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Inhibition of Amino Acid Incorporation into Protein of Human Neutrophils by Phagocytosis

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The incorporation of uniformly labeled ^{14}C-amino acids into total protein of human polymorphonuclear leukocytes is inhibited when the cells are challenged with heat-killed *Escherichia coli* or latex particles. Parallel studies of amino acid incorporation and glucose oxidation indicate that the inhibition is dependent upon ingestion of the particles. It is suggested that the process of phagocytosis might inhibit the transport of amino acids into the cell either by competing for available energy or by internalizing specific transport sites.

During the process of phagocytosis by a polymorphonuclear leukocyte, there are profound alterations in membrane structure, resulting in the formation of a phagocytic vesicle with the internalization of as much as 35% of the plasma membrane (9). There is a concomitant increase in lipid turnover, particularly of phospholipids (3, 5, 8), which is presumably needed for the alterations in the membrane.

Since the cell membrane in phagocytes contains nearly equal amounts of protein and lipid (6), an increased turnover of protein during phagocytosis might be expected. This is not the case. The present communication demonstrates that there is a net decrease in the incorporation of radioactive amino acids into total cellular protein during phagocytosis.

MATERIALS AND METHODS

Isolation of cells. Leukocytes were isolated from the blood of apparently healthy volunteer subjects by a method described previously (1). The isolated cells were suspended in Dulbecco phosphate-buffered saline (PBS; containing both magnesium and calcium ions) and adjusted to a final concentration of 5×10^6 phagocytes per ml by the addition of PBS. Phagocytes are defined here as segmented neutrophils, band neutrophils, eosinophils, and monocytes. Lymphocytes accounted for less than 10% of the isolated cell suspension.

Glucose oxidation. The oxidation of glucose-*1*-^{14}C to $^{14}CO_2$ was determined by modification of a previously described method (4). Each flask contained 0.20 μCi of glucose-*1*-^{14}C (New England Nuclear Corp., Boston, Mass.; specific activity 54.2 mCi/mmol) and 1.66 μmol of unlabeled glucose. Phagocytosis was initiated in appropriate flasks by the addition of 0.15 ml of latex particles prepared as previously described (2), or by the addition of 1.0 ml of a standard suspension of heat-killed bacteria. The particle:cell ratio was approximately 10,000:1 in both cases. The bacteria were incubated overnight in soy broth to the stationary phase of growth and heat-killed by immersion in a boiling-water bath for 20 min. The bacteria were collected by centrifugation, washed three times with 0.9% sodium chloride, and suspended in PBS to a standard absorbance of 1.00 at 525 nm on a Beckman DU spectrophotometer. Dialyzed human serum (pooled, type O, 0.10 ml) was added to flasks where indicated. A 10-ml amount of human serum was dialyzed overnight against 1 liter of PBS, pH 7.4, at 0 C. The final volume of all flasks was adjusted with PBS to give 3.0 ml after the addition of cells. Reaction was initiated by the addition of 1.0 ml of isolated phagocytes (5×10^6 cells).

The reaction was allowed to proceed for 1 h at 37 C and was terminated by the addition of 1.0 ml of 5% trichloroacetic acid. The $^{14}CO_2$ released during the course of the incubation was collected in 0.50 ml of hyamine hydroxide and counted in a liquid scintillation spectrometer as described previously (4).

Amino acid incorporation. A 100-μCi amount of uniformly labeled ^{14}C-amino acids (New England Nuclear Corp., Boston, Mass.; 100 μCi/ml) was evaporated to dryness under a stream of nitrogen gas. The residue was dissolved in 10.0 ml of PBS to give a stock solution of 10 μCi/ml, which was stored frozen. A 1:10 dilution of this stock solution was prepared fresh for each experiment to give a working solution of 1.0 μCi/ml. A typical experiment involved incubating 0.40 μCi of labeled amino acids with 5×10^6 phagocytes in a total volume of 3.0 ml (the volumes were adjusted by the addition of appropriate quantities of PBS). Phagocytosis was initiated by the addition of the same particles described above; 0.10 ml of dialyzed human serum was added to some flasks. Dialysis of the serum was required to remove endogenous amino acids which lowered the specific activity of the isotope and, thus, the degree of incorporation observed. Incubation was generally performed for 1 h at 37 C.

The reaction was usually stopped by the addition of 3.0 ml of 5% trichloroacetic acid. The precipitate obtained was washed two times with 5 ml of trichloroacetic acid and once with acetone. Excess acetone was removed by heating in a 60 C water bath for 20 min. The dried pellet was digested in 0.50 ml of 0.2 N NaOH for 2 h at 60 C: the solution was neutralized with 0.20 ml of 3% acetic acid, and 0.50 ml of water was added. A 1.0-ml sample was added to 10 ml of Aquasol (New England Nuclear Corp., Boston, Mass.), and the radioactivity was determined in a liquid scintillation spectrometer.

Alternatively, the reaction was stopped in some cases by the addition of 5.0 ml of ice-cold PBS, followed by immediate centrifugation at 0 C. The cell pellet was washed three times with 5 ml of cold PBS by repeated suspension and centrifugation. Excess PBS was removed by overnight drying in a 60 C water bath. The resulting pellets were digested, neutralized, and counted as previously described.

RESULTS

Some of the characteristics of the incorporation of labeled amino acids into total leukocytic protein are illustrated in Fig. 1. Under the assay conditions employed, the incorporation progresses in a linear fashion for at least 120 min and is dependent upon the isotope concentration up to at least 1.0 μCi of radioactive amino acids. The effect of phagocytosis of polystyrene spherules upon the incorporation of amino acids into protein is described in Table 1. Very little incorporation is observed when the reaction is terminated at zero time or when it is allowed to incubate for 60 min at 0 C. On the other hand, a significant amount of isotope is incorporated into trichloroacetic acid-precipitable material by resting cells; this incorporation is only slightly enhanced by the inclusion of glucose in the medium. A significant and reproducible inhibition of this incorporation is observed when latex particles are included in the incubation mixture. This inhibition was evident at two concentrations of isotope, and was not appreciably altered by the inclusion of exogenous glucose in the medium. Control experiments demonstrated that this inhibition by latex could not be explained by either adsorption of isotope onto the latex or a quench effect of latex in the scintillation counter. Five times the concentration of latex used in the present experiment failed to adsorb a measurable amount of isotope and to decrease the counting rate when added to a standard amount of labeled amino acids in a scintillation vial (data not shown). Thus, the inhibition was presumed to be dependent upon the phagocytosis of the particles by the cells.

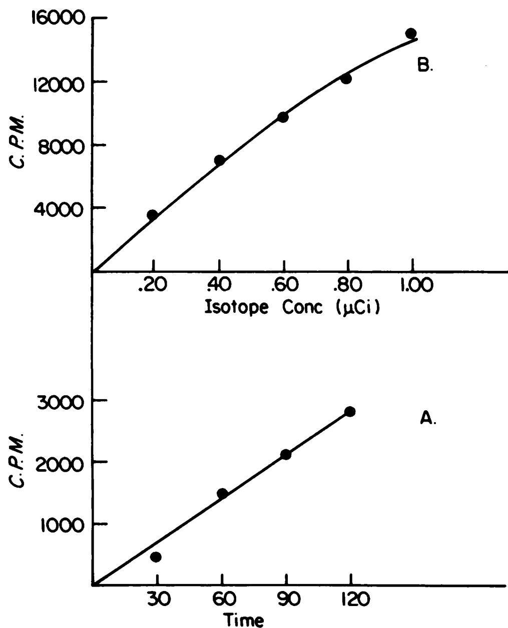

FIG. 1. *Incorporation of labelled amino acids into phagocytic protein. Each point represents the mean of three determinations. A, Effect of incubation time (minutes); B, effect of isotope concentration (microcuries).*

The correlation between inhibition of amino acid incorporation and phagocytosis was investigated in several experiments employing more than one type of particle. The degree of ingestion of a particle was estimated indirectly by the increase in glucose-*1*-^{14}C oxidation when the cell was challenged with a specific particle (Table 2). In the absence of serum, latex is ingested adequately, as seen by the fivefold

TABLE 1. *Incorporation of ^{14}C-amino acids into leukocyte protein*[a]

Expt conditions	Amino acid incorporation (counts/min)	
	Expt 1	Expt 2
Zero time incubation	68	206
Incubation at 0 C	144	327
Complete system	6,968	13,463
Complete system + glucose	7,831	
Complete system + latex	2,927	5,926
Complete system + glucose + latex	3,172	

[a] The complete system consisted of 5 × 10^6 polymorphonuclear leukocytes plus isotope in a volume of 3.0 ml of PBS. A 0.40-μCi amount of labeled amino acid was used in experiment 1; 1.00 μCi was used in experiment 2. Glucose was added, where indicated, to give a final concentration of 0.10 mg/ml. Each value represents the mean of closely agreeing triplicate determinations.

TABLE 2. *Parallel study of amino acid incorporation and glucose-1-^{14}C oxidation*[a]

Description	Amino acid incorporation (counts/min)		Glucose-*1*-^{14}C oxidation (counts/min)	
	− Serum	+ Serum	− Serum	+ Serum
Experiment 1				
Zero time incubation	293	193	77	104
− Particles	6,562	6,454	2,099	860
+ Latex	3,485	4,517	10,286	14,934
+ *E. coli*	5,765	3,947	3,089	22,163
Experiment 2				
Zero time incubation	693	686	121	103
− Particles	16,090	17,146	2,321	866
+ Latex	7,808	12,056	16,178	17,980
+ *E. coli*	12,609	8,186	4,417	28,444

[a] Each value represents the mean of closely agreeing triplicate determinations. Experiment 1 was stopped by the addition of trichloroacetic acid; experiment 2 was stopped by the addition of cold buffer and centrifugation.

increase in glucose oxidation. Under the same conditions, there is a 50% inhibition of amino acid incorporation into the cell. *Escherichia coli*, on the other hand, is very poorly ingested in the absence of serum, as indicated by the small stimulaton (1.5-fold) in glucose oxidation. There is, at best, a marginal inhibition of amino acid incorporation into protein. However, when serum is present, the *E. coli* is ingested very well, as indicated by a marked stimulation of glucose oxidation. There is now a marked inhibition of amino acid incorporation into protein. The presence of serum seems to reduce the latex-induced inhibition of amino acid incorporation (there is 47% inhibition of incorporation in the absence of serum and 30% inhibition when serum was added). The reason for this effect is unknown, but it is quite reproducible and suggests that the inhibition observed with *E. coli* in the presence of serum represents a minimal value. Similar results were observed regardless of whether the reaction was terminated with trichloroacetic acid or cold PBS. The amount of protein released to the medium was the same for resting or phagocytizing cells, so there was a decrease in the specific radioactivity of the cells during phagocytosis.

The inhibition of amino acid incorporation by latex is seen at all time periods examined and can be observed as early as 10 min after the initiation of the reaction (Fig. 2).

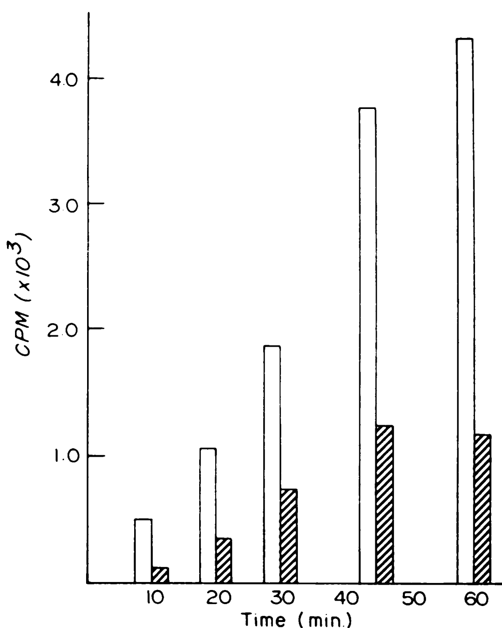

FIG. 2. *Effect of latex on the incorporation of labelled amino acids into phagocytic protein. Open bars, resting cells; hatched bars, latex present. Each point represents the mean of three determinations.*

DISCUSSION

Sbarra and Karnovsky (8) reported previously that the ingestion of starch particles by guinea pig peritoneal polymorphonuclear leukocytes depressed the incorporation of leucine-^{14}C into total cellular protein by 15%, but they observed no effect when latex was used as the challenging particle. The discrepancy between their observations and the present communication might reflect methodological differences or the differences in cell type employed. Certainly the data in Tables 1 and 2 strongly suggest that there is decreased net incorporation of amino acid into protein during phagocytosis. Further, this decrease is observed with at least two types of particles.

The data in Table 2 suggest that this phenomenon is closely related to the actual ingestion of particles. Under conditions in which particles are not ingested (e.g., *E. coli* in the absence of serum), there is no significant inhibition of incorporation. If serum is present, the particles are ingested as determined by the increase in glucose-*1*-^{14}C oxidation, and there is a concomitant inhibition of the incorporation of amino acid into protein. On the other hand, latex is ingested well in the absence of serum and, likewise, inhibits the incorporation of amino acids under these conditions. The addition of serum with latex does not greatly increase ingestion and does not cause further inhibition of amino acid incorporation. In fact, serum itself seems to alleviate the inhibition to some extent. The mechanism of this serum effect is not known. The use of glucose oxidation as a measure of degree of ingestion is valid, provided that normal cells are employed and no inhibitors of leukocyte metabolism are present.

Since the incorporation experiments were routinely performed using a 60-min incubation period, it might be argued that the inhibition of incorporation by latex is due simply to cell damage which accompanies phagocytosis. The data in Fig. 2 do not support such a conclusion; inhibition of incorporation was observed at the earliest time periods examined when relatively little cell damage could be expected. Furthermore, greater than 90% of both resting and phagocytizing cells were observed to exclude trypan blue dye after incubation periods of as great as 1 h, indicating no differences in viability between the resting and phagocytizing cells.

The incorporation of amino acids into protein by intact cells is a complex process which is dependent upon both uptake of the amino acids and the subsequent metabolic events involved in protein synthesis. Rosenberg and Downing (7) have demonstrated that amino acids are transported actively into phagocytes by a system requiring the expenditure of energy. Winkler has demonstrated that the incorporation of a single amino acid into phagocytic protein proceeds in a linear fashion for 3 to 4 h (11), in accord with the data presented in Fig. 1 for a mixture of amino acids. He has demonstrated further that extracellular amino acids are used preferentially to internal pool amino acids in protein synthesis, and concluded that the velocity of amino acid transport through the cell membrane is rate limiting in terms of the incorporation rate into protein. The present results are compatible with such an explanation. Figure 1 demonstrates that the rate of incorporation is directly dependent upon the extracellular concentration of isotope, suggesting that availability of the labeled amino acids is rate limiting. The data in Table 2 further substantiate this hypothesis. The inhibition of amino acid incorporation by phagocytosis was observed equally when the reaction was terminated by the addition of trichloroacetic acid or by the addition of cold PBS and centrifugation. If the inhibition by phagocytosis were on protein synthesis per se and the uptake of amino acids were not rate limiting, then there should be no inhibition when the reaction was stopped with PBS, since that procedure measures the total intracellular concentration of label regardless of whether it is actually incorporated into protein. The fact that inhibition is seen under these conditions suggests that uptake of the amino acids is rate limiting.

Phagocytosis could inhibit amino acid uptake in several ways. Since both phenomena are active processes requiring the expenditure of energy, the available energy might be shunted away from amino acid transport and used preferentially for phagocytosis. Alternatively, the phagocytic process might internalize a portion of the membrane containing transport sites for the amino acids. Tsan and Berlin (9) have shown that several transport mechanisms for nucleosides in rabbit phagocytes are unaffected even after 35% of the membrane has been internalized. However, it is likely that multiple transport sites are involved for the different amino acids, and the present system might be expected to be much more sensitive to internalization of the membrane. Different transport systems for some of the amino acids have been demonstrated in both alveolar macrophages (10) and polymorphonuclear leukocytes (7).

The blockage of entry of a single amino acid into the cell could reduce the incorporation of all amino acids into protein.

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LITERATURE CITED

1. DeChatelet, L. R., and M. R. Cooper. 1970. A modified procedure for the determination of leukocyte alkaline phosphatase. Biochem. Med. 4:61-68.
2. DeChatelet, L. R., D. Qualliotine-Mann, R. Caldwell, C. E. McCall, and J. P. Gusdon. 1973. Effects of promethazine-hydrochloride on human polymorphonuclear leukocytes. Infect. Immunity 7:403-408.
3. Elsbach, P. 1972. Lipid metabolism by phagocytes. Sem. Hemat. 9:227-239.

4. Holmes, B., P. G. Quie, D. B. Windhorst, and R. A. Good. 1966. Fatal granulomatous disease of childhood—an inborn abnormality of phagocytic function. Lancet 1:1225-1228.
5. Karnovsky, M. L., and D. F. H. Wallach. 1961. The metabolic basis of phagocytosis. III. Incorporation of inorganic phosphate into various classes of phosphatides during phagocytosis. J. Biol. Chem. **236**:1895-1901.
6. Nachman, R. L., B. Ferris, and J. F. Hirsch. 1971. Macrophage plasma membranes. I. Isolation and studies on protein components. J. Exp. Med. **133**:785-806.
7. Rosenberg, L. E., and S. Downing. 1965. Transport of neutral and dibasic amino acids by human leukocytes: absence of defect in cystinuria. J. Clin. Invest. **44**:1382-1393.
8. Sbarra, A. J., and M. L. Karnovsky. 1960. The biochemical basis of phagocytosis. II. Incorporation of C^{14}-labelled building blocks into lipid, protein, and glycogen of leukocytes during phagocytosis. J. Biol. Chem. **235**:2224-2229.
9. Tsan, M., and R. D. Berlin. 1971. Effect of phagocytosis on membrane transport of nonelectrolytes. J. Exp. Med. **134**:1016-1035.
10. Tsan, M., and R. D. Berlin. 1971. Membrane transport in the rabbit alveolar macrophage. The specificity and characteristics of amino acid transport systems. Biochim. Biophys. Acta **241**:155-169.
11. Winkler, K. 1972. Protein synthesis in human leukocytes II. Kinetics of the flow of amino acids from the extracellular space and the intracellular pools resulting in protein synthesis. Hoppe-Seyler's Z. Physiol. Chem. **353**:782-786.
12. Winkler, K., G. Heller-Schoch, and R. Neth. 1972. Protein synthesis in human leukocytes. IV. Mutual inhibition of amino acids in cell suspensions and cell-free systems. Hoppe-Seyler's Z. Physiol. Chem. **353**:787-792.