Intraphagosomal pH of Human Polymorphonuclear Neutrophils[1] (34810)

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It has been postulated that the pH in the membrane-bound phagocytic vacuole (the phagosome) containing an ingested particle is lower than that of the rest of the cell. This is of importance because many of the antibacterial systems operative in polymorphonuclear neutrophils (PMN) are markedly influenced by pH (1–4). The qualitative *in vivo* studies of mouse phagocytes by Rous in 1925 (5), and mouse and guinea pig phagocytes by Sprick in 1956 (6), suggested that the intraphagosomal pH may be as acid as 3.0 to 5.5.

Materials and Methods. We used an *in vitro* technique whereby PMN were separated from heparinized peripheral blood by dextran sedimentation (7) and resuspended in Hanks' balanced salt solution at a pH of 7.4. This solution also contained 10% autologous human serum. Heat-killed *Candida albicans* were stained with one of nine different indicator dyes by boiling 10^{10} washed candida and 2 ml of water with 16 mg of dye for 2 hr. Five $\times$ 10^6 leukocytes per ml in Hanks' balanced salt solution with 10% human serum

[1] Supported in part by The Philip H. Hiss, Jr., Fund and Grant AI-09504-01 from the NIH. The author is indebted to Miss B. MacLean and Mrs. T. Sterrett for expert technical assistance.

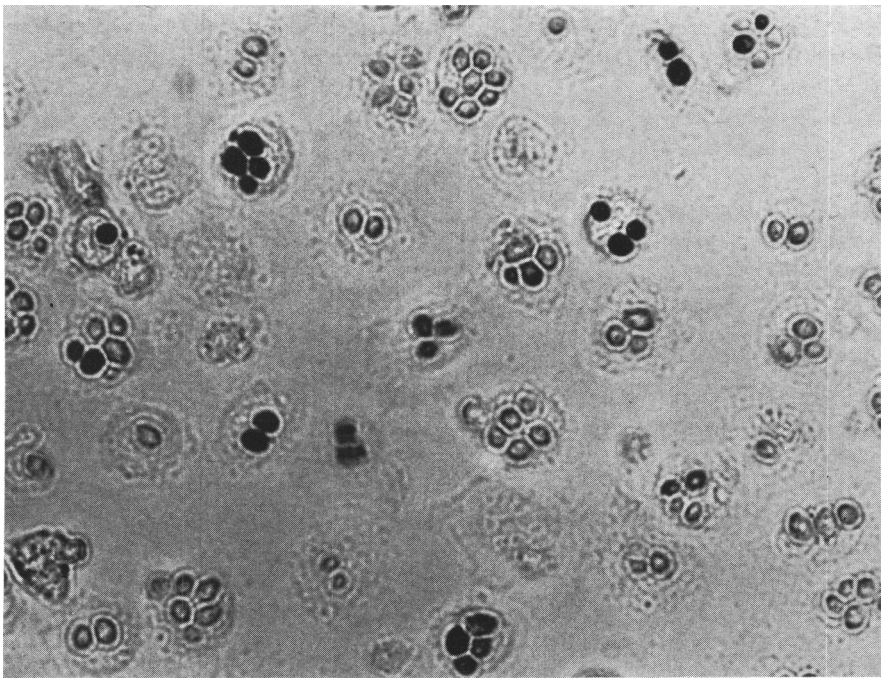

FIG. 1. Light photomicrograph of living human PMN containing neutral red-strained candida. The dark (red) color of some of the intracellular candida indicates a pH below 6.6 (original magnification $\times$400).

TABLE I. Results of Experiments with Indicator Dye-Stained Candida and Human Polymorphonuclear Neutrophils.[a]

Indicator dye (10)	Basic color and pH at which dye-stained candida appeared this color	Acid color and pH at which dye-stained candida appeared this color	Color of extracellular candida (Media at pH 7.4)	Color of intracellular candida
Bromphenol blue	Blue 4.5	Yellow 2.9	Blue	Blue
Bromcresol green	Blue-green 5.5	Yellow 3.7	Blue green	Blue-green
Methyl red	Red 6.2	Yellow 4.2	Red	Red
Lacmoid	Red 6.3	Blue 4.4	Red	Red
Chlorphenol red	Red 6.4	Yellow 4.8	Red	Red
Bromcresol purple	Blue 6.8	Yellow 5.2	Blue	Blue
Bromthymol blue	Blue 7.5	Yellow 5.9	Blue	Yellow-green
Brilliant yellow	Red 7.7	Yellow 6.5	Yellow-red	Yellow
Neutral red	Amber 8.2	Red 6.6	Amber	Red
Phenol red	Red 8.4	Yellow 6.8	Yellow	Yellow

[a] Dye-stained candida showed the colors noted at the stated pH. Forty to sixty per cent of neutral red-stained candida turned red in the phagosomes of living PMN within 60 min. None of the extracellular neutral red-stained candida turned red.

were incubated on round coverslips in a 5% CO_2 in air incubator at 37° for 15 min to allow PMN to adhere. The coverslips were dipped in 37° Hanks' solution to wash off nonadhering cells. Washed dye-stained candida, suspended in Hanks' solution with 10% serum were then overlaid to provide a ratio of five candida per PMN. The coverslips were incubated for 15 min at 37° in a 5% CO_2 in air incubator to allow phagocytosis to take place. The coverslips were then placed in a Sykes-Moore apparatus (8), the chambers filled with Hanks' solution and 10% serum, and, in some instances, the chemical to be tested, and observed with light and phase-contrast microscopy on a 37° stage. The color of the extracellular candida was noted, and all intracellular candida within 50 PMN were counted and classified according to their color, every 30 min for 2 hr. All experiments were performed in quadruplicate.

A second series of experiments was performed to test the effect of various metabolic inhibitors and other chemicals on the processes relating to the decrease in intraphagosomal pH. Neutral red-stained candida showed the most definite intracellular color changes (Fig. 1), and were used for these experiments.

Results. The results of the study are shown in Table I. The intracellular intraphagosomal pH was found to be about 6.0–6.5.

The fall in intraphagosomal pH was prevented by 20 m*M* sodium fluoride, 0.4 m*M* iodoacetate, and refrigeration at 5°. 1.0 m*M* potassium cyanide, the absence of glucose, 5.5 m*M* deoxyglucose, 2.1 m*M* hydrocortisone-21-succinate, 0.25 m*M* colchicine, 0.2 m*M* sodium arsenate, and 1% dimethylsulfoxide all failed to influence the intraphagosomal pH change.

Discussion. This modest change in intraphagosomal pH may be related to the increase in lactic acid production known to accompany phagocytosis (9). Inhibition of the intraphagosomal pH fall by glycolytic inhibitors tends to support this hypothesis.

Summary. Candida albicans were stained with one of nine different indicator dyes which encompassed a pH range of 2.5–8.0. Living polymorphonuclear neutrophils were observed microscopically, and color changes of intracellular dye-stained candida were noted. Intraphagosomal pH decreased to the 6.0–6.5 range and this pH change was prevented by certain glycolytic inhibitors.

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Received Feb. 12, 1970. P.S.E.B.M., 1970, Vol. 134.