase the density of the heat-conducting fillers, heat transfer might be expected to increase as well. However, in 38 determinations on 12 samples of down, polyester, and polyolefin we have found no significant effect of compression on thermal insulating properties at reductions in loft to 52 percent of the control value.

Burton and Edholm (*3*) observed that the principal function of the filling material is "merely to immobilize the enclosed air, preventing convection currents and making it effectively 'dead air.' " Our findings, gathered by a single method comparing the thermal insulating qualities of fillers in the form they are used by the consumer, confirm this statement. None of the synthetic fillers has a superior ability to provide "warmth" in clothing. It seems that factors other than thermal insulation, such as weight, drapability, durability, and cost should be the major factors in selecting outerwear containing thermal insulation materials. The effects of moisture and bellows action produced by the wearer's activity may outweigh thermal insulative values in the wearer's perception of warmth.

W. C. KAUFMAN
D. BOTHE
S. D. MEYER
Department of Human Biology, University of Wisconsin, Green Bay 54302

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Haptoglobin: A Natural Bacteriostat

Abstract. *The combination of bacteria and blood in a wound can have lethal consequences, probably because hemoglobin iron supports prolific bacterial growth. Rats inoculated intraperitoneally with pathogenic* Escherichia coli *and small amounts of hemoglobin die. Simultaneous administration of haptoglobin, a naturally occurring hemoglobin-binding protein, fully protects against lethality. Therefore, haptoglobin may not only accelerate the clearance of free hemoglobin, but also limit its utilization by adventitious bacteria. Haptoglobin may have therapeutic potential in the treatment of life-threatening, hemoglobin-driven bacterial infections.*

Physicians have long been aware that the coincidence of blood and bacteria in a wound may engender life-threatening infection (*1*). Blood or free hemoglobin has a synergistic effect on the lethality of intraperitoneal or subcutaneous inocula of bacteria such as *Escherichia coli* (*2*). The effective component of hemoglobin is iron, and various soluble iron compounds exert an equivalent adjuvant effect (*3, 4*). It has been suggested that hemoglobin interferes with phagocytic destruction of the bacteria (*5*). We have, however, been unable to detect such interference. Instead, we found the following (*6*):

1) The phagocytosis and killing of *E. coli* by neutrophils in vitro is unaffected by the presence of hemoglobin.

2) The migration of neutrophils to the peritoneal cavity in response to intraperitoneal inoculation with *E. coli* is equally rapid and effective whether hemoglobin is present or not.

3) The rate of clearance of heat-killed, radioactively labeled bacteria from the peritoneal cavity is unchanged by simultaneous injection of hemoglobin.

4) Even if live *E. coli* are entrapped in 0.2-μm Nuclepore chambers implanted intraperitoneally, injected hemoglobin still exerts an adjuvant effect.

Thus, hemoglobin does not appear to reduce the phagocytosis and killing of *E. coli*. We therefore hypothesized that hemoglobin might serve simply as a source of nutritional iron, promoting the growth of inoculated *E. coli*. Although the total iron concentration in mammalian body fluids is $2 \times 10^{-5}M$, almost all of the iron is tightly associated with specialized, iron-binding proteins, leaving a free iron concentration of $10^{-18}M$ (*7*). Most aerobic bacteria require $\sim 10^{-6}M$ iron for growth (*7–9*). Thus, as Weinberg (*9*) argued, the availability of iron may be the major nutritional limitation to the replication of many pathogenic bacteria in vivo.

In the experiments reported here, we used a single strain of pathogenic *E. coli* first isolated from an infected patient. The techniques for the routine culture and passage of this organism have been described elsewhere (*5*). When we cultured the organisms in synthetic, iron-

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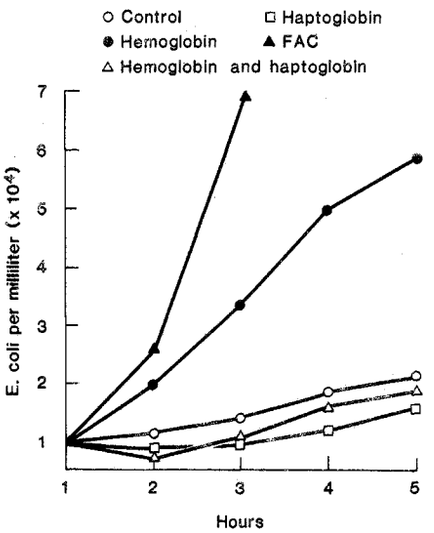

Fig. 1. Rate of growth of *E. coli* at 37°C in iron-poor synthetic medium or medium to which $5 \times 10^{-6}M$ hemoglobin (as tetramer), $5 \times 10^{-6}M$ hemoglobin and $1.2 \times 10^{-5}M$ haptoglobin (sufficient to bind all free hemoglobin), $1.2 \times 10^{-5}M$ haptoglobin, or $10^{-5}M$ ferric ammonium citrate (*FAC*) was added. Hemoglobin-binding capacity of various haptoglobin preparations was assessed as described by Roy *et al.* (*16*). The data are means for two independent experiments.

poor medium (*10*), their rate of replication was extremely low over a 5-hour incubation period (Fig. 1). When they were cultured in medium with added free hemoglobin, their rate of replication was markedly higher (Fig. 1). A similar high rate of *E. coli* replication in complement-depleted human serum with added hemoglobin was reported earlier (*11*). Indeed, we found that, whereas *E. coli* in serum alone divide very slowly, the addition of hemoglobin or iron salts triggers enhanced bacterial growth. These observations suggest that the basis of the hemoglobin adjuvant effect is promotion of unrestricted bacterial growth by hemoglobin iron.

Such hemoglobin-driven bacterial growth may be rare in vivo because of the presence of haptoglobin, a naturally occurring hemoglobin-binding protein. The stable complex formed by haptoglobin and hemoglobin (dissociation constant, $2 \times 10^{-7}M$) (*12*) is rapidly cleared by the reticuloendothelial system (*13*). The concentration of haptoglobin in plasma increases dramatically in response to both acute and chronic infections (*14*), suggesting a bacteriostatic role for this protein.

In confirmation of the idea that haptoglobin might prevent bacterial utilization of hemoglobin iron, we found that when enough purified haptoglobin (*15*) was added to bind all the hemoglobin in the medium, enhanced bacterial replication was blocked (Fig. 1). This was not due to a toxic effect of haptoglobin, since haptoglobin does not affect the replication of *E. coli* in iron-poor medium supplemented with heme or ferric ammonium citrate. Rather, haptoglobin probably makes hemoglobin iron unavailable for incorporation into bacterial iron-binding proteins.

These results suggested that haptoglobin might similarly suppress hemoglobin-driven *E. coli* peritonitis. The experimental model (*3, 5*) involves intraperitoneal inoculation of male Sprague-Dawley rats with 2.5×10^7 *E. coli* alone or mixed with stroma-free human hemoglobin (20 mg), haptoglobin (30 mg), or ferric ammonium citrate (2.3 mg) (total volume, 1 ml) immediately before injection. The rats were observed for 72 hours. As shown in Fig. 2, > 85 percent of the rats injected with *E. coli* plus either hemoglobin or ferric ammonium citrate died within the observation period, whereas almost all animals injected with *E. coli* alone or with *E. coli*, hemoglobin, and haptoglobin survived. Thus, formation of the haptoglobin and hemoglobin adduct renders harmless the otherwise lethal intraperitoneal inoculum of *E. coli* and hemoglobin.

The mechanism whereby haptoglobin blocks the utilization of hemoglobin iron by *E. coli* is not known. Preliminary experiments indicate that hemoglobin complexed with haptoglobin is protected against proteolytic attack by the bacterium, whereas free hemoglobin is rapidly degraded. The binding of hemoglobin by haptoglobin may simply block assimilation of hemoglobin iron by the bacterium.

Heretofore, it was assumed that haptoglobin functions principally as a vehicle for the clearance of free hemoglobin in cases of intravascular hemolysis (*13*). The hemoglobin and haptoglobin complex is rapidly cleared by the reticuloendothelial system, and the nephrotoxic danger posed by free hemoglobin may be averted. Haptoglobin almost certainly does serve this purpose. However, our results suggest an additional role for haptoglobin, that of preventing the utilization of hemoglobin iron by various pathogenic bacteria, especially those which may be present in abscesses and those which escape into the peritoneal cavity from a perforated viscus. The presence of free, uncomplexed hemoglobin may explain the lethality of such conditions as hemorrhagic pancreatitis, secondary bacterial peritonitis, and, perhaps, the so-called toxic shock syndrome. Our studies suggest the possibility that haptoglobin might be used in the prevention or treatment of potentially fatal hemoglobin-supported bacterial infections.

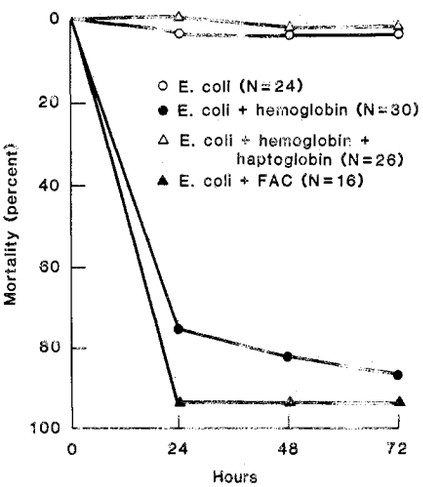

Fig. 2. Mortality of male Sprague-Dawley rats (125 g) inoculated intraperitoneally with 2.5×10^7 *E. coli*; *E. coli* and 20 mg of stroma-free hemoglobin; *E. coli*, 20 mg of stroma-free hemoglobin, and 30 mg of human haptoglobin (as estimated from hemoglobin binding capacity) (*16*); or *E. coli* and 2.3 mg of FAC. Significance of differences between groups at 24 hours: *E. coli* alone versus *E. coli* plus hemoglobin, χ^2 (1) = 28.48, $P < .001$; *E. coli* alone versus *E. coli* plus hemoglobin and haptoglobin, χ^2 (1) = 0.002, $.98 > P > .95$; *E. coli* plus hemoglobin versus *E. coli* plus hemoglobin and haptoglobin, χ^2 (1) = 25.52, $P < .001$; *E. coli* alone versus *E. coli* plus FAC, χ^2 (1) = 28.48, $P < .001$. All tests were done with Yates' correction for continuity as a control for small sample sizes (*17*).

JOHN W. EATON
PAUL BRANDT
JOHN R. MAHONEY
Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis 55455
JAMES T. LEE, JR.
Department of Surgery, University of Minnesota

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Primary Changes of Membrane Currents During Retention of Associative Learning

Abstract. *A single identified neuron was repeatedly isolated by axotomy from the central nervous system of the nudibranch mollusk* Hermissenda crassicornis. *An early voltage-dependent outward K^+ current of this neuron was reduced and more rapidly inactivated for animals previously trained with paired but not randomized light and rotation. Since this current change can affect interneuron and motorneuron output via known synaptic pathways, it helps explain a long-lasting behavioral change that shows the defining features of vertebrate associative learning.*

During the daylight period of its light-dark cycle the nudibranch mollusk, *Hermissenda crassicornis* moves toward a light source, that is, up a light-intensity gradient (*1*). After 1-hour training sessions with paired light and rotation on three successive days, this positive phototaxis is markedly reduced (*1, 2*). This behavioral change increases as a function of practice, persists for several days, and is pairing-specific (that is, does not follow randomly associated light and rotation) and is stimulus-specific (*2, 3*). Changes of individual neurons were found to be closely correlated with the behavioral change (*3–5*), which shares defining features of associative learning as demonstrated for vertebrates. The data available indicated that primary (*6, 7*) neuronal changes occurred within the type B photoreceptors (of which there are three in each eye). After axotomy, which eliminated all impulse activity and synaptic interactions, type B cells from animals trained with paired light and rotation (but not control procedures) showed an increased input resistance and an enhanced depolarizing response after light steps (*3–5*).

We hypothesized that these changes of type B properties, observed 1 and 2 days after the last days of associative training, could be due to long-term reduction of voltage-dependent K^+ currents across the type B photoreceptor membrane (*4, 5, 8*). Two such currents were determined (*9*) for this membrane (in the absence of light): an early, rapidly inactivating voltage-dependent K^+ current (I_A) [compare (*10*)] and a late, slowly inactivating voltage-dependent K^+ current (I_B) [compare (*11*)]. It has been possible to separate these currents pharmacologically: I_A is preferentially blocked by 10 m*M* 4-aminopyridine and I_B by tetraethylammonium ion (*12*). Residual I_{Na} or $I_{Ca^{2+}}$ were not detected (for the dark membrane) after treatments that would eliminate K^+ currents, enhance inward currents, or both.

We have tested the hypothesis of a reduced I_A or I_B during retention of the associatively learned behavior. Using a blind (*13*) experimental procedure, type B neurons axotomized immediately before recording were removed from animals previously exposed to training with paired light and rotation ("paired" group) or with randomly associated light and rotation ("random" group). These neurons were voltage-clamped at −60 mV holding potential with two microelectrodes (*8, 14*) 1 and 2 days after the third day of training. The peak amplitude (*15*) of I_A in response to 5.0-second positive step command pulses to −10 or 0 mV was significantly lower for the cells from paired animals than for cells from random or control animals (which had received no training) (Fig. 1 and Table 1). No significant differences were found for I_B. There were no significant differences between groups for "leak" current, holding current, or resting potential.

We have previously shown that I_A completely and rapidly becomes inactivated after a 5.0-second positive command pulse to 0 mV (*9, 12*). The time course of this exponential inactivation was measured here in two ways. In the first, we used a preceding pulse of variable duration to −30 mV followed by a test pulse to 0 mV. After 30 μsec, a test pulse alone was given. The ratio of peak I_A amplitude with a preceding pulse to peak I_A without a preceding pulse was calculated and plotted as a function of the duration of the preceding pulse. The half-time of decay of this curve, t_{pp}, was used as the measure of inactivation (*16*) rate of the preceding pulse potential. In the second method, we used twin, 5-second pulses to 0 mV with a 120 msec return to −60 mV between them. The ratio of peak I_A during the second pulse to the peak during the first was used as a measure of I_A inactivation.

These two measures were positively correlated with one another ($r_S = +.54$, $P < .02$). This twin pulse ratio for I_A was significantly lower for the paired cells than for random or control cells (Fig. 1 and Table 1). The half-time of decay (t_{pp}) for the paired cells was significantly lower only when compared with the control cells that were not significantly different from random cells (Table 1). All the significant differences (I_A peak amplitudes and inactivation ratios) were found for one of the three type B neurons in each eye, the medial cell (Fig. 1). The lateral and intermediate type B cells did not show differences with a small sample size and were not further analyzed here (*17*).

Since the peak amplitudes of I_A and the twin pulse ratios for random cells were not different from the naïve cells, the significant differences of I_A for paired cells are truly a result of the pairing of light with rotation rather than a direct result of phototransduction itself. This separation of phototransduction effects and conditioning effects on I_A and I_B was further demonstrated by an additional experiment. The I_A peak amplitude was unchanged during presentation of a light step, whereas I_B peak amplitude was markedly reduced (Fig. 1). The conditioning procedure, unlike light, changes I_A but leaves I_B unaltered.

The observed effect of repeated stimulus pairing on the type B photoreceptor during and immediately after acquisition of the associative learning behavior is prolonged membrane depolarization (*4, 18*). This prolonged depolarization probably arises from a synaptic enhancement of a light-induced, voltage-dependent Ca^{2+} current (*8*). Enhancement of this current is a direct consequence of paired

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