tagonistic field mediated by amacrine cells; activity in both regions is elicited exclusively by spatiotemporal change.

Another major class of ganglion cells in the mudpuppy, the on-center and off-center units, generate a sustained discharge in response to maintained contrast across their concentric, antagonistic receptive fields (*1*). The central response in these units was reduced by the presence of the windmill, but they were not further affected by its spin, much like the bipolar cells described above. It appears, therefore, that the dichotomy between sustained and transient activity in the different classes of ganglion cells applies to both the center and antagonistic surround. Units driven by steady central illumination are antagonized by a steady surround; those responding to change at the center are antagonized only by change in the surround (*7*).

The retinas of animals with elaborate synaptic connections at the inner plexiform layer display many movement-sensitive functions at the ganglion cell level. For example, in frog (*8*), rabbit (*9*), and pigeon (*10*) there is a class of ganglion cells that responds best to small moving targets. These units represent a good example of a movement-activated receptive field with antagonistic zones; activity is suppressed when the size of the moving targets is increased past a critical dimension. My results suggest that the large targets extend beyond the central excitatory zone of the ganglion cell, so their movement elicits activity in peripheral amacrine cells which in turn antagonize the central response to movement.

The directionally selective movement detectors described in pigeon (*10*), rabbit (*9*), and mudpuppy (*3*), to mention only a few animals, represent an even more complex movement-sensitive system. It has been shown in each of these systems that directional selectivity is mediated by inhibition when the target is moving in the "null" direction and that, in the mudpuppy, the inhibition is measured as a hyperpolarization of the ganglion cell membrane. The mechanisms described above, however, are not sufficient to account for the directionally selective function; this represents a problem for further investigation.

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Lack of Enhanced Oxygen Consumption by Polymorphonuclear Leukocytes on Phagocytosis of Virulent Salmonella typhi

Abstract. *Human polymorphonuclear leukocytes exhibit an enhanced rate of oxygen consumption during phagocytosis of relatively avirulent strains of* Salmonella typhi *or* Staphylococcus aureus. *However, phagocytosis of a virulent strain of* Salmonella typhi *is not associated with augmented oxygen consumption. The ability of a bacterial strain to alter the postphagocytic rate of oxygen consumption of polymorphonuclear leukocytes may be related to its in vivo virulence.*

Phagocytosis and bacteriocidal activity by polymorphonuclear leukocytes have been classically associated with an enhanced rate of their oxygen consumption. The biochemical mechanisms responsible for this increased rate are not clearly defined, although several schemes have been proposed which involve the hexose monophosphate shunt, glutathione peroxidase, and pyridine nucleotide oxidases which are cyanide-insensitive (*1*). The virulence of certain *Salmonella typhi* strains has been attributed to an interaction between specific antigenic determinants of the bacterial cell wall and factors associated with serum bacteriocidal activity (*2*). However, the relation between antigenic virulence factors and cellular host defense mechanisms has not been clearly established although the influence of *Salmonella* cell wall structure upon phagocytosis has been studied (*3*). We report that phagocytosis of a virulent *Salmonella* strain (Quailes) is not accompanied by an augmented oxygen consumption but ingestion of either a less virulent strain (O-901) or of the relatively avirulent *Staphylococcus aureus* 502A is associated with the classical postphagocytic increase in oxygen uptake. This alteration of the normal metabolic bacteriocidal reactions of phagocytes may contribute to the in vivo virulence of *S. typhi*.

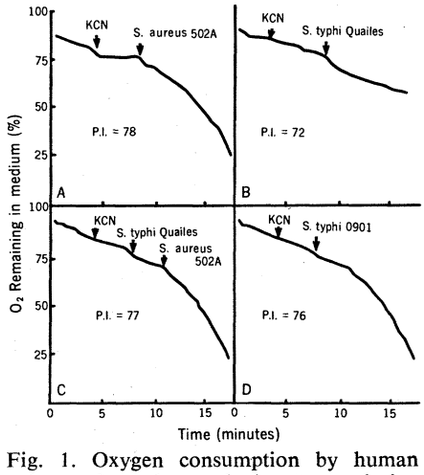

Fig. 1. Oxygen consumption by human polymorphonuclear leukocytes during phagocytosis of *Staphylococcus aureus* 502A, *Salmonella typhi* Quailes, and *Salmonella typhi* O-901. Phagocytic index (*P.I.*) is the percentage of polymorphonuclear leukocytes containing bacteria.

Normal human polymorphonuclear leukocytes were isolated from blood by the technique of dextran-enhanced sedimentation of erythrocytes. The leukocytes were then washed with modified Hanks solution (HBG) (*4*) by two low-speed (180*g*) centrifugations. The contaminating erythrocytes were eliminated by hypotonic lysis and centrifugation (*5*). The leukocyte pellet was then suspended in HBG, the cells were enumerated in a standard hemocytometer and in smears with Wright stain, and the cell suspension was adjusted with HBG to a final concentration of 10^7 polymorphonuclear leukocytes per milliliter.

The *S. aureus* 502 A, *S. typhi* Quailes, and *S. typhi* O-901 were grown for 18 hours in trypticase soy broth. They were washed and suspended twice in Hanks balanced salt solution with bicarbonate (HBSS), and they were then enumerated indirectly by turbidimetry in a Spectronic 20 spectrophotometer at 525 nm. Serum was obtained from a normal AB+ donor to provide nonspecific factors affecting opsonification and phagocytosis.

Oxygen consumption during phagocytosis was measured at 37°C by a polarographic technique employing a Clark electrode. The phagocytic mixture consisted of 0.75 ml of HBG, 0.75 ml of HBSS, 0.1 ml of serum, and 0.5 ml of the leukocyte suspension. This resulted in a leukocyte concentration of 2.4×10^6 cell/ml. After 5 to 10 minutes of temperature equilibration and the establishment of an endogenous rate of oxygen uptake, KCN was added to a final concentration of 2 m*M*. When a baseline rate of oxygen consumption was established in the presence of KCN, phagocytosis was initiated by the addition of 50 μl of the appropriate bacterial suspension, resulting in a bacteria to phagocyte ratio of 2 : 1 to 3 : 1. During the polarographic evaluation, 10-μl samples were withdrawn from the polarograph, were stained with methylene blue, and the smears were examined to quantify phagocytosis.

The result of a typical polarographic experiment with *S. aureus* 502A as the test bacteria is shown in Fig. 1A. The inhibition by cyanide of the endogenous leukocyte consumption of oxygen is clearly demonstrated as is the postphagocytic enhancement in the rate of oxygen uptake. However, in a similar experiment with *S. typhi* Quailes (Fig. 1B), the postphagocytic increase in oxygen consumption is not seen after the addition of this virulent strain to the phagocytic system. Despite the difference in oxygen consumption, phagocytosis of both *S. aureus* and *S. typhi*, to similar degrees, was confirmed by examination of stained smears of the phagocytic preparation: 78 percent of the polymorphonuclear leukocytes contained *S. aureus* and 72 percent of the leukocytes contained *S. typhi*, in the respective studies. The leukocytes, after phagocytosis of *S. typhi*, are still viable and functional as demonstrated by a normal postphagocytic increase in oxygen consumption following ingestion of *S. aureus* 502A subsequent to a prior challenge with *S. typhi* Quailes (Fig. 1C). The response of increased oxygen consumption was observed after the phagocytosis of the relatively avirulent *S. typhi* O-901 (Fig. 1D) at a rate comparable to that with the other organisms.

These studies suggest that a virulent strain of *S. typhi* does not stimulate oxygen consumption in normal human polymorphonuclear leukocytes after being ingested by them. This observation is compatible with in vitro studies of leukocyte bacteriocidal function and with several clinical states of infection.

It has been observed in in vitro studies that phagocytosis can occur under anaerobiosis (*6*) or in the presence of substances, such as phenylbutazone (*7*), hydrocortisone (*8*), and colchicine (*5*), that inhibit the oxidative pathway of the hexose monophosphate shunt. This inhibition of oxygen consumption is generally associated with a depression of bacteriocidal activity.

Individuals with genetic defects in hexose monophosphate oxidative metabolism in leukocytes, such as deficiencies in glucose-6-phosphate dehydrogenase (*9*), in pyridine nucleotide oxidase (*10*), or in glutathione peroxidase (*11*), are highly susceptible to infection. The leukocytes of these patients do not demonstrate a postphagocytic enhancement of oxygen consumption in vivo.

An altered leukocyte metabolism after phagocytosis of *S. typhi* may be related to the following clinical observations. Sickle cell disease is associated with an increased incidence of *Salmonella* infections and the leukocytes of patients with this disorder do not exhibit a postphagocytic increase in oxygen consumption (*12*). A persistent *Salmonella* infection has been documented in a female carrier of chronic granulomatous disease. Her blood has a leukocyte population composed of both normal and defective cells (*13*). The leukocytes of patients with clinical typhoid fever and bacteremia do not demonstrate an abnormal reduction of nitroblue tetrazolium (*14*). Thus, there is a relation between altered oxidative metabolism in the hexose monophosphate shunt of polymorphonuclear leukocytes and the presence of *Salmonella* infections.

The failure of *S. typhi* Quailes to enhance oxygen consumption in this in vitro study may be related to the virulence of the strain, which in turn may be dependent on surface antigenic properties. The stimuli, presented by particles, that cause a polymorphonuclear leukocyte to initiate phagocytosis and subsequent metabolic events are unknown. There is generally believed to be an interaction between the surface of the particle and the cell membrane of the leukocyte. If this is correct, *S. typhi* Quailes possesses the stimuli required for phagocytosis, but not those required to initiate hexose monophosphate shunt activity. Consequently, the chain of metabolic events leading to maximum bacteriocidal activity is not initiated, and although the bacteria are ingested, they are not all killed. Thus, some survive as intracellular parasites, and by virtue of this survival, express virulence. The failure of an organism to initiate an enhanced postphagocytic rate of oxygen consumption in normal human polymorphonuclear leukocytes may be a sensitive in vitro indicator of virulence in vivo.

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