ethyl nortestosterone ester had a value very close to these (3.74), and that the 2 testosterone esters had practically identical values to each other (3.96, 4.02) and to the 3 nortestosterone values. Thus, deletion of the methyl group, presence of the 17-ethyl group, esterification at the 17 position, esterification at both the 3 and 17 positions and the nature of the esterifying groups did not seem to affect the common action of these compounds which is being expressed as an increase in uptake of the labelled amino acid. What proportion of uptake of the AIB is attributable to the anabolic effect and what proportion to the androgenic effect of these steroids cannot be deduced from these data. Nevertheless, the degree of agreement among the 5 esterified compounds suggests that the various compounds have the same effect on the uptake or concentrating mechanism or that their metabolic breakdown results in a common compound causing the effect noted.

Summary. 1. A test for determination of the anabolic potential of steroids using increase in uptake of a C^{14} labelled non-metabolizable amino acid by the levator ani muscle of the rat is described. 2. This test is considerably more rapid than the 2 standard levator ani weight increment tests. Reproducibility of the method within a given test is quite good as is reproducibility in serial tests with a given steroid. The sensitivity of this test is 5- to 7-fold that of the weight increment test. 3. The reduction in urinary AIB excretion observed even before any nitrogen retention becomes established suggests the possibility of using this reduction as an aditional or alternate indicator of the anabolic activity of steroids. 4. The close agreement in uptake ratios with 5 different steroid esters suggests that the metabolic product ultimately effective in causing the increase in uptake of the labelled compound is the same or that the various steroids have the same effect on the uptake mechanism.

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Effect of Size and Concentration of Latex Particles on Respiration of Human Blood Leucocytes.* (26743)

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Recent workers on physical and biochemical aspects of phagocytosis have used suspensions of polystyrene latex particles as the test substance(1,2). Using guinea pig exudate leucocytes and polystyrene latex particles 1.171 μ in diameter, Sbarra and Karnovsky concluded that phagocytosis requires energy, and that the oxygen consumed by the test cells is directly proportional to the number of particles ingested by them(2). Strauss and Stetson have noted the effect of 0.802 μ polystyrene latex particles and solutions of endotoxins of Gram negative bacteria on the respiratory activity of leucocytes of human peripheral blood(1).

The present paper describes effect of size and concentration of polystyrene latex particles upon oxygen consumption of leucocytes of heparinized human peripheral blood.

Materials and methods. Blood. Normal human peripheral blood was drawn and heparinized as described elsewhere(1). Blood

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TABLE I. Physical Properties of Suspensions of Polystyrene Latex Particles.

	Dow Lot #	Size of particle (μ)	Vol of particle (ml)	% solids by wt	s.g. of suspension	Concentration particles/ml
1	LS-040A	.088	3.61×10^{-16}	9.42	.982	2.66×10^{14}
2	LS-055A	.188	3.48×10^{-15}	10.30	.966	3.06×10^{13}
3	LS-057A	.264	9.63×10^{-15}	10.20	.964	1.10×10^{13}
4	LS-061A	.365	2.55×10^{-14}	9.99	.962	4.07×10^{12}
5	LS-063A	.557	9.05×10^{-14}	12.91	.964	1.48×10^{12}
6	LS-066A	.814	2.84×10^{-13}	11.20	.984	4.00×10^{11}
7	LS-067A	1.171	8.43×10^{-13}	10.26	.956	1.27×10^{11}

from one donor was used for all experiments in the interest of consistent quantitative responses. However, various donors responded similarly in a qualitative sense. *Polystyrene latex particles.* Samples of 7 graded sizes of particles were obtained from Dow Chemical Co., Midland, Mich., through the courtesy of Dr. John W. Vanderhoff. Each sterile sample contained particles of uniform diameter suspended in a buffered saline solution, and was stored at 4°C. From data supplied by Dow Chemical Co., the concentration of each stock suspension was calculated§ (Table I). However, investigators should be aware that suspensions must not be used after storage over many months, for there will be fewer particles present than originally, owing to destructive action by bacteria which have gained entry when vials are opened to withdraw samples. Different concentrations of latex suspensions were prepared by dilution with physiologic saline. *Manometric determinations.* Oxygen consumption was measured by the direct method of Warburg, and as in previous experiments(1) 2 ml whole heparinized blood was used per flask. 0.2 ml latex suspension was pipetted into the sidearm of triplicate sets of flasks. After a 90 minute equilibration period, the suspensions were added. The value at 30 minutes after addition is used for interpretation because recent investigations(1,3,4) suggest that the significant portion of the phagocytic event occurs within this time period.

Results. Series A: Different sized particles were studied in a final concentration of 6.35 $\times 10^{10}$ particles/ml. Control values for blood without latex were constant at 7 μl $\pm$ 0.5 μl oxygen uptake per 2 ml of blood at 30 minutes. Stimulation of oxygen uptake was directly proportional to the size of the polystyrene latex particle within the range 0.264 μ to 1.171 μ. Particles smaller than 0.264 μ produced no significant increment in leucocyte respiration (Table II).

Series B: Experiments were designed to determine the relationship between concentration and oxygen uptake for 3 different sizes of particles and also to confirm observations of series A (Table III). When size was held constant, increase in oxygen uptake was directly proportional to the concentration of the three sizes of particles tested. On the other hand when concentration of particles was held constant, larger particles result in a greater increase in oxygen uptake over control values, as in series A.

Further experiments using particles 0.088 μ, 0.188 μ and 0.264 μ have shown no stimulation of respiration by the first 2 sizes, and erratic stimulation of respiration by the last size, even when undiluted stock suspensions were tested.

Discussion. Polystyrene latex particles

§ $$\frac{\text{No. particles}}{\text{ml}} = \frac{\text{g solids}}{\text{ml suspension}} \times \frac{1}{\text{s.g. suspension}} \times \frac{1}{\text{vol each particle}}$$

TABLE II. Effect of Size of Particles upon Leucocyte Respiration. Concentration constant at 6.35 $\times 10^{10}$ particles/ml.

Group	No. of determinations	Size of particle (μ)	μl increase in O_2 uptake/2 ml blood over control values at 30 min.
1	2	.088	.5 $\pm$ .7
2	2	.188	.5 $\pm$ .7
3	2	.264	3.3 $\pm$ 1.1
4	3	.365	7.4 $\pm$ 2.4
5	3	.557	12.3 $\pm$ 1.3
6	3	.814	38.8 $\pm$ 1.4
7	2	1.171	46.6 $\pm$ 3.5

TABLE III. Effect of Concentration of Particles upon Leucocyte Respiration for 3 Sizes of Particles.*

Group	Size of particle (μ)	μl increase in O_2 uptake over control values at 30 min. Concentration particles/ml, 12.7×10^{10}	Concentration particles/ml, 6.35×10^{10}	Concentration particles/ml, 1.05×10^{10}
1	.365	13.3 ± 1.1	7.4 ± 1.7	1.5 ± 1.9
2	.814	45.7 ± 3.7	37.5 ± 4.7	8.8 ± 2.3
3	1.171	47.7 ± 2.3	48.0 ± 5.0	13.9 ± 1.4

* Each oxygen uptake value represents mean of 3 experiments.

possess physical properties which lend themselves to investigation of the mechanics of phagocytosis. They have been characterized as inert(2). They are available in rigidly standardized suspensions containing particles highly uniform in diameter. With the exception of the 3 smallest sizes they are easily visible with the phase microscope.

Preliminary phase microscopy observations have not resolved the problem of how many latex particles enter the leucocytes. It is extremely difficult to count the number of particles within cells owing to the presence of optically similar cytoplasmic constituents. While Table III shows a direct proportionality between particle concentration and respiration for particles of 3 selected sizes, the failure of the 2 smallest sizes of particles to be associated with significant increases in oxygen uptake by leucocytes probably indicates that little or no phagocytosis of these particles occurred. Thus a critical particle size is defined in this system; below it, addition of particles to whole blood regardless of particle concentration fails to stimulate leucocyte respiration. This may explain earlier results in which heat denatured bovine albumin failed to stimulate leucocyte respiration (1).

Latex particles have proved useful in quantitating some mechanical aspects of phagocytosis. The information gained in regard to size and concentration now lends itself to the study of factors involved in the phagocytic event which are not strictly mechanical in nature. The system described permits further studies in phagocytosis in which the role of certain opsonic factors may be assessed; latex particles coated with various globulin fractions are being used at present to delineate this role.

Summary. Rigorously standardized polystyrene latex particles of graded sizes were added to whole blood, and oxygen uptake by leucocytes as they ingested these particles was measured by the direct method of Warburg. The importance of both size and concentration of particles was established.

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