STUDIES ON THE EFFECT OF CERTAIN MACROMOLECULAR SUBSTANCES ON THE RESPIRATORY ACTIVITY OF THE LEUCOCYTES OF PERIPHERAL BLOOD*

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There have been several reports of the increased respiratory activity and other metabolic properties (1–4) exhibited by leucocytes during the process of phagocytosis of particulate materials. In these studies, the test cells usually have been "exudate" leucocytes derived from the peritoneal cavity after the intraperitoneal injection of solutions of glycogen or other macromolecular material. It seemed of interest to study this phenomenon under more physiologic circumstances, using the leucocytes of peripheral blood in their usual environment. It has been found that the leucocytes of several mammalian species show marked stimulation of respiratory activity in the presence of antigen-antibody complexes and endotoxins, and the results suggest that this approach may be useful in studying the nature of the biological activity of these and other macromolecular substances.

Materials and Methods

Blood.—Human blood was drawn from the antecubital vein into a syringe to which had been added heparin in an amount sufficient to give a final concentration of 3 units per ml. of whole blood. Blood from rabbits, rats, dogs, guinea pigs, monkey (*Cercopithecus callitrichus*), chicken, and bull was obtained by cardiac puncture, carotid cannulation, or femoral artery puncture, and heparin (3 units/ml.) was added. Syringes and glassware used in these equipments were sterilized but not siliconized. *Lymphocyte suspensions* were prepared from human blood by passage through a 10.0 by 2.1 cm. siliconized glass column into which there had been packed tightly 2 gm. of No. 3950 pyrex glass wool (obtained from the Fisher Chemical Co., New York) which had been siliconized by exposure to the vapor produced by the passage of air through a water-soluble silicone preparation. The effluent samples of blood contained intact lymphocytes in 90 to 95 per cent of the original number, but usually contained only a few polymorphonuclear leucocytes and platelets.

Endotoxin.—Lipopolysaccharide endotoxin derived from *Escherichia coli*, strain 0111: B4 and *Salmonella typhosa* endotoxin (obtained from Difco Laboratories, Inc., Detroit) were

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used. *E. coli* and *S. typhosa* polysaccharide fractions were kindly supplied by Dr. Anna Marie Staub of the Institut Pasteur. A "toxic lipid fraction" prepared from *E. coli* endotoxin was obtained through the courtesy of Dr. Harvey J. Robinson, Merck and Co., Rahway.

Antigen-Antibody Complexes.—Ovalbumin (twice recrystallized) was obtained from the Worthington Biochemical Corporation, Freehold, New Jersey. Normal albino rabbits of either sex were immunized by repeated weekly intradermal and intravenous injections of this antigen in doses which ranged from 1 to 50 mg. Serum obtained from the immunized ani-

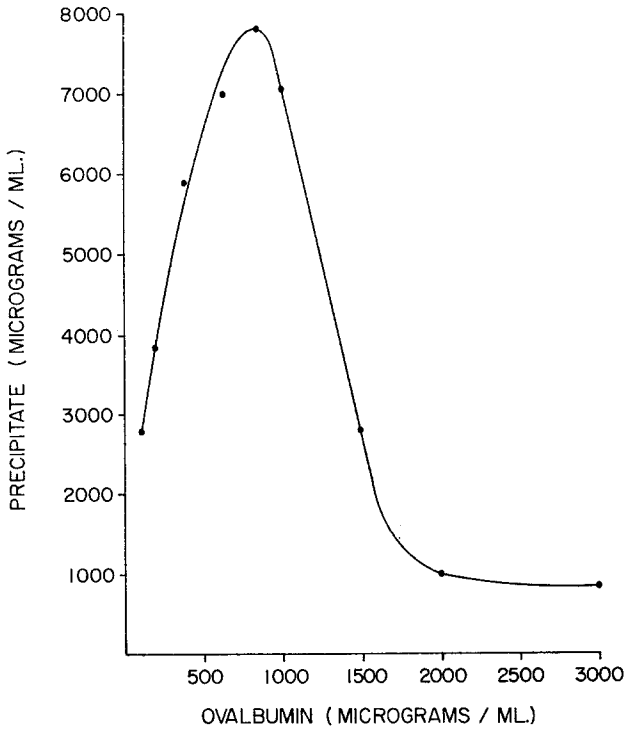

FIG. 1. Antigen titration curve for the antiovalbumin serum used in these experiments. Results are expressed in micrograms of total protein in the immune precipitate obtained by mixing 1.0 ml. of serum with 1.0 ml. of antigen solution at various antigen concentrations.

mals was pooled under sterile conditions and maintained in the frozen state until used. After removal from storage the serum was allowed to come to room temperature and was then inactivated for 30 minutes at 56°C. Antigen titrations were carried out as the first step in an eventual testing of the precipitates and the supernates formed in the antigen-excess and antibody-excess regions of the precipitin curve. Fig. 1 illustrates the precipitin curve obtained with the rabbit antiovalbumin used in these experiments. The equivalence point of the antiovalbumin serum was found to be approximately 0.9 mg. of antigen per ml. of serum. For preparation of antigen-antibody complexes in the region of antigen excess, 1.5 mg. of ovalbumin dissolved in 1.0 ml. of saline was mixed with 1.0 ml. of antiserum.

Polystyrene Latex Particles.—Samples of Dow Lot LS 449-E were obtained from Difco Laboratories, Inc., Detroit. Each particle measured 0.802 microns in diameter, and the stock

suspension contained 8×10^9 particles per ml. For use in the manometric determinations 3 ml. of the concentrated stock suspension were diluted to 10 ml. with Krebs-Ringer solution or physiologic saline solution.

Heat-Denatured Serum Albumin.—A 1 per cent bovine serum albumin solution in physiologic saline solution was heated at pH 7.0 to 75°C. for 20 minutes, and the denatured albumin obtained by this method was then stored at 4°C. until used.

Manometric Determinations.—For measurement of O_2 consumption, the direct method of Warburg was used throughout. 0.2 ml. of 5 per cent KOH was added to the center well of each 15 ml. flask, the fluid phase was 2.0 ml. whole blood (with exceptions noted below) and the gas phase was room air. The Warburg respirometer used subjected the flasks to shaking at 37.5°C. at 156 oscillations/minute. In all the experiments, duplicate or triplicate sets of flasks were used.

After drawing the blood as described above, 2.0 ml. aliquots were immediately pipetted into Warburg flasks and then equilibrated with room air by shaking in the respirometer at 37.5°C. Ten or 15 minutes usually elapsed between venipuncture and subsequent oxygenation of the blood at constant temperature in the Warburg respirometer. Extension of this equilibration period to more than an hour had no effect on the character or magnitude of the reactions to be described.

Thoracic Duct Lymphocytes.—Fresh dog lymph was kindly supplied by Drs. J. H. Jacobson and E. Bergofsky of Presbyterian Hospital, New York. Normal adult mongrel dogs of either sex were placed under general anesthesia, the thoracic duct was cannulated, and, after a steady rate of flow of lymph was established, aliquots were collected through a polyethylene catheter. Lymphocyte counts were performed on the samples, and 2.0 ml. aliquots were pipetted into Warburg flasks for manometric determinations of respiratory rates.

EXPERIMENTAL

Oxygen Uptake of Normal Human Venous Blood.—Fig 2 illustrates the pattern of oxygen uptake by normal human venous blood. After a rapid initial uptake due to the oxygenation of hemoglobin, the oxygen uptake stabilized at 8 to 12 μliters of oxygen per 2.0 ml. of blood per hour and thereafter a steady rate of oxygen consumption was observed for many hours. In order to determine which of the components of blood was concerned with this uptake, whole blood was separated by centrifugation into red blood cell, plasma, and buffy coat fractions, and the oxygen uptake of each of these fractions was measured. Neither plasma alone nor erythrocytes resuspended in plasma exhibited any uptake after the initial period of equilibration. While the buffy coat resuspended in plasma showed some oxygen uptake, this was much reduced in comparison to that of equivalent amounts of whole blood. Evidence that the process of centrifugation and resuspension had damaged the respiratory capacity of the leucocytes was furnished by the finding that the oxygen uptake of leucocytes harvested in their own plasma after spontaneous sedimentation of erythrocytes duplicated faithfully the values obtained with whole blood.

The addition to the center well of $Ca(CN)_2$ (0.64 M) as described by Robbie (5) caused inhibition of the oxygen uptake of whole blood and is consistent with the interpretation that the oxygen consumption observed in these experiments was due to the respiratory activity of the leucocytes of the whole blood samples.

The rate of oxygen uptake of whole blood did not appear to be a simple function of the total leucocyte count or of the total polymorphonuclear leucocyte or lymphocyte counts. While it was not possible to collect, isolate, and test uninjured polymorphonuclear leucocytes, lymphocytes obtained from the blood of normal humans according to the technique of Johnson and Garvin (6) and canine lymphocytes obtained by thoracic duct cannulation were found to take up approximately 6 μliters of oxygen per flask per hour. The numbers of lymphocytes in these lymph samples were approximately the same (2000 to 3000 cells/

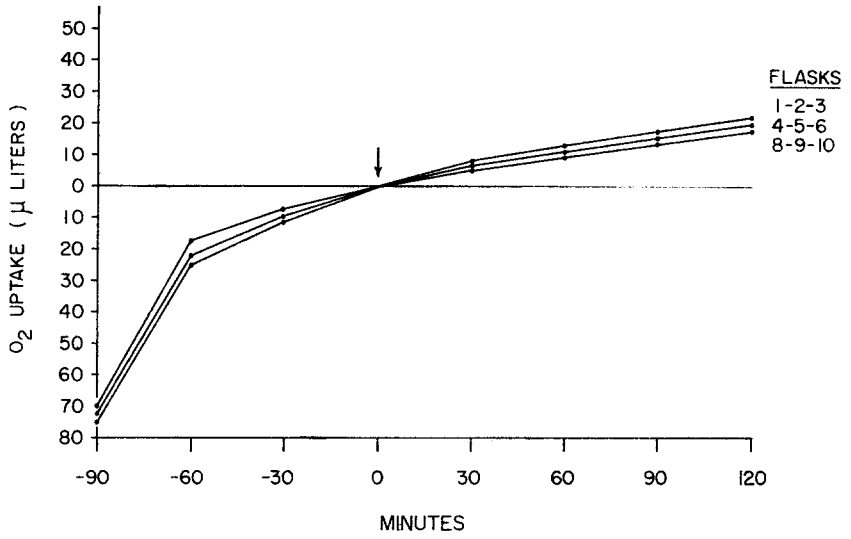

FIG. 2. Oxygen uptake by normal human blood. In this and in the following figures the points represent the average values of triplicate sets of flasks. Each flask contained 2.0 ml. whole blood and 0.2 ml. 5 per cent KOH in the center wells. Blood was collected from a single donor for each experiment. The arrow represents the point at which equilibration had been achieved and at which additions were made from flask sidearms.

mm.^{3}) as the number of lymphocytes in the whole blood samples tested, and it therefore seems likely that a substantial portion of the oxygen uptake of human whole blood reflects the respiratory activity of its lymphocytes.

Examination of stained smears prepared from blood samples removed after 6 or 8 hours in the respirometer revealed intact red blood cells with normal contour or in various stages of crenation. In the leucocyte series, the lymphocytes after incubation appeared similar morphologically to the lymphocytes of freshly drawn blood, but the polymorphonuclear leucocytes were altered: the cytoplasm of the cells was abnormally pale and contained numerous vacuoles while the lobes of the nuclei had become distorted and dense. Platelets tended to agglutinate and were identified after incubation only as amorphous masses.

Effect of Addition of Endotoxin to Normal Human Whole Blood.—Upon addition of endotoxin from the sidearms of the flasks, a striking change in the rate of oxygen uptake occurred (Fig. 3). A marked increase in oxygen uptake oc-

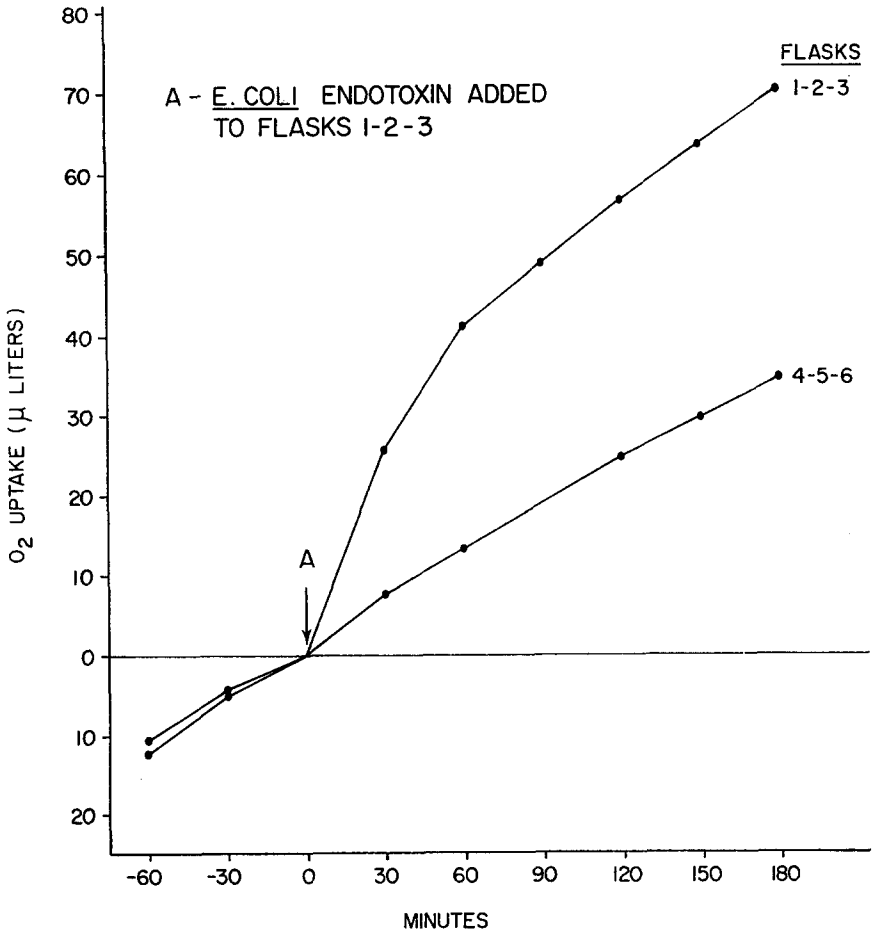

FIG. 3. The effect of addition of *E. coli* endotoxin to normal human blood. The endotoxin was dissolved in 0.2 ml. of Krebs-Ringer solution, placed in the sidearms of the flasks, and tipped into the blood at point *A*. Control flasks (4–5–6) similarly received 0.2 ml. Krebs-Ringer solution.

curred over a period of approximately an hour and a half with most of the extra oxygen being consumed during the 1st hour; by the end of an hour or two the accelerated oxygen consumption had generally subsided. In experiments in which manometric measurements were made at 5 minute intervals during the first 60 minutes after the addition of endotoxin, it was found that during the

first few minutes there existed no appreciable increase in the rate of oxygen uptake, but thereafter the rate of uptake was markedly increased.

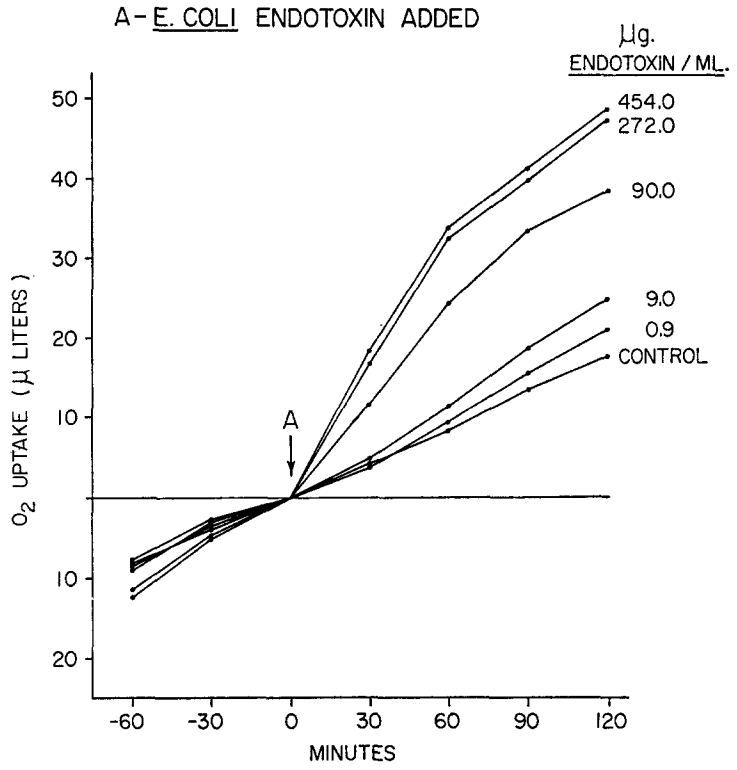

FIG. 4. Dose response of normal human blood to additions of *E. coli* endotoxin. Each experimental flask contained 2.0 ml. whole blood, 0.2 ml. 5 per cent KOH in the center well, and 0.2 ml. Krebs-Ringer solution containing various amounts of endotoxin in the sidearm. At point *A* the contents of the sidearms were tipped into the blood, the control flasks similarly receiving 0.2 ml. Krebs-Ringer solution without endotoxin. The increased rate of oxygen consumption occurring during the next hour is evident, as is the dose-response relationship and the existence of a maximum response beyond which larger doses of endotoxin produced no further effect.

The dose-response relationship was studied by the addition of endotoxin in various concentrations, and the results are illustrated in Fig. 4 and Table I. Some stimulation of respiration occurred even with doses of endotoxin as low as 10^{-7} gm./ml., and it was calculated that at this concentration the system contained on the order of 100 molecules of endotoxin per leucocyte. It was found that blood samples from several donors behaved similarly in a qualitative sense but showed considerable quantitative variation; that is, blood from some donors exhibited a twofold rise in rate of uptake for every tenfold increase in endotoxin

concentration, while blood from other donors showed as much as a fourfold rise in uptake for each tenfold increase in endotoxin concentration. However, the magnitude of response was quite consistent from day to day for any given individual.

The stimulation of oxygen uptake upon the addition of endotoxin was also observed with leucocyte-platelet suspensions in plasma from which the erythrocytes had been allowed to settle out spontaneously, but it was not observed with erythrocytes suspended in plasma or with plasma alone and therefore did not reflect an altered affinity of hemoglobin or plasma proteins for oxygen. It was found that the addition of NaF (in final concentration of 3.7×10^{-3} M) had no appreciable effect on the rate of oxygen consumption by normal whole blood, but completely suppressed the extra oxygen consumption usually produced by the addition of endotoxin; similar results were obtained with iodoace-

TABLE I

Effect of Endotoxin Concentration on Magnitude of Increased Oxygen Uptake

Endotoxin concentration*	No. of determinations	Percentage increase in O_2 uptake at 1 hr. over control values
0.09	3	+12
0.90	9	+25
9.00	9	+68
90.0	9	+192
272.0	3	+247
454.0	3	+262

* Final concentration in μg./ml. blood.

tate. Both of these substances are known to inhibit the glycolysis of leucocytes (7), and these observations further indicate that the observed phenomenon was a reflection of an effect of endotoxin on the carbohydrate metabolism of blood leucocytes.

In these experiments, it was not possible to identify the cell type involved in the increased O_2 consumption. Earlier work (1–4) suggests that the polymorphonuclear leucocytes were probably involved. It seemed unlikely that the lymphocytes of whole blood were responsible for the phenomenon, since human lymphocytes and canine thoracic duct lymphocytes did not respond to the addition of endotoxin with any detectable change in oxygen uptake.

In similar experiments, the oxygen uptake of canine blood and monkey blood was stimulated by the addition of endotoxin. While guinea pig blood also responded, the magnitude of the response was small. In marked contrast, no detectable change in O_2 uptake occurred when endotoxin was added to rabbit, bovine, rat, or chicken blood. The possible significance of this species variation will be discussed below.

The most likely explanation of the phenomenon is that it represents a stimulation of the respiration of polymorphonuclear leucocytes analogous to that reported earlier (1, 2, 4). Two chief possibilities exist to account for such a stimulation of respiration. The first is an increased rate of utilization of glycogen or other intracellular substrates while a second possibility is that plasma glucose or other exogenous substrate is transported into the cells or utilized by them at a more rapid rate. The first of these seemed attractive, since the amount of glycogen in normal human polymorphonuclear leucocytes is of the proper order of magnitude (1.93 to 6.2 mg. per leucocytes of 100 ml. of blood (8)) to account for the increased oxygen consumption observed. However, in preliminary experiments (Table II) no detectable decrease in leucocyte glycogen was found after incubation with endotoxins. The alternative hypothesis, that there is an

TABLE II

Glycogen Content of Leucocytes

The results are expressed as milligrams of glycogen per leucocytes of 100 ml. of whole blood. Endotoxin was added to a final concentration of 100 μg./ml. blood and the mixture was incubated at 37°C. with shaking for 1 hour. The data do not indicate that appreciable glycogen breakdown occurred under these conditions which were essentially those under which the manometric determinations were carried out, although the possibility is not excluded that initial breakdown and resynthesis occurred, as in the experiments of Cohn and Morse (1).

Experiment No.	Before incubation with endotoxin	After incubation with endotoxin
1	2.26	2.08
2	2.84	3.06
3	3.03	3.33

increased utilization of glucose or other substrate from plasma, is being explored with isotopic techniques.

In all of the experiments described thus far, heparin in a final concentration of 3 units per ml. was used to prevent coagulation. Larger amounts of heparin (up to 60 units per ml. of blood) were found to inhibit oxygen uptake, perhaps owing to the toxic effect of this material on the respiring cells. When sodium citrate or ethylenediaminetetraacetate was added to human blood in anticoagulant amounts, or when the blood was first passed through a fenwal ion-exchange column, the characteristic increase in oxygen uptake seen upon subsequent addition of endotoxin failed to occur. Although it seemed likely that the binding of calcium or other divalent cations was responsible for the effect of these agents, restoration of calcium and/or magnesium prior to tipping endotoxin into the system did not result in restoration of the effect seen with normal whole blood. On the other hand, blood defibrinated by shaking with glass beads did exhibit an increased oxygen uptake upon the addition of endotoxin, although the magnitude of this increase was relatively small.

It was noted incidentally that the heparinized blood in the flasks containing endotoxin clotted over several hours after the close of an experiment, whereas blood in the control flasks remained fluid. This observation may be related to the tendency of heparinized whole blood samples from rabbits which had

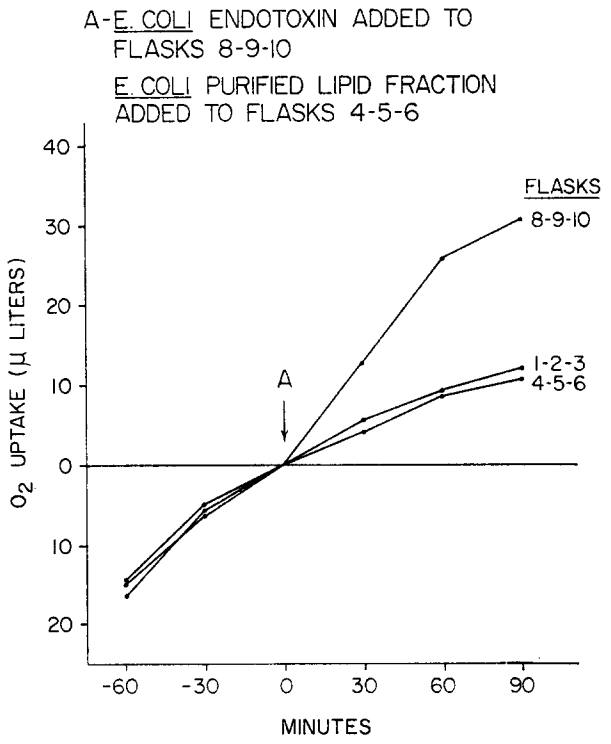

FIG. 5. Effect of addition of *E. coli* purified lipid fraction to normal human blood. In this experiment, after suitable equilibration, into flasks 8-9-10 was tipped the complete *E. coli* lipopolysaccharide endotoxin (90 μg. per ml. of blood), while flasks 4-5-6 each received 0.2 ml. (100 μg.) of the lipid fraction alone. Flasks 1-2-3 served as controls, receiving only Krebs-Ringer solution. The purified toxic lipid had no effect on leucocyte respiration.

been given intravenous injections of endotoxin to clot more readily than the blood of normal control rabbits (9).

Since the complex macromolecules of bacterial endotoxins can be degraded and fractionated to yield an antigenic but non-toxic polysaccharide and a toxic lipid (10), it was of interest to learn whether such fractions exerted an effect on the respiration of leucocytes. Neither the purified toxic lipid of *E. coli* endotoxin nor the purified polysaccharide fraction of *S. typhosa* endotoxin caused an increase in oxygen consumption above control levels when added to whole blood, whereas the addition of the complete lipopolysaccharide endotoxin

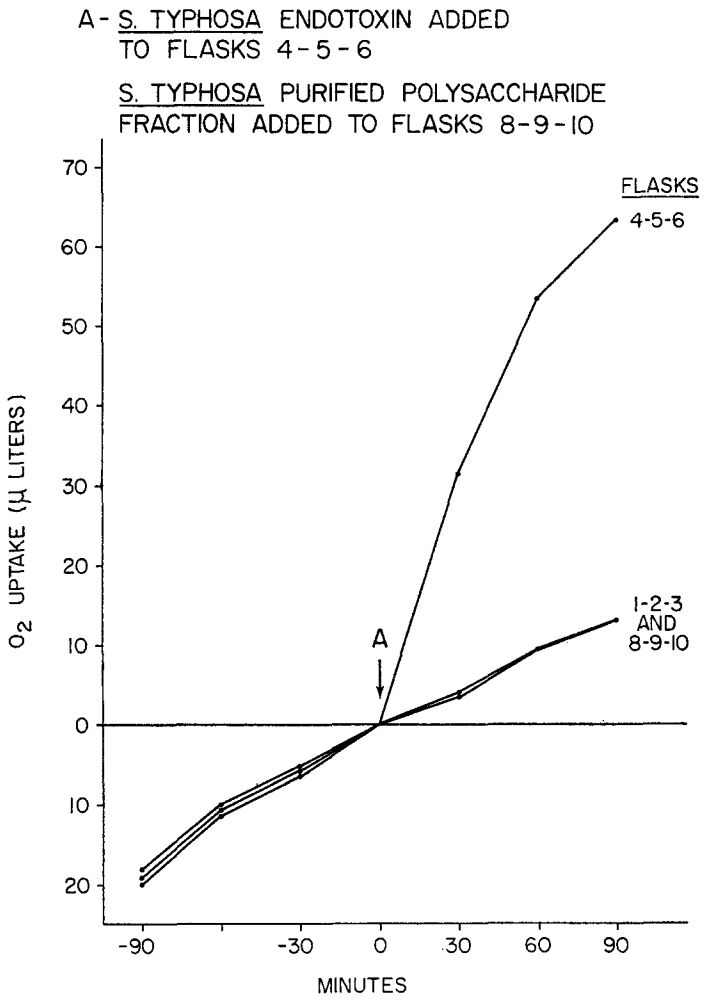

FIG. 6. Effect of addition of *S. typhosa* purified polysaccharide fraction to normal human blood. The experimental conditions are similar to those of Fig. 5; however, the complete endotoxin lipopolysaccharide in this case was derived from *S. typhosa*, as was the polysaccharide used. The final concentration of each was 90 μg./ml. whole blood, and the polysaccharide failed to produce an increase in leucocyte respiration.

was in each instance accompanied by a marked increase in oxygen consumption. These data are illustrated in Figs. 5 and 6 respectively.

The Effect on Oxygen Uptake of Whole Blood of Addition of Antigen-Antibody Complexes and Particulate Material.—It has recently been found that the coagulation mechanism is affected *in vitro* by the addition of soluble antigen-antibody complexes and that a similar effect can be produced by endotoxins, perhaps by virtue of the reactions of these antigens with "natural antibodies"

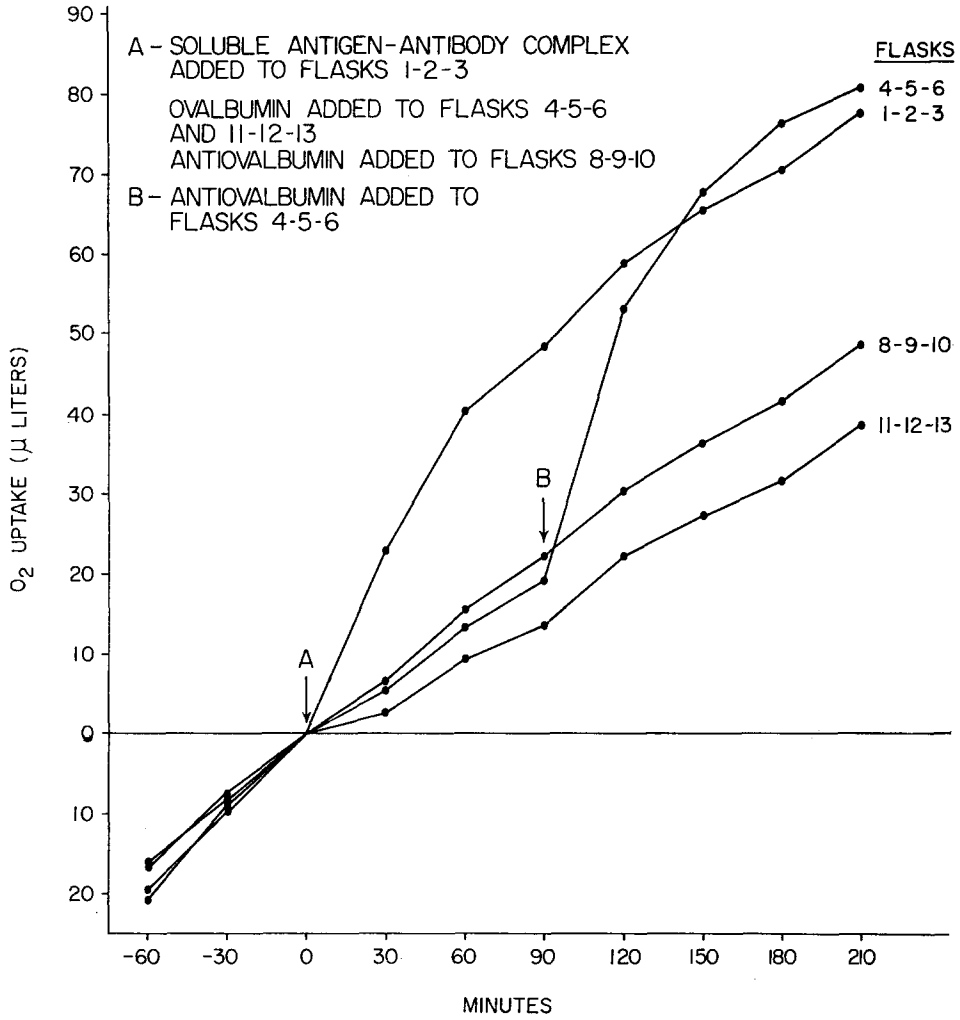

FIG. 7. Stimulation of oxygen uptake by antigen-antibody complexes. The flasks each contained 2.0 ml. normal human blood, with 0.2 ml. of 5 per cent KOH in the center wells, and 0.2 ml. reagent in the sidearms. The sidearms in flasks 1-2-3 contained 0.2 ml. of the supernatant of an ovalbumin-antiovalbumin mixture prepared in antigen excess. Flasks 4-5-6 were double sidearm vessels, with 0.2 ml. undiluted antiovalbumin rabbit serum in one sidearm and 0.2 ml. ovalbumin (1.5 mg./ml. Krebs-Ringer solution) in the other. Sidearms of flasks 8-9-10 contained 0.2 ml. undiluted antiserum and those of flasks 11-12-13 contained 0.2 ml. ovalbumin (1.5 mg./ml.).

in the test system (11). It was therefore considered possible that the phenomenon under study might represent an antigen-antibody effect, and to this end the effect of the addition of antigen-antibody complexes to human whole blood was studied. It was found that soluble ovalbumin-antiovalbumin com-

plexes prepared in antigen excess produced an increase in oxygen uptake of familiar nature and degree (Fig. 7). It will be observed that blood samples to which rabbit antiserum or ovalbumin alone had been added maintained a

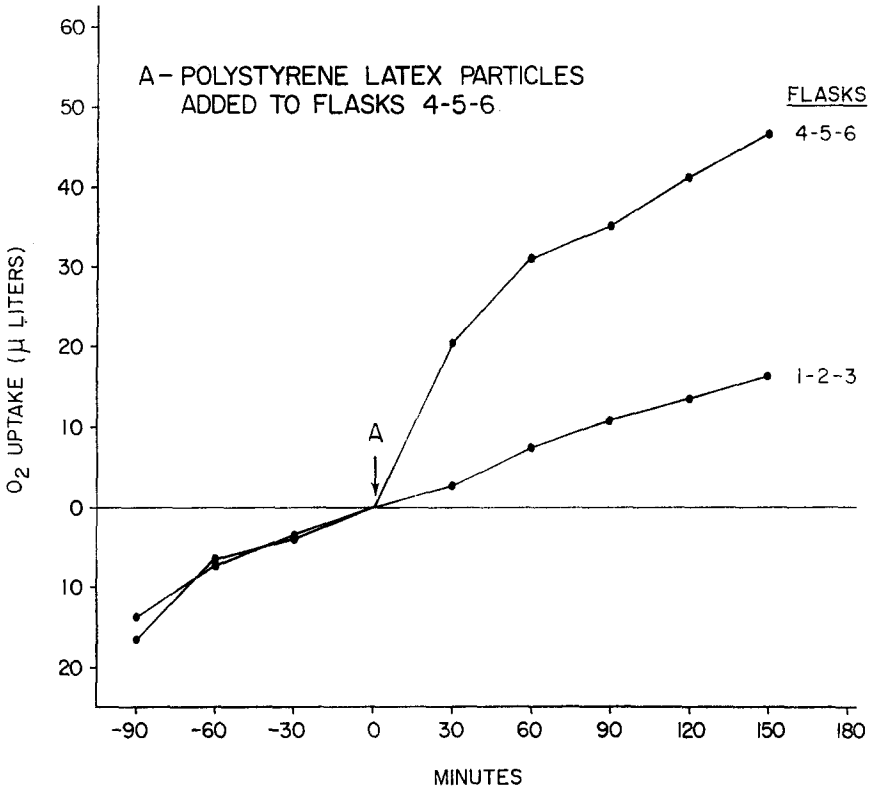

FIG. 8. Effect of addition of polystyrene latex particles to normal human blood. The stock solution of polystyrene latex particles was diluted with either normal saline or Krebs-Ringer solution, so that there were 2.4×10^9 particles per ml. of solution. 0.2 ml. of this diluted material were added to 2.0 ml. of blood and thus the final concentration of latex was 4.8×10^7 particles per ml. of blood. The white blood count was 8400 per $mm.^3$ or 8400×10^6 cells per ml. of blood, and was the equivalent of 5.7 particles per white blood cell. Compare this figure with Fig. 3.

steady rate of oxygen uptake, unaltered by the addition of these heterologous proteins. In marked contrast was the sudden change in oxygen uptake by blood samples to which had been added soluble antigen-antibody complexes. The rate of uptake was considerably accelerated during the 1st hour, after which the rate returned to the normal control level. When antiserum was subsequently added to flasks containing antigen, a similar effect was produced.

Active phagocytosis is known to be an energy-requiring phenomenon and

an increased leucocytic oxygen uptake has been shown to be a reflection of the metabolic requirements for phagocytosis of particulate or macromolecular material (1–4). Accordingly, two types of particles, heat-denatured serum albumin and polystyrene latex, were added to human whole blood. Heat-denatured albumin when added in final concentration of 1.0 mg./ml. of blood failed to result in any increase in oxygen consumption above control values.

TABLE III

The Effect of The Addition of Latex Particles on the O_2 Uptake of Human Whole Blood in The Presence of Metabolic Inhibitors

All blood samples in these experiments were taken from the same donor. Blood was added to a test tube containing the appropriate concentration of 2,4-dinitrophenol, NaF, or iodoacetate, the contents mixed, and the blood was pipetted into the Warburg vessels. In each experiment, four sets of triplicate flasks were used. The first three sets contained 2.0 ml. normal blood in the main compartment and 0.2 ml. of normal saline, inhibitor, or latex respectively in the sidearms. The fourth set carried blood preincubated with the specific metabolic inhibitor in the main compartment and latex in the sidearms.

Experiment No.	WBC	Inhibitor	Concentration in flasks	μliters of O_2 taken up at 5 hr.			
				Blood alone	Blood plus added inhibitor	Blood plus added latex	Blood incubated with inhibitor; then latex added
			M				
1	6850*	2,4-dinitrophenol	1×10^{-4}	6.0	7.4	40.9	34.8
2	8900	Sodium fluoride	2×10^{-2}	8.9	13.0	37.4	7.6
3	8000	Sodium fluoride	2.87×10^{-3}	10.9	10.9	39.5	29.0
4	6340	Sodium fluoride	3.64×10^{-4}	8.2	11.2	31.8	26.6
5	8260	Iodoacetate	3.64×10^{-4}	9.7	6.6	34.7	10.0
6	10,350	Iodoacetate	2.57×10^{-4}	8.4	7.4	30.1	11.7
7	6600	Iodoacetate	1×10^{-4}	7.9	6.3	39.6	15.8

* Total leucocytes per c. mm. blood.

While it is known that aggregates of denatured albumin are phagocytosed by macrophages (12–14), it has not yet been established that the polymorphonuclear leucocytes of the blood can phagocytose this material. However, it will be seen in Fig. 8 that the addition of polystyrene latex particles to human whole blood produced a response which was qualitatively and quantitatively similar to that produced by endotoxin or antigen-antibody mixtures. It should be noted in passing, that the magnitude of this effect was many fold greater than that reported to have occurred when guinea pig exudate leucocytes were chosen as the test cells (4). Microscopic examination of smears prepared from

flask contents revealed active phagocytosis of the latex particles by the polymorphonuclear leucocytes.

Table III illustrates the effect of various metabolic inhibitors on the response to the addition of latex particles to normal human blood. Both NaF and iodoacetate suppressed the response, while 2,4-dinitrophenol was without effect in the concentration tested. Microscopic examination of samples from these flasks revealed that phagocytosis of latex particles had not occurred in flasks which showed inhibition of oxygen uptake. The significance of these observations will be discussed below.

Addition of latex particles to heparinized rabbit whole blood failed to result in an increase in oxygen uptake. As described above, rabbit blood also had consistently failed to exhibit an increase in oxygen consumption in the presence of endotoxin or antigen-antibody complexes. Examination under the phase microscope of heparinized rabbit and human whole blood to which had been added polystyrene latex particles revealed rapid ingestion of particles by actively ameboid polymorphonuclear leucocytes in the case of human blood, but ingestion of only a few particles by the leucocytes of rabbit blood. In neither instance did lymphocytes ingest particles.

DISCUSSION

In the present study, the leucocytes of the circulating blood have been shown to undergo a marked change in metabolic behavior in the presence of particulate and macromolecular material. It seems likely that the effects observed are analogous to those described earlier for guinea pig (4) and rabbit (1, 2) exudate leucocytes.

The marked stimulation of respiration described represents a sensitive method for demonstration of an effect of endotoxin and antigen-antibody complexes on living cells *in vitro*. The reactivity of leucocytes to these substances appears to involve a relatively fragile mechanism, since the capacity to react is lost upon centrifugation and resuspension of these cells. Since the phenomenon was produced under relatively physiologic circumstances in these experiments, it may be that a similar effect occurs *in vivo*. It will be of interest to further study the relationship of this phenomenon to various *in vivo* and *in vitro* immune reactions.

While these experiments were in progress the studies of Sbarra and Karnovsky (4) appeared reporting the stimulation of oxygen uptake by guinea pig exudate leucocytes during the phagocytosis of inert particles. They concluded that the ingestion of particles requires energy and that this energy is provided by glycolysis. The present experimental findings differ in some respects from those of Sbarra and Karnovsky (4). Thus, these authors found that NaF (2×10^{-2} M) resulted in a greatly increased respiration of resting (control) exudate leucocytes, while in the present experiments with whole blood

only a small increase was observed. Guinea pig exudate leucocytes exposed to NaF (2×10^{-2} M) in the presence of added latex particles exhibited a further increase in O_2 uptake (4) while whole blood incubated with NaF failed to respond to subsequent additions of either antigen-antibody complexes or latex particles with the expected increase in oxygen uptake. The human blood leucocytes and guinea pig exudate leucocytes also apparently differ in their response to 2,4-dinitrophenol.

A particularly interesting possibility is that the phenomenon under study may be a reflection of the energy-yielding process postulated by Mongar and Schild (15) as being involved in the release of histamine. Several considerations seem to make this hypothesis worth investigation. For example, all or nearly all of the histamine of human, dog, and monkey is found in the leucocytes (16–18); in contrast, the histamine of rabbit blood is found predominantly in the platelets, and under the influence of shaking or pipetting there is an almost quantitative release of platelet histamine into the plasma (16). The presence of the major portion of the blood histamine in the leucocytes of man, dog, and monkey correlates roughly with the marked effect described above with the blood of these species; the distribution of histamine between leucocyte and platelet fractions in the guinea pig correlates roughly with the intermediate reactivity of its blood; while in the rabbit, in contrast, virtual absence of histamine from the leucocytes is correlated with the lack of response to endotoxin and other test materials. Finally, the fact that oxygen lack and metabolic inhibitors such as iodoacetate block the release of histamine from guinea pig lung in anaphylaxis as described in the *in vitro* experiments of Mongar and Schild (15), indicating that an energy-requiring process is involved in histamine release, is pertinent here. While release of histamine was not measured in the experiments described in this report, increased oxygen consumption by leucocytes in the presence of endotoxin was blocked by both iodoacetate and fluoride ions, both of which interfere with aerobic glycolysis. The experiments described have all been carried out under conditions in which histamine is known to be liberated, and it will be of considerable interest to determine the kinetic relationships between the release of histamine into the plasma and the increased respiratory activity of the cells.

It is possible that a common denominator exists in the interaction between human leucocytes and the various macromolecular materials tested, in that gamma globulin may in each case be required to opsonize the macromolecule or particle for ingestion. Endotoxin molecules may interact as antigens with natural antibodies in the test system, while polystyrene latex particles are known to adsorb gamma globulin non-specificially to their surfaces. In preliminary experiments, it has been found that the oxygen uptake of the blood of patients with agammaglobulinemia is only weakly stimulated by the addition of polystyrene latex particles, while the addition of latex particles previously

incubated with human gamma globulin is followed by an increase in oxygen consumption entirely comparable to that seen with normal blood. Furthermore, these globulin-coated latex particles produce a distinct increase in the oxygen uptake of rabbit blood, which failed to react to additions of uncoated particles or of human gamma globulin alone. This effect of gamma globulin is currently the subject of an investigation, the results of which will be reported subsequently.

SUMMARY

The oxygen uptake of lightly heparinized human blood was found to increase markedly upon the addition of bacterial endotoxins, soluble antigen-antibody complexes, or polystyrene latex particles. The effect apparently reflects a transitory stimulation of respiratory activity of the blood leucocytes.

The effect did not occur in the presence of anticoagulant amounts of citrate or ethylenediaminetetraacetate, was inhibited by iodoacetate and fluoride ions, and may be related to the energy-yielding processes involved in histamine release or phagocytosis.

BIBLIOGRAPHY

1. Cohn, Z. A., and Morse, S. I., Functional and metabolic properties of polymorphonuclear leucocytes. I. Observations on the requirements and consequences of particle ingestion, *J. Exp. Med.*, 1960, **111,** 667.
2. Cohn, Z. A., and Morse, S. I., Functional and metabolic properties of polymorphonuclear leucocytes. II. The influence of a lipopolysaccharide endotoxin, *J. Exp. Med.*, 1960, **111,** 689.
3. Baldridge, C. W., and Gerard, R. W., The extra respiration of phagocytosis, *Am. J. Physiol.*, 1933, **103,** 235.
4. Sbarra, A. J., and Karnovsky, M. L., The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leucocytes, *J. Biol. Chem.*, 1959, **234,** 1355.
5. Robbie, W. A., The use of cyanide in tissue respiration studies, *Methods Med. Research*, 1948, **1,** 307.
6. Johnson, J. M., and Garvin, J. E., Separation of lymphocytes in human blood by means of glasswool column, *Proc. Soc. Exp. Biol. and Med.*, 1959, **102,** 333.
7. Martin, S. P., McKinney, G. R., and Green, R., The metabolism of human polymorphonuclear leucocytes, *Ann. New York Acad. Sc.*, 1955, **59,** 996.
8. Valentine, W. N., Follette, J. H., and Lawrence, J. S., The glycogen content of human leucocytes in health and various disease states, *J. Clin. Inv.*, 1953, **32,** 251.
9. Thomas, L. Physiologic and pathologic alterations produced by the endotoxins of gram-negative bacteria, *A. M. A. Arch. Int. Med.*, 1958, **101,** 452.
10. Westphal, O., and Luderitz, O., Chemische erforschung von lipopolysacchriden gram negativer backterien, *Angew, Chem.*, 1954, **66,** 407.
11. Robbins, J., and Stetson, C. A., Jr., An effect of antigen-antibody interaction on blood coagulation, *J. Exp. Med.*, 1959, **109,** 1.

12. Benacerraf, B., Halpern, B. N., Stiffel, C., Cruchaud, S., and Biozzi, G., Phagocytose d'une fraction serum chauffe et iode par le systeme reticuloendothelial et comportment consecutif de ses cellules a l'egard d'autres colloides, *Ann. Inst. Pasteur*, 1955, **89,** 601.
13. Biozzi, G., Halpern, B. N., Benacerraf, B., Stiffel, C., and Mouton, D., Influence de la quantite d'iode fixee sur les proteins seriques normales et modifies par la chaleur sur la phagocytose de ces colloides par les cellules du s.r.e., *Ann Inst. Pasteur*, 1957, **92,** 89.
14. Benacerraf, B., Biozzi, G., Halpern, B. N., Stiffel, C., and Mouton, D. Phagocytosis of heat denatured human serum albumin labelled with I^{131} and its use as a means of investigating liver blood flow, *Brit. J. Exp. Path.*, 1957, **38,** 35.
15. Mongar, J. L., and Schild, H. O., Inhibition of the anaphylactic reaction, *J. Physiol.*, 1957, **135,** 301.
16. Code, C. F., The histamine content of white blood cells, *in* Blood Cells and Plasma Proteins, Their State in Nature, (J. L. Tullis, editor), New York, Academic Press, Inc., 1953.
17. Lowry, O. H., Graham, H. T., Harris, F. B. Priebat, M. K., Marks, A. R., and Brigman, R. U., The chemical measurement of histamine in blood plasma and cells., *J. Pharmacol. and Exp. Therap.*, 1954, **113,** 116.
18. Humphrey, J. H., and Jacques, R., The histamine and serotonin content of the platelets and polymorphonuclear leucocytes of various species, *J. Physiol.*, 1954, **124,** 305.