The Effect of Chloramphenicol on Human Leukocyte Phagocytosis and Respiration* (33668)

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The ingestion of particles by polymorphonuclear (PMN) leukocytes, the subsequent metabolic events and their significance to host defense have been the subject of extensive recent investigation (1–6). The most striking metabolic alteration associated with normal phagocytosis is a sharp increase in oxygen consumption (1, 2) due to the increased metabolism of glucose through the hexose monophosphate (HMP) shunt (3–5). There also is increased anaerobic glycolysis, glycogenolysis, and lipid turnover (3, 4, 6). Until recently, it was felt that the increase in oxidative glycolysis provided both the essential energy for phagocytosis, and secondarily resulted in stimulation of the HMP shunt (5). This probably is not the case, however, since phagocytosis was observed in the absence of any increase in oxygen consumption. Malawista and Bodel demonstrated dissociation of phagocytosis from increased oxygen consumption in human leukocytes incubated with colchicine (7). Baehner and Nathan (8, 9) and Holmes *et al.* (10) reported that the leukocytes of children with chronic granulomatous disease have little or no increase in oxygen consumption and HMP shunt activity during particle uptake. In patients with granulomatous disease intracellular killing of the ingested microorganisms also is severely impaired (11). This is believed due to the failure of leukocyte degranulation following phagocytosis. Selvaraj and Sbarra (12) postulated that the metabolic events associated with phagocytosis are separated into two events: (i) particle ingulfment which is dependent upon anaerobic glycolysis for energy, and which is inhibited by the glycolytic inhibitors sodium fluoride and iodoacetate; and (ii) degranulation followed by subsequent degradation by the cell of the ingested particle with increased oxygen consumption and HMP shunt activity. These studies strongly suggest that the respiratory burst associated with phagocytosis is related to intracellular killing of the ingested bacteria and not to ingestion per se. It was of interest, therefore, when we observed that human PMN leukocytes in the presence of chloramphenicol failed to show the expected increase in oxygen consumption following particle ingestion.

Materials and Methods. Approximately 25 ml of venous blood anticoagulated with 4 units of heparin/ml were obtained from each of 29 subjects for leukocyte phagocytosis and respiration studies. Leukocyte phagocytosis was evaluated by means of latex particle uptake, and leukocyte respiration was measured with a Gilson respirometer by modification of the Strauss and Stetson technique (2). Subjects studied included healthy adults and patients with a moderate polymorphonuclear leukocytosis associated with mild infection or surgery. Total leukocyte counts ranged from 5000 to 20,000/mm^3. Thirty-three experiments were performed on the 29 subjects, with 4 persons studied on 2 separate occasions.

Double sidearm Warburg reaction flasks were prepared with 0.2 ml of 10% KOH and a filter paper wick in each center well. Two tenths ml of a 3% suspension in saline of polystyrene latex particles, 0.79 μ diameter (Dow Chemical Company Lot No. LS449E) were placed in both side arms of 2 of the 4 flasks containing crystalline chloramphenicol and 2 of the 4 control flasks which did not contain chloramphenicol. Three ml of whole blood were then added to each flask. Thus,

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all experiments were internally controlled so that the results of duplicate flasks containing chloramphenicol, both with and without latex particles, could be compared to the results of similar flasks in which chloramphenicol was absent. The concentrations of chloramphenicol studied were 6×10^{-3}, 2×10^{-3}, and $2 \times 10^{-4} M$. The effect of $6 \times 10^{-3} M$ chloramphenicol on the resting respiration and response to particles of suspensions of pure red cells and pure platelets also was determined. The reaction vessels were attached to the respirometer where they were allowed to equilibrate with room air for a period of 15–30 min. The manometer readings during subsequent 15-min intervals for the first hour were recorded. The polystyrene latex particles in the side arms then were added, and the manometer readings were continued every 15 min for another hour. Oxygen consumption was expressed in microliters of oxygen consumed per hour per 10^8 neutrophilic polymorphonuclear leukocytes. The oxygen consumed during the first hour prior to the addition of particles was considered the resting oxygen consumption. The phagocytizing oxygen consumption was the difference in oxygen consumption during the second hour between flasks which contained particles and those without particles. The data were analyzed by means of the paired *t* test.

At the termination of each experiment, blood smears were made from each flask containing latex particles. They were stained with Wright's stain and 100 PMN leukocytes were evaluated for the degree of phagocytosis under oil immersion magnification. A cell was considered to have adequate phagocytosis if there were more than 30 particles/cell. This was expressed as the percentage of neutrophilic polymorphonuclear leukocytes with adequate phagocytosis.

Results. The resting and phagocytizing oxygen consumptions and the percentage of PMN with adequate phagocytosis are shown in Tables I, II, and III. Although the oxygen consumptions were calculated for 10^8 PMN leukocytes, there was considerable variability from one experiment to another. This is partially due to variability among subjects, but probably is primarily due to the crowding effect artifact which is described as a decrease in the respiration per cell as the number of cells increases. The resting respiration declined slowly over the 2-hr period of observation from a mean of 200 μl during the first hour to a mean of 71 μl during the second hour for all of the controls in the 33 experiments. With phagocytosis, however, the mean respiration during the second hour was 312 μl which is a 4.5-fold increase over the second hour resting respiration.

With 6×10^{-3} Molar chloramphenicol, the mean first hour resting oxygen consumption was 224 μl while the control was 243 μl. This small difference was statistically significant. The phagocytizing oxygen consumption for the chloramphenicol exposed cells, however, was only 11 versus 246 μl for the control. In spite of this severe impairment of the respiratory burst; of the 20 experiments performed, phagocytosis per se was in the normal range in 5, partially inhibited in 11, and essentially absent in 5 (Table I). There was no chloramphenicol effect at this concentration on the respiration of plasma suspensions of either red cells or platelets.

The most significant results were found at chloramphenicol concentrations of 2×10^{-3} *M*. Resting oxygen consumption was not significantly changed from the control. The mean phagocytizing oxygen consumption, however, was only 85 μl in comparison to the mean control value of 214 μl. Although the mean phagocytizing oxygen consumption was reduced by greater than 60% phagocytosis per se was unimpaired in all experiments (Table II).

Both oxygen consumption and phagocytosis were in the control range in all experiments using 2×10^{-4} *M*olar chloramphenicol (Table III).

Discussion. The dissociation of phagocytosis from increased oxygen consumption in the presence of chloramphenicol supports the hypothesis that activation of the HMP shunt occurs subsequent to particle ingestion as a separate phenomenon. The similarity of the leukocyte responses of children with chronic

TABLE I. The Effects of $6 \times 10^{-3} M$ Chloramphenicol on Human Leukocyte (PMN) Phagocytosis and Respiration.

Expt. no.	Resting O_2 consumption (μl O_2/hr/10^8 PMN)		Phagocytizing O_2 consumption[a] (μl O_2/hr/10^8 PMN)		PMN with adequate phagocytosis (%)[b]	
	Chloro (+)	Chloro (−)	Chloro (+)	Chloro (−)	Chloro (+)	Chloro (−)
1	446	473	−26	315	0	93
2	234	295	13	228	0	90
3	257	268	13	350	8	100
4	191	205	−2	200	3	99
5	279	272	−26	305	39	100
6	331	377	26	300	54	96
7	135	152	3	225	48	100
8	275	314	51	342	48	90
9	184	167	−11	131	39	100
10	153	167	−10	127	31	94
11	152	153	35	113	56	90
12	428	475	18	472	49	100
13	124	138	9	282	41	100
14	138	139	4	306	25	100
15	66	101	−4	193	58	98
16	426	372	122	297	96	100
17	210	255	1	307	89	100
18	68	70	−1	57	84	80
19	65	79	−1	67	74	71
20	165	225	5	300	80	100
Mean	224	243	10.9	246		
	$p = <.01$		$p = <.001$			

[a] Δ respiration between phagocytizing and resting cells during the second hour.
[b] Thirty or more latex particles/cell.

granulomatous disease, which show a deficiency of NADH oxidase, and our observations using chloramphenicol suggest that leukocyte nucleotide oxidase may be the site of inhibition. Studies with normal leukocytes (12, 13) also have stressed the role of the reduced pyridine nucleotide oxidases for activation of the HMP shunt. Preliminary experiments in our laboratory with methylene blue demonstrated reversal of the oxygen consumption inhibition associated with chloramphenicol. Thus: the HMP shunt is operable when oxidized nucleotides are made available.

Chloramphenicol was reported to be an inhibitor of protein synthesis both in bacterial and in mammalian cells (14, 15). It also was described as an inhibitor of xanthine oxidase activity in rat liver homogenates (16). The duration of our experiments probably was too short, however, to invoke the mechanism of diminished protein synthesis as a primary mechanism for our findings. Previous studies failed to implicate xanthine oxidase inhibition as the mechanism for the effect of chloramphenicol on leukocyte respiration (16).

Follette *et al.* (16) demonstrated severe inhibition of leukocyte homogenate respiration by chloramphenicol, but not by chlortetracycline, oxytetracycline, penicillin, or streptomycin using concentrations of from 1×10^{-3} to $6.1 \times 10^{-3} M$. These concentrations,while much larger than those found *in vivo*, are comparable to the concentrations of other inhibitors commonly used *in vitro* such as iodoacetate. Using comparable concentrations of chloramphenicol, we observed little suppression of resting respiration of intact

TABLE II. The Effects of 2×10^{-3} *M* Chloramphenicol on Human Leukocyte (PMN) Phagocytosis and Respiration.

	Resting O_2 consumption (μl O_2/hr/10^8 PMN)		Phagocytizing O_2 consumption[a] (μl O_2/hr/10^8 PMN)		PMN with adequate phagocytosis (%)[b]	
Expt. no.	Chloro (+)	Chloro (−)	Chloro (+)	Chloro (−)	Chloro (+)	Chloro (−)
1	75	93	32	119	80	91
2	93	90	100	315	95	96
3	99	122	43	244	95	97
4	84	81	242	388	90	92
5	73	66	74	96	95	89
6	58	58	70	226	91	94
7	76	77	36	107	91	92
Mean	80	87	85	214		
	$p = <.4$		$p = <.01$			

[a] Δ respiration between phagocytizing and resting cells during the second hour.
[b] Thirty or more latex particles/cell.

leukocytes in whole blood, suggesting that the intact cell membrane may serve as a barrier to the intracellular diffusion or transport of chloramphenicol.

The concentrations of chloramphenicol used in our *in vitro* studies were many times those usually achieved in the plasma of patients treated with chloramphenicol, and therefore our results should not be directly extrapolated to events which occur in man. The data, nevertheless, demonstrate chloramphenicol interference with another area of intracellular function and serve to further illustrate the complex nature of the metabolic alterations associated with the phenomenon of phagocytosis.

Summary. The respiratory burst associated with phagocytosis can be significantly inhibited by high concentrations of chloramphenicol without impairment of phagocytosis per

TABLE III. The Effects of 2×10^{-4} *M* Chloramphenicol on Human Leukocyte (PMN) Phagocytosis and Respiration.

	Resting O_2 consumption (μl O_2/hr/10^8 PMN)		Phagocytizing O_2 consumption[a] (μl O_2/hr/10^8 PMN)		PMN with adequate phagocytosis (%)[b]	
Expt. no.	Chloro (+)	Chloro (−)	Chloro (+)	Chloro (−)	Chloro (+)	Chloro (−)
1	413	493	388	354	95	99
2	131	97	312	284	99	96
3	119	106	200	217	92	89
4	132	124	270	265	100	97
5	124	134	306	302	88	87
6	81	77	51	72	87	89
Mean	167	172	255	249		
	$p = <.8$		$p = <.6$			

[a] Δ respiration between phagocytizing and resting cells during the second hour.
[b] Thirty or more latex particles/cell.

se. The impaired respiratory burst reflects decreased activation of the HMP shunt probably through the mechanism of enzyme inhibition.

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Growth Hormone Releasing Activity in the Hypothalamus of Rats Subjected to Prolonged Heat Stress* (33669)

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Evidence for the existence of a neurohumor, designated as growth hormone releasing factor (GHRF), in the hypothalamic extracts of several animal species has been established and recently reviewed (1, 2). Hypothalamic control of the secretion of pituitary growth hormone (GH) is mediated by GHRF since crude hypothalamic extracts have been shown to stimulate the release of GH by the pituitary *in vitro* (3) and also *in vivo* (4). Highly purified preparations of pig GHRF have also been shown to cause depletion of the pituitary GH content in rats (5) with simultaneous increase in plasma GH. Such a response to GHRF represents an increased release of GH from the pituitary into the blood (6, 7).

Although several experimental investigations have produced evidence that a variety of nonspecific stimuli induce alterations in growth hormone secretion (8, 9), only a few studies (10) indicated variations in hypothalamic GHRF content due to stress.

Mueller and co-workers (10) found no significant difference from normal controls in growth hormone releasing activity (GHRA) of hypothalami, GH content in pituitaries, or GHRA of plasma in rats after 5 and 60 min subcutaneous injection of 0.5 ml of 10% formalin. No difference was produced in rats exposed to cold (4°) for 5 min. However, rats exposed to cold for 1 hr showed a significant increase in GHRA of the hypothalamus, with a simultaneous depletion of GH in pi-

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