CHANGES IN pH OF THE CYTOPLASM DURING PHAGOCYTOSIS OF MICROORGANISMS STAINED WITH INDICATOR DYES

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Some bacteria are capable of developing inside phagocytes whose cytoplasm becomes their habitat. The hydrogen ion concentration in the intact cytoplasm of the somatic cells of mammals and unicellular organisms is close to neutral, with a slightly acid tendency (pH 6.6-6.8) [1]. Meanwhile, granules of indicator dyes ingested by guinea pig's phagocytes gave a much more acid reaction (pH 3.0) [3]. In other experiments, microorganisms stained with the same dyes were injected intraperitoneally into guinea pigs and albino mice, and macrophages subsequently extracted from the animal's peritoneal cavity were examined under the microscope. In this case the character of the staining indicated a less acid reaction around the phagocytosed microorganisms (pH 4.7-7.5) [4]. This reaction, however, differs considerably from the reaction of the cytoplasm. The problem of the chemical composition of the cytoplasm surrounding phagocytosed microorganisms has become of considerable importance at the present time in connection with the study of the action of chemotherapeutic substances on intracellular bacteria.

In the present investigation a comparative study was made of the changes in staining of particles of indicator dyes and of bacteria stained by the same dyes inside the phagocytes.

EXPERIMENTAL METHOD

Chemically stable indicator dyes, not changing their color on oxidation or reduction, were used for staining. They included bromphenol blue (pH 3.0-4.6; pK 4.0), bromcresol green (pH 3.8-5.4; pK 4.7), and bromcresol purple (pH 5.2-6.8; pK 6.3). Their full acid color is yellow and their full alkaline color blue. A 24-h culture of Staphylococcus aureus (strain Zhaev), grown on meat-peptone broth, and 2-week culture of Mycobacterium tuberculis var. hum. H_{37} Rv and M. tuberculosis var. avium 430, grown on liquid Sutton's medium with the addition of 10% ox serum and 0.05% Tween-80, were used for staining. The microorganisms were sedimented by centrifugation and killed by heating to 70°. The staphylococci were placed in a saturated solution of the dye in 0.9% NaCl solution and the mycobacteria in a 1% solution of the same dye in 95% ethanol with the addition of 0.5% HCl. Staining

TABLE 1. Changes in Staining of Microorganisms in Buffer Solution

pH of buffer solution	Bromphenol blue	Bromcresol green	Bromcresol purple
2.04	Yellow	Yellow	0
4.0	Greenish	Yellow-greenish	0
4.3	Blue-green	Greenish-yellow	0
4.5	"	Light green	0
4.7	Blue	Green	0
5.0	"	Dark green	Yellow
5.3	"	Blue-green	Yellow-greenish
5.5	"	Blue	"
5.7	0	"	Yellow-green
6.0	0	"	"
6.5	0	"	"
6.85	0	"	Blue

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TABLE 2. Changes in Color of Particles of Indicator Dyes and Bacteria Stained with Them inside and outside the Phagocytes of an Albino Mouse

Color observed	Suspension of indicator dye						Microorganisms stained with indicator dyes											
	bromphenol blue		bromcresol green		bromcresol purple		bromphenol blue				bromcresol green							
							staphylococci		M. tuberc. var. hum. H_{37} Rv		M. tuberc. var. avium 430		staphylococci		M. tuberc. var. hum. H_{37} Rv		M. tuberc. var. avium 430	
	p	o/p	p	o/p	p	o/p	p	o/p	p	o/p	p	o/p	p	o/p	p	o/p	p	o/p
Blue	+	+	+	+	+	+	+	+	+	+	+	+						
Blue-green	-	-	+	+	-	-	-	-	-	-	-	-						
Green	-	-	-	-	-	-	-	-	-	-	-	-						
Dark green	-	-	-	-	-	-	-	-	-	-	-	-						
							-	-	-	-	-	-	+	+	+	+	+	+
							-	-	-	-	-	-	+	+	+	+	+	+
							-	-	-	-	-	-	*	*	-	-	*	*
							-	-	-	-	-	-	-	-	-	-	-	-

Legend: p – phagocytosed particles; c/p particles lying outside the phagocytes. Asterisks denote single cases.

took place at 20°. For the staphylococci it lasted 1 day and for the mycobacteria 14 days. The suspension of stained microorganisms in phosphate buffer (pH 7.0) was injected intraperitoneally into albino mice weighing 28-30 g, which had previously received intraperitoneal injections of 3% peptone solution on two successive days. The peritoneal exudate was aspirated 2.5 or 24 h after injection of the microorganisms and investigated under the microscope by the hanging drop method. In other experiments, instead of the suspension of microrganisms, the mice were injected with a suspension of the indicator dyes in phosphate buffer (pH 7.0).

To assess the reaction of the intracellular medium a system of phosphate buffers was prepared at pH intervals of 0.2-0.3. The stained microorganisms were placed in these buffers and examined macroscopically and microscopically. The changes in color are given in Table 1.

EXPERIMENTAL RESULTS

It is clear from Table 2 that the phagocytosed particles of dye most commonly indicated a neutral or weakly acid reaction of the cytoplasm. In a few cases the color corresponded to a lower pH value. The change in the color of the staphylococci and mycobacteria was the same and indicated a much higher hydrogen ion concentration in their neighborhood. Even heat-killed microorganisms are known to retain their pathogenic properties and, in particular, their ability to cause necrobiosis of the tissue in the region into which they are introduced. In this case the acid reaction in the neighborhood of the microorganisms may be regarded as a manifestation of local injury to the cytoplasm.

The irritant properties of the particles of dye were much weaker, but even so they cannot be regarded as completely inactive. In this case great care must be exercised when assessing the changes in color of these particles indicating an acid or even a weak acid reaction, because these changes may be the result of disturbance of the normal physicochemical equilibrium of the cytoplasm. Be that as it may, there is no doubt that around the phagocytosed bacteria lies a zone with a hydrogen ion concentration of 4.7-5.2. For such a reaction to persist inside the cell, a limiting membrane is essential. It has recently been shown that phagocytosed bacteria are surrounded by a special membrane which, in the living cell, is relatively inpermeable to the tetracyclines [2]. This membrane may assist in the preservation of differences in the level of the hydrogen ion concentration between areas of the cytoplasm in the immediate vicinity of the phagocytosed bacteria and other parts.

LITERATURE CITED

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