

# THE DEPRESSION OF PHAGOCYTOSIS BY EXOGENOUS CYCLIC NUCLEOTIDES, PROSTAGLANDINS, AND THEOPHYLLINE

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## ABSTRACT

The effects of agents that elevate intracellular cyclic adenosine 3',5'-monophosphate (cAMP) have been studied with respect to phagocytosis by guinea pig polymorphonuclear leukocytes. The investigation depends upon the use of a precise method for following ingestion. Theophylline, dibutyryl cAMP, and prostaglandins inhibited the phagocytosis of starch particles. The inhibitions caused by prostaglandins  $E_1$ ,  $E_2$ , and  $F_{2\alpha}$  ( $PGE_1$ ,  $PGE_2$ , and  $PGF_{2\alpha}$ ) were synergistic with that due to theophylline. Inhibition by  $PGA_1$  and  $PGA_2$  was not. At equal concentrations the order of increasing inhibition of phagocytosis (assayed at 10 min) by the prostaglandins was  $PGE_1 < PGF_{2\alpha} < PGE_2 < PGA_1 = PGA_2$ . Our results are consistent with the hypothesis that increased intracellular levels of cAMP impair the phagocyte's ability to ingest particles. The mechanism of the inhibition has not been defined. The increment in oxidation of  $[1-^{14}C]$ glucose to  $^{14}CO_2$  that normally accompanies phagocytosis was found to be depressed in the presence of  $PGE_1$  or theophylline, together or individually as expected from the inhibition of phagocytosis. Paradoxically, oxygen consumption although depressed by theophylline or  $PGE_1$  plus theophylline, was stimulated by  $PGE_1$  alone.

## INTRODUCTION

The ubiquitous role of cyclic adenosine 3',5'-monophosphate (cAMP)<sup>1</sup> as a regulatory agent involved in the control of a number of cell processes is well documented (Robison et al., 1971). Included among cAMP-sensitive cellular processes are a number of exo- and endocytotic phenomena. Thus, increased intracellular cAMP levels have

<sup>1</sup> *Abbreviations used in this paper are:* cAMP—cyclic adenosine 3',5'-monophosphate; KRP—Krebs-Ringer phosphate medium, pH 7.4;  $PGE_1$ ,  $PGE_2$ ,  $PGA_1$ ,  $PGA_2$ ,  $PGF_{2\alpha}$ —prostaglandins  $E_1$ ,  $E_2$ ,  $A_1$ ,  $A_2$ , or  $F_{2\alpha}$ ; PMN—polymorphonuclear leukocytes.

been correlated with increased insulin release from pancreatic islets (Sussman and Vaughan, 1967), increased ACTH, thyroid-stimulating hormone (TSH), and growth hormone release from the anterior pituitary (Fleischer et al., 1969; Bowers et al., 1968; Gagliardino and Martin, 1968) and release of thyroid hormone and calcitonin by thyroid cells (Ensor and Munro, 1969; Care et al., 1969). Phagocytosis of latex beads by thyroid cells (Kowalski et al., 1972) was also enhanced. These observations together with recent evidence implicating cAMP in the regulation of several leukocyte functions such as allergic histamine release

(Lichtenstein and Margolis, 1968), phagocytosis (Stossel et al., 1972; Bourne et al., 1971; Weissmann et al., 1971), candidacidal activity (Bourne et al., 1971), iodide metabolism (Stolc, 1972), release of lysosomal enzymes (May et al., 1970) and lymphocyte transformation (Smith et al., 1971 *a, b*) prompted us to examine the effects of cAMP and the prostaglandins on phagocytosis by polymorphonuclear leukocytes (PMN). It has been established that the PMN contain the enzymatic machinery for the synthesis and degradation of cAMP and that prostaglandins increase the intracellular accumulation of cAMP in these cells (Butcher and Baird, 1968; Scott, 1970; Bourne et al., 1971; Bourne and Melmon, 1971; Stossel et al., 1970; Stolc, 1972; Woodin, 1972; Bourne et al., 1973).

We have found that agents reported to effect an increase in intracellular cAMP levels inhibit the ingestion of starch particles by guinea pig PMN in a dose-related manner. This was true whether the increase in intracellular cAMP was brought about by the exogenous nucleotide in the form of the dibutyryl derivative, by the phosphodiesterase inhibitor theophylline, or by activators of adenyl cyclase such as the prostaglandins. The inhibition of phagocytosis brought about by PGE<sub>1</sub>, PGE<sub>2</sub> and PGF<sub>2α</sub> was potentiated by theophylline, i.e., was beyond the level expected if each agent acted independently. Although not conclusive, this evidence is consistent with the probability that inhibition of phagocytosis by the prostaglandins is a result of their stimulation of adenyl cyclase.

## MATERIALS AND METHODS

### *Chemicals*

All chemicals used were of reagent grade and were purchased from commercial sources. cAMP, theophylline (Mann Research Lab. Inc., N. Y.), and dibutyryl cAMP (Sigma Chemical Co., St. Louis, Mo.) were dissolved in Krebs-Ringer phosphate solution, pH 7.4 (KRP), containing 7.5 mM glucose. These solutions were freshly prepared for each experiment. Prostaglandins E<sub>1</sub>, E<sub>2</sub>, A<sub>1</sub>, A<sub>2</sub>, and F<sub>2α</sub> were kindly supplied by Dr. J. Pike of the Upjohn Co., Kalamazoo, Mich. Stock solutions at 1.0 mg/ml were prepared by dissolving the free acids in 95.0% ethanol, 0.002 M sodium carbonate 1:9, and adjusting the pH to 6.7. A solution containing similar proportions of carbonate and ethanol and adjusted to pH 6.7 was used as a control.

### *Preparation of Cellular Monolayers of Guinea Pig Polymorphonuclear Leukocytes*

Elicited peritoneal exudate PMN were collected and washed in Krebs-Ringer Phosphate medium as described in previous publications from this laboratory (Oren et al., 1963). Cell monolayers were prepared by incubation of 1.0 ml of a cell suspension at a concentration of  $1.0 \times 10^7$  cells/ml in plastic culture dishes as detailed by Michell et al., 1969).

A very brief summary of the procedure is as follows: Cells were obtained from the peritoneal cavities of guinea pigs 18 h after the injection of neutral caseinate solution (12% in physiological saline). The cells were centrifuged, washed once in KRP, resuspended in KRP, and counted in a hemocytometer. After appropriate dilution 1-ml aliquots were pipetted into small Petri dishes for tissue culture (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles) and incubated for 1 h. Excess cells were then decanted and the dishes washed in three successive beakers containing KRP.

### *Measurements of Rates of Phagocytosis*

Ingestion of radioactively labeled starch particles by cellular monolayers was employed as a measure of phagocytic activity as described by Michell et al. (1969). Approximately  $1.0 \times 10^7$  cells were used in the preparation of each monolayer resulting in the recovery of 0.4–0.6 mg of cellular protein. After a 30.0 min preincubation with drug-containing or control solution, the monolayers were exposed for a specified time to 5.0 mg of starch labeled with <sup>14</sup>C. The period of phagocytosis was 5.0–60.0 min in preliminary time-course experiments, and 10.0 min in all other uptake experiments. To terminate phagocytosis, the monolayers were washed free of excess particles through six beakers of 0.9% NaCl. The amount of starch ingested was determined by counting the residual radioactivity in air-dried monolayers on a gas flow counter (Michell et al., 1969). Uptake of starch particles was expressed as milligrams ingested starch (based on the specific activity of the labeled particles) per milligram of cellular protein, determined by assaying appropriate aliquots of the NaOH-digested monolayers by the method of Lowry et al. (1951). The amount of phagocytosis exhibited by duplicate drug-treated monolayers was expressed as the percentage of that in duplicate control monolayers.

### *Metabolic Studies*

Oxygen consumption was measured by Warburg respirometry concomitantly with the oxidation of

[1-<sup>14</sup>C]glucose to <sup>14</sup>CO<sub>2</sub> in 15.0-ml capacity flasks containing 3.0 ml of medium and 0.2 ml of KOH in the center-well as previously described (Sbarra and Karnovsky, 1959).

## RESULTS

### *Effect of Various Agents that Increase Intracellular cAMP Levels on the Time Course of Phagocytosis*

Phagocytosis is a saturable process which shows an initial linear rate and then levels off at maximal cellular capacity (Michell et al., 1969; Stossel et al., 1972). We first screened the agents for their effects at various times during the uptake process since it was conceivable that rate of phagocytosis or capacity of the cells for particles might be selectively affected. In Fig. 1, a representative experiment is depicted on the effects of PGE<sub>1</sub> (20.0 μg/ml; 5.6 × 10<sup>-5</sup> M), theophylline (5.0 mM), and dibutyryl cAMP (1.0 mM and 5.0 mM) on the time course of phagocytosis. The insert depicts the kinetics of uptake for untreated (control) PMN. It is clear that all of the agents employed diminished phagocytosis substantially at early

times when the rate of uptake was maximal (Michell et al., 1969). At later times when the capacity for uptake by control cells had nearly reached its maximum, the inhibition of phagocytosis tended to be relieved. Although the amount of inhibition produced by 5.0 mM theophylline and 5.0 mM dibutyryl cAMP remained significant even 60.0 min after cells had been presented with particles, that brought about by PGE<sub>1</sub> and 1.0 mM dibutyryl cAMP had all but disappeared after 15.0 and 30.0 min respectively.

The effects thus depend upon critical timing and concentrations. Several other experiments of this type demonstrated slight shifts in optimal timing, optimal concentrations of agents, and the degree of inhibition observed with different batches of cells. The prostaglandins are compared in Fig. 2. Once again it can be seen that the inhibitions are maximal during the period of linear uptake in control cells (<15 min) (see Fig. 1). Not only PGE<sub>1</sub>, but also PGE<sub>2</sub>, PGA<sub>1</sub>, PGA<sub>2</sub>, and PGF<sub>2α</sub> were found to inhibit phagocytosis in PMN. PGA<sub>1</sub> and PGA<sub>2</sub> were more potent inhibitors than PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2α</sub> in that their effects were more sustained at later times.

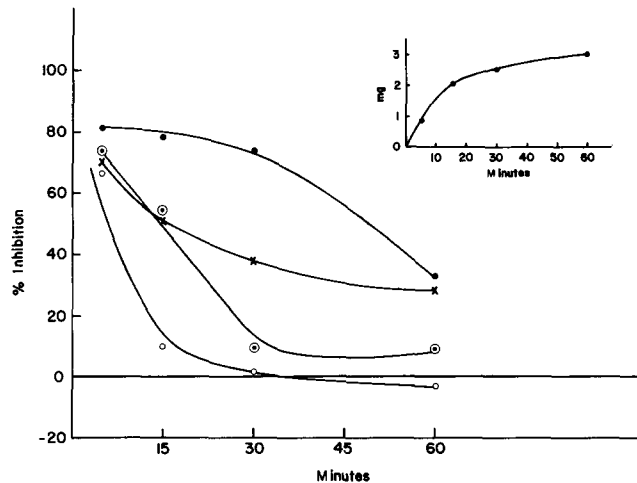


FIGURE 1 Time course of inhibition of starch uptake by agents that affect intracellular levels of cAMP. The ordinate refers to uptake of [<sup>14</sup>C]starch (expressed as % inhibition), and the abscissa represents the period of exposure of cells to particles. Monolayers were exposed to the drugs at 37°C for 30 min before particles were added. The points are mean values from a representative experiment done in duplicate. Deviation from the mean averaged ±2.0% for the theophylline (5 mM) curve (●); ±3.2% for the 5.0 mM dibutyryl cAMP curve (×); ±3.3% for the 1.0 mM dibutyryl cAMP curve (○), and 5.0% for the PGE<sub>1</sub> (5.6 × 10<sup>-5</sup> M) curve (○). The insert depicts the kinetics of starch uptake for the untreated cells. The ordinate refers to uptake of [<sup>14</sup>C]starch (expressed as milligrams of starch per milligram of monolayer protein) and the abscissa, to time of exposure to 5.0 mg of [<sup>14</sup>C]starch. The individual values deviated from the mean by no more than 5.0%.

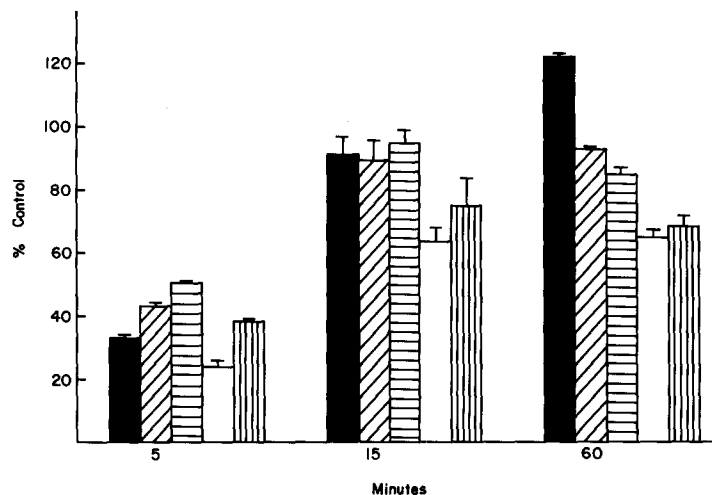


FIGURE 2 Inhibition of phagocytosis by prostaglandins at various times subsequent to the addition of [<sup>14</sup>C]starch. The ordinate refers to uptake of [<sup>14</sup>C]starch particles, expressed as % control. On the abscissa is indicated the period of exposure of the monolayers to 5.0 mg of starch particles. The cells were preincubated for 30.0 min at 37°C in the presence or absence of drugs before the addition of [<sup>14</sup>C]starch. The bars represent the average deviation of triplicate samples in a single experiment. All prostaglandins were applied at a concentration of 20.0 μg/ml = 5.6 × 10<sup>-5</sup> M. PGE<sub>1</sub> (solid bar); PGE<sub>2</sub> (slashed hatching); PGF<sub>2α</sub> (horizontal hatching); PGA<sub>1</sub> (open bar); PGA<sub>2</sub> (vertical hatching).

PGE<sub>1</sub> appeared to exhibit a stimulation of phagocytic capacity at later times.

*Comparison of Agents that Increase Intracellular cAMP Levels as Inhibitors of the Rate of Phagocytosis*

The inhibitory effect of various agents at different concentrations was compared for the most sensitive part of the normal ingestion curve, i.e., when maximal ingestion rates pertained. The experiments are summarized in Table I. All of the agents examined were inhibitory when an appropriate concentration was selected. Dibutyryl cAMP was a more potent inhibitor of phagocytosis than cAMP itself. It is generally assumed that the higher efficiency of dibutyryl cAMP in many cAMP-responsive systems is due to better permeation into cells of the less polar dibutyryl derivative, or to its higher resistance to phosphodiesterase (Robison et al., 1971). However, some doubt has been cast on this explanation by Szabo and Burke (1972) who concluded that the effects of dibutyryl cAMP on thyroid cells were predominantly due to the substituted nucleotide itself or to N<sub>6</sub>-monobutyryl cAMP rather than to cAMP formed by deacylation.

*Demonstration of Synergism by Theophylline and PGE<sub>1</sub> or PGE<sub>2</sub> in Depressing Phagocytosis*

All of the prostaglandins we examined were able to inhibit phagocytosis with variations merely in degree, yet only PGE<sub>1</sub> and PGE<sub>2</sub> have been shown so far to be effective activators of leukocyte adenylyl cyclase (Bourne et al., 1971; Scott, 1970). The question thus arose as to whether or not the prostaglandin-induced inhibition of phagocytosis depended upon changes in intracellular cAMP levels, or whether there were some other effects peculiar to the chemical nature of prostaglandins. If the prostaglandins were indeed inhibiting phagocytosis by virtue of their ability to activate adenylyl cyclase, then it should be possible to potentiate the inhibition with a phosphodiesterase inhibitor such as theophylline. The possibility that theophylline might potentiate the effects of the prostaglandins was examined using a combination of the two drugs at concentrations that individually produced small effects. For theophylline, particularly, concentrations were below 5 mM, which it may be noted in Fig. 1 caused a high degree of inhibition.

In Table II the inhibitory activity of theo-

TABLE I  
*Inhibition of Phagocytosis of Starch Particles by Guinea Pig PMN Monolayers Due to Agents that Increase Intracellular cAMP Concentrations\**

Agent	Concentration	% inhibition	<i>P</i>
	<i>mM</i>		
cAMP	10.0	13.3 ± 3.9 (17)	<0.005
Dibutyryl cAMP	2.0	47.3 ± 2.8 (6)	<0.001
	25.0	87.4 ± 1.0 (2)	—
Theophylline	1.25	8.6 ± 5.6 (4)	<0.3
	2.5	29.0 ± 3.9 (11)	<0.001
	4.0	48.7 ± 3.1 (6)	<0.001
	10.0	80.7 ± 1.2 (3)	<0.001
	<i>μM</i>		
PGE <sub>1</sub>	56.3	19.7 ± 10.2 (6)	<0.10
PGE <sub>2</sub>	2.82	17.3 ± 12.1 (8)	<0.2
	28.2	34.4 ± 7.9 (9)	<0.005
	56.3	47.1 ± 5.6 (4)	<0.005
PGA <sub>1</sub>	14.1	34.5 ± 8.6 (3)	<0.05
	56.3	57.3 ± 6.3 (5)	<0.001
PGA <sub>2</sub>	2.82	35.7 ± 11.4 (8)	<0.02
	28.2	35.2 ± 6.3 (9)	<0.001
	56.3	56.1 ± 7.6 (5)	<0.005
PGF <sub>2α</sub>	21.0	9.8 ± 5.6 (8)	<0.1
	42.0	32.5 ± 10.4 (11)	<0.01
	105.0	33.6 ± 10.0 (5)	<0.025

\* The monolayers were preincubated at 37°C for 30.0 min in the presence of drug or control solutions before the addition of 5.0 mg of radioactively labeled starch. Phagocytosis was allowed to proceed for 10.0 min. The data are presented as percentage inhibition ± SE. The number in parenthesis refers to the number of paired experiments performed. Each experiment was done in duplicate. For the prostaglandins 1 μg/ml corresponded to 2.82 μM, 20 μg/ml to 56.0 μM. *P* is the probability value for the significance of the inhibitions.

phylline and prostaglandins combined is compared with the inhibition predicted if each agent inhibited at a site independent of the other. The data are segregated into three groups: one in which the inhibition predicted for independent sites was between 0 and 50%, averaging at approximately 35%, a second in which predicted inhibitions fell between 50 and 75%, averaging at about 65%, and a third in which predicted inhibitions were above 75%, averaging near 80%. The range of drug concentrations used to obtain predicted levels of inhibition <75% were as follows: theophylline, 1.0–3.0 mM; PGE<sub>1</sub>, 10.0–20.0 μg/ml (2.8–5.6 ×

10<sup>-5</sup> M); PGA<sub>1</sub>, PGE<sub>2</sub>, and PGA<sub>2</sub>, 1.0–10.0 μg/ml; PGF<sub>2α</sub>, 10.0–50.0 μg/ml. For predicted inhibitions >75% the following concentrations of drugs were required: theophylline, 2.5–4.0 mM; PGE<sub>1</sub>, 20.0 μg/ml; PGE<sub>2</sub>, PGA<sub>1</sub>, and PGA<sub>2</sub>, 10.0–20.0 μg/ml; PGF<sub>2α</sub>, 20.0–50.0 μg/ml. A range of theophylline and prostaglandin concentrations was utilized in each experiment in order to obtain inhibitions by the separate agents such that synergism could be observed in combination. This strategy was adopted because of variations in response to separate agents that were manifested by different batches of cells. When the predicted

TABLE II  
*Potentiation by Theophylline of the Inhibitory Effect on Phagocytosis Produced by Prostaglandin\**

Range of predicted inhibition	Inhibition predicted ‡	Inhibition observed	Potentiation §	P
0-50%				
PGE <sub>1</sub>	36.5 ± 4.2 (4)	71.4 ± 8.7 (4)	196.4 ± 10.3 (4)	<0.005
PGE <sub>2</sub>	37.8 ± 1.6 (5)	54.9 ± 6.2 (5)	148.5 ± 22.0 (5)	<0.025
PGA <sub>1</sub>	32.0 ± 6.5 (6)	33.8 ± 6.2 (6)	115.0 ± 17.1 (6)	NS
PGA <sub>2</sub>	37.1 ± 8.6 (4)	37.1 ± 11.2 (4)	91.1 ± 18.1 (4)	NS
PGF <sub>2α</sub>	34.2 ± 4.1 (8)	46.1 ± 4.7 (8)	143.6 ± 15.7 (8)	<0.05
50-75%				
PGE <sub>1</sub>	—	—	—	—
PGE <sub>2</sub>	64.9 ± 2.4 (8)	66.8 ± 3.3 (8)	103.6 ± 5.6 (8)	NS
PGA <sub>1</sub>	65.3 ± 3.6 (6)	65.3 ± 8.0 (6)	97.1 ± 13.4 (6)	NS
PGA <sub>2</sub>	65.3 ± 1.5 (10)	65.1 ± 5.0 (10)	98.9 ± 5.9 (10)	NS
PGF <sub>2α</sub>	63.7 ± 2.0 (12)	62.2 ± 2.1 (12)	97.1 ± 6.2 (12)	NS
75-100%				
PGE <sub>1</sub>	76.6 ± 1.1 (2)	72.6 ± 9.7 (2)	94.6 ± 11.3 (2)	NS
PGE <sub>2</sub>	78.9 ± 5.3 (3)	66.8 ± 8.4 (3)	84.3 ± 8.5 (3)	NS
PGA <sub>1</sub>	82.1 ± 2.2 (2)	82.7 ± 3.4 (2)	101.0 ± 6.8 (2)	NS
PGA <sub>2</sub>	84.0 ± 2.1 (4)	65.5 ± 8.9 (4)	77.7 ± 10.1 (4)	<0.1
PGF <sub>2α</sub>	82.5 (1)	65.2 (1)	79.0 ± (1)	—

\* The amount of inhibition of phagocytosis obtained when prostaglandin and theophylline were applied together during a 30-min preincubation period is compared with the amount of inhibition predicted from that observed when the monolayers were preincubated with each agent alone. Data are entered as percent plus or minus SE. Numbers in parentheses indicate number of experiments. The significance of the differences between the experimental values obtained and the predicted values was determined by application of the Student *t* test and is entered as *P*.

‡ Predicted inhibition was calculated as follows: Predicted inhibition = inhibition by prostaglandin (PG) + (100 - inhibition by PG) (inhibition by theophylline/100).

§ Potentiation was calculated in paired experiments as follows: Potentiation = 100 (Inhibition observed/Inhibition calculated)

inhibition was within the range of 0-50%, it could be seen that the inhibition of phagocytosis brought about by PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2α</sub> was clearly potentiated by theophylline in a significant fashion. When predicted inhibitions were between 50% and 75%, no potentiation was demonstrable and the drugs behaved as though they were acting at independent sites. At levels of inhibition predicted to be >75%, combinations of drugs led to inhibitions of phagocytosis that were slightly below the predicted levels. The inhibition by PGA<sub>1</sub> and PGA<sub>2</sub> was not potentiated by theophylline even at concentrations where the predicted amount of inhibition was within the range where it has been possible to demonstrate synergism in the case of PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2α</sub>.

#### *Effects of PGE<sub>1</sub> and Theophylline on Metabolism*

Since the increment in oxygen consumption and glucose oxidation associated with phagocytosis varies directly with the rate of phagocytosis over a broad range (Sbarra and Karnovsky, 1959; Michell et al., 1969; Stossel et al., 1972), it was expected that the prostaglandins and theophylline would inhibit these metabolic functions secondarily to their inhibition of the uptake process itself. This turned out to be the case for the most part. However, we were surprised to find that rather than inhibiting respiration during phagocytosis, PGE<sub>1</sub> stimulated it. From Fig. 3 *a* it can be seen that neither PGE<sub>1</sub> nor theophylline had much effect on "resting" respiration but preparations containing

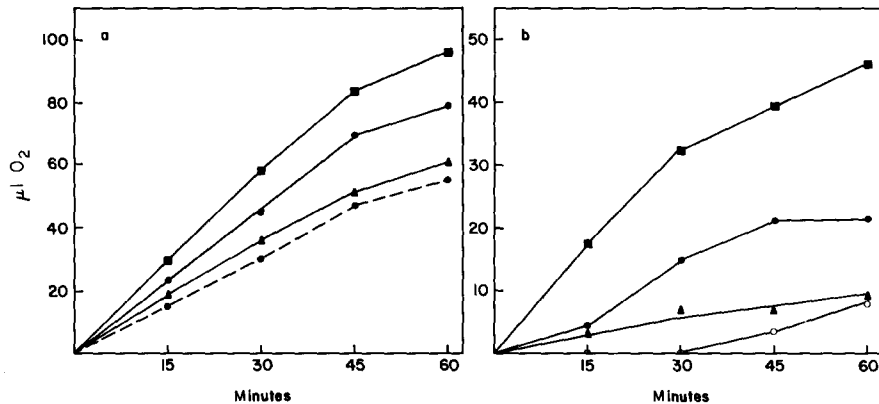


FIGURE 3 Effects of PGE<sub>1</sub> + theophylline on oxygen consumption in resting and phagocytizing guinea pig PMN. The ordinate represents the amount of oxygen consumed in Fig. 3 a and the difference in oxygen consumption between phagocytizing and resting cells in Fig. 3 b, as  $\mu\text{l O}_2$ . The abscissa refers to the time of incubation for both Fig. 3 a and Fig. 3 b. The cells were exposed to drugs for 30 min before recording of oxygen consumption. Each flask contained  $1.0 \times 10^8$  PMN. The points represent the mean of duplicate flasks in a single experiment. The broken line (---) in Fig. 3 a represents resting oxygen consumption by control PGE<sub>1</sub>-treated, theophylline-treated, or PGE<sub>1</sub> + theophylline-treated cells. Resting oxygen consumption after 30 min with or without drugs deviated from the dotted line by an average of 13%. The solid lines in Fig. 3 a represent O<sub>2</sub> consumption by phagocytizing cells. Deviation from the mean after 30 min averaged 7% for the control curve (●), 5% for the PGE<sub>1</sub> curve (■), and 6% for the theophylline curve with or without PGE<sub>1</sub> (▲). In Fig. 3 b the respiratory burst during phagocytosis is given for: control cells (●), PGE<sub>1</sub>-treated cells (■), PGE<sub>1</sub> + theophylline-treated cells (○), and theophylline treated cells (▲). Theophylline concentration was 2.5 mM, PGE<sub>1</sub> concentration was  $5.6 \times 10^{-5}$  M.

drug diverged from the control with respect to respiration during ingestion. This is more dramatically brought out in Fig. 3 b where the *difference* between phagocytic and resting respiration is plotted as a function of time. Theophylline alone clearly eliminates the respiratory burst that normally accompanies phagocytosis, as might be expected (see above) whereas PGE<sub>1</sub> alone stimulates it even though phagocytosis per se is possibly slightly depressed (see Table I and Fig. 2). These effects were not additive when the two drugs were applied together, in which instance the inhibitory effect of theophylline predominated. The increase in oxygen consumption during phagocytosis is thought to be due to the activation of a cyanide-insensitive NADH-oxidase which is present in these cells (Cagan and Karnovsky, 1964). Direct assay of this enzyme in the presence of PGE<sub>1</sub> showed no stimulation (data not shown). The increase in oxygen consumption of phagocytizing PGE<sub>1</sub>-treated cells relative to phagocytizing controls is abolished in the presence of cyanide, further eliminating cyanide-insensitive oxidases as the locus of PGE<sub>1</sub> stimulation of respiration.

The effects of these drugs on the oxidation of [<sup>14</sup>C]glucose to <sup>14</sup>CO<sub>2</sub> are shown in Fig. 4. Both drugs (PGE<sub>1</sub> and theophylline) alone and in combination inhibited the phagocytic stimulation of glucose oxidation, probably due to inhibition of ingestion per se. Inhibition of the resting level of glucose oxidation was minimal except in the case of the combination of drugs. All metabolic measurements were made for a period of phagocytosis of 60 min.

#### DISCUSSION

It is apparent from the data we have presented that dibutyryl cAMP, theophylline, and prostaglandins—all compounds that would be expected to elevate intracellular cAMP levels—bring about an inhibition in phagocytosis of starch particles by guinea pig PMN. The fact that cAMP itself was the least potent inhibitor may be due to its slow permeation into cells or to rapid degradation by phosphodiesterases at the cell surface. At least two other reports pertinent to our observations have appeared in the literature. Stossel et al. (1972) reported briefly that their new method for assaying

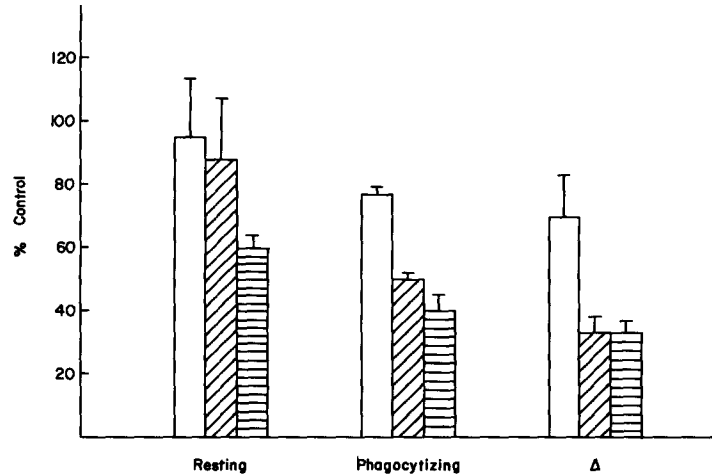


FIGURE 4 Effect of PGE<sub>1</sub> and theophylline separately and together on the oxidation of [1-<sup>14</sup>C]glucose to <sup>14</sup>CO<sub>2</sub>. The ordinate refers to the amount of <sup>14</sup>CO<sub>2</sub> recovered from [1-<sup>14</sup>C]glucose during a 60-min incubation (expressed as % of control). The state of the cells (resting or phagocytizing) is indicated; Δ represents the difference in <sup>14</sup>CO<sub>2</sub> production between phagocytizing and resting cells. The cells were preincubated with drug for 30 min before the addition of [1-<sup>14</sup>C]glucose. Bars indicated average deviation between duplicate experiments each done in triplicate. PGE<sub>1</sub> (5.6 × 10<sup>-5</sup> M), open bar; theophylline (2.5 mM), slashed hatching; PGE<sub>1</sub> (5.6 × 10<sup>-5</sup> M) plus theophylline (2.5 mM), horizontal hatching.

rates of phagocytosis by use of albumin-stabilized paraffin emulsions containing Oil Red O was able to detect a diminution of phagocytosis by theophylline or dibutyryl cAMP. Bourne et al. (1971) claimed that preincubation of neutrophils for 30 min with 3.0 mM dibutyryl cAMP, 1.0 × 10<sup>-5</sup> M PGE<sub>1</sub>, or 3.0 mM theophylline produced a "slight but consistent" inhibitory effect on ingestion of radioactively labeled yeasts which they deemed insufficient to account for the reduced candidacidal activity observed in response to these same drugs. We note, however, that in those experiments there were inhibitions of ingestion in the range of 25–40%. Further, Weissmann et al. (1971) have demonstrated that cAMP, dibutyryl cAMP, and theophylline inhibit the uptake of [<sup>125</sup>I]BSA by mouse macrophages in culture.

We found that not only PGE<sub>1</sub> but also PGE<sub>2</sub>, PGA<sub>1</sub>, PGA<sub>2a</sub> inhibited phagocytosis. PGE<sub>1</sub> and PGE<sub>2</sub> are the only prostaglandins that have been shown thus far to elevate intracellular levels of cAMP in leukocytes. PGF<sub>1a</sub> specifically did not and PGA<sub>1</sub>, PGA<sub>2</sub>, and PGF<sub>2a</sub> have not been studied in this regard (Scott, 1970). We noted that the behavior of prostaglandin-theophylline combinations on the phagocytic response varied according to the amount of inhibition expected if theophylline and prostaglandins were acting at unrelated sites, as follows: First, if the amount of

inhibition predicted was less than 50%, the inhibitory effects of PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2a</sub> were potentiated by theophylline. Here the data are in accord with a synergistic effect brought about by action of the two drugs on the same system. In view of the well-documented effects of theophylline and certain prostaglandins on cAMP metabolism in a number of different tissue and cell types including the PMN (Butcher and Baird, 1968; Scott, 1970; Bourne et al., 1971; Bourne and Melmon, 1971; Stossel et al., 1970; Stolc, 1972; Bourne et al., 1973), these observations would tentatively implicate a mechanism of inhibition of phagocytosis by prostaglandins that involves the adenylyl cyclase system. It should be noted however that under these same conditions, the inhibition of phagocytosis effected by PGA<sub>1</sub> and PGA<sub>2</sub> was *not* potentiated by theophylline. Second, when the predicted inhibition of phagocytosis was between 50 and 75%, the observed values were the same as those predicted. This sort of behavior is consistent with action of the two drugs on independent systems. The situation with PGA<sub>1</sub> and PGA<sub>2</sub> mentioned above might be viewed in this regard, i.e., PGA<sub>1</sub> and PGA<sub>2</sub> could inhibit phagocytosis by a mechanism distinct from one involving cAMP. Third, when inhibitions in excess of 75% were predicted, a response was obtained which was less than that predicted on the basis of independent



sites. It is possible that the concentrations of drugs required to bring about inhibitions of phagocytosis in excess of 75% approached a threshold required for them to act as surface active agents and thus stimulated phagocytosis to some degree. The prostaglandins in particular would be suspect in this regard. Stimulation of endocytosis by surface active agents has been reported (Cohn and Morse, 1960; Graham et al., 1967). In a complex system such as a whole cell, combinations of the mechanisms suggested above may well be operative as well as the phenomenon of competitive interaction, i.e., the possibility that theophylline might displace prostaglandin from a given site and vice versa.

We believe that the degree to which dibutyl cAMP, theophylline, and the prostaglandins inhibited phagocytosis in our hands might well have been sufficient to account for observations made by others on decreased candidacidal activity (Bourne et al., 1971), decreased phagocytic release of  $\beta$ -glucuronidase (May et al., 1970), and decreased iodide metabolism (Stolc, 1972) in response to these drugs. Likewise the inhibition of metabolism in the presence of particles observed by ourselves and others (Bourne et al., 1971; May et al., 1970; Weissmann et al., 1972) could be secondary to the inhibition of phagocytosis itself. The paradoxical stimulation of the phagocytic respiratory burst elicited by PGE<sub>1</sub> remains unaccounted for. It appears not to involve the cyanide-insensitive oxidase system present in PMN and thought to be responsible for the increment in respiration during phagocytosis. This effect is probably not mediated via the adenylate cyclase system since theophylline did not potentiate it, although both drugs were at concentrations at which potentiation by theophylline of inhibitions of phagocytosis by PGE<sub>1</sub> were discerned.

Phagocytosis by leukocytes stands alone as an example of a bulk transport process that is inhibited by cAMP. In other such systems studied, cAMP either stimulated the process or had no effect. Not only endocytosis (phagocytosis) but also exocytosis as reflected by allergic histamine release is inhibited by cAMP in leukocytes (Lichtenstein and Margolis, 1968). One could speculate that in the phagocytic PMN, increased intracellular levels of cAMP, brought about by particle internalization, might serve as a signal for the cell to stop phagocytizing in order that energy supplies may be replenished and surface membrane replaced. It is

as yet uncertain whether or not an alteration of cAMP concentrations is one of the biochemical manifestations of the phagocytic response. It was reported by Park et al. (1971) that the cAMP content of human peripheral blood leukocytes is increased several fold approximately 5 min after the initiation of phagocytosis. However, others were not able to confirm these results (Stolc, 1972). It has been suggested that the increase in cAMP observed by Park et al. (1971) could have occurred in cells other than PMN since the leukocytes used had not been fractionated (Manganiello et al., 1971). It is frequently difficult to correlate changes in a particular physiological response with changes in total cellular cAMP concentration because of complications arising from compartmentalization and local ionic environment.

The *mechanism* whereby cAMP might act to control phagocytosis is an intriguing question. In other systems and especially in case of the cAMP-stimulated activation of skeletal muscle phosphorylase, the cAMP effect is thought to involve the stimulation of a protein kinase (Walsh et al., 1970). Indeed, Greengard et al. (1969) have suggested that all the varied effects of cAMP may result from the phosphorylation of a protein secondary to the stimulation of a protein kinase. A report on the presence of a cAMP-dependent protein kinase in human PMN has recently appeared (Tsung et al., 1972). Whether or not the inhibition of phagocytosis effected by elevated intracellular cAMP is the consequence of a protein kinase-mediated phosphorylation of some component essential for phagocytosis is a point of conjecture.

The authors are deeply indebted to Dr. Robert L. Jungas for a critical examination of this manuscript.

This work was supported by U. S. Public Health Service Grant no. A1-03260 from the National Institute of Allergy and Infectious Disease.

Received for publication 14 May 1973, and in revised form 2 July 1973.

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