SUMMARY

1. The yields of monosaccharides obtained by the hydrolysis of extracts of oat-coleoptile cell-wall material are reported. The material has a high content of hot water-insoluble arabinose, probably mainly derived from arabinoglucuronoxylans.

2. Changes in amounts of monosaccharides that occur during elongation of isolated oat-coleoptile cylinders were determined. When sugar was supplied in the medium, a general increase in cell-wall monosaccharides occurred. When elongation occurred without sugar, a decrease in non-cellulosic glucose and galactose and an approximately compensating increase in α-cellulose glucose was observed.

The investigation was begun at the Low Temperature Research Station, Cambridge, while the author was a member of the Society of Fellows, Harvard University. I thank Dr L. W. Mapson and Dr F. A. Isherwood for providing facilities and for advice and help. Subsequent work was supported by National Science Foundation Grant G-8705. I thank Miss Rosalie Hunt, Mr D. Rottenberg and Mr A. Ruesink for technical assistance.

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Particle Uptake by Polymorphonuclear Leucocytes and Ehrlich Ascites-Carcinoma Cells

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It is now well known that the rate of oxygen uptake by guinea-pig polymorphonuclear leucocytes is accelerated by the presence of particles that are engulfed by these cells; for example, insoluble starch particles, polystyrene-latex spherules, or particles of carbon and sulphur (Sbarra & Karnovsky, 1959; Iyer, Islam & Quastel, 1961), or bacteria such as *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Escherichia coli* (Stähelin, Suter & Karnovsky, 1956*a*, *b*; Iyer *et al.* 1961). It is also known that the phagocytic stimulation of respiration by the particles taken up is proportional to the amount of particles present [e.g. with starch (Sbarra & Karnovsky, 1959) or with killed *E. coli* (Iyer *et al.* 1961)] until a maximum value is reached. According to Iyer *et al.* (1961), phagocytic stimulation of metabolism is proportional, within certain limits, to the amount of dead bacteria engulfed by the leucocytes and to the number of leucocytes used. It was suggested that the process of phagocytic stimulation might be used for an assay method for phagocytosis.

However, since phagocytosis takes place under anaerobic conditions as well as aerobically, and the presence of substances that suppress respiration may be without effect on the phagocytic process, it is unlikely that a satisfactory assay of the extent of phagocytosis can be based solely on measure-

ments of the rates of oxygen consumption by phagocytizing leucocytes.

It is essential for the further study of the mechanisms involved in phagocytosis to have a reliable method for the measurement of the quantity of particles engulfed by phagocytes. So far, most determinations have been based on direct counts of particles taken up, a procedure both tedious and inaccurate.

We have used a new method for the assay of phagocytosis. This is based on the uptake of polystyrene particles by phagocytes, extraction of the polystyrene from the washed cells with dioxan and the determination of the polystyrene with a Beckman spectrophotometer. The method gives a reliable assay of the amount of polystyrene taken up.

The present paper is concerned with a description of this technique and of some of the results that have been obtained with it.

EXPERIMENTAL

Materials

Bovine γ-globulin (fraction II) was obtained from Nutritional Biochemicals Corp. The polystyrene spherules were kindly supplied by the Dow Chemical Co. The spherule suspensions were dialysed against water and then stored in aqueous suspension.

Methods

Polymorphonuclear leucocytes were prepared by injecting guinea pigs intraperitoneally with 20 ml. of 12% (w/v) sodium caseinate and collecting the peritoneal exudates 17–20 hr. later (Iyer *et al.* 1961). Microscopic examination of this preparation showed the following differential count: neutrophils (mature), 74%; neutrophils (immature), 10%; eosinophils, 7%; monocytes, 4%; lymphocytes, 5%.

Ehrlich ascites-carcinoma cells were grown in Swiss white mice after intraperitoneal injection. Ascitic fluid was withdrawn with a hypodermic syringe after 6 days of growth and the cells were separated by centrifuging (Tenenhouse & Quastel, 1960). They were washed three or four times with 8–10 vol. of cold 0·9% NaCl solution to remove blood elements and soluble ascitic constituents. They were diluted with 60 times their packed volume of Ringer medium (composed as follows: NaCl, 120 mM; KCl, 4·9 mM; $CaCl_2$, 1·7 mM; $MgSO_4$, 1·2 mM; KH_2PO_4, 2·7 mM; Na_2HPO_4, 13·3 mM) containing glucose (10 mM).

Human leucocytes were isolated from the blood of a patient with acute leukaemia by differential centrifuging of whole heparin-treated blood. The leucocytes were washed twice with Ringer medium from which $CaCl_2$ had been omitted, and were then suspended in Ringer medium containing glucose (10 mM).

Incubation and extraction techniques. The phagocytosis experiments were carried out in 25 ml. Erlenmeyer flasks immersed in a temperature-controlled water-bath shaker at 37°. Each flask usually contained 5 ml. of Ringer medium, glucose (10 mM), 10 mg. of γ-globulin, 50 mg. wet wt. of cells, and polystyrene particles where indicated. The incubation was carried out for 20 min. At the end of the experiment, the cells were collected by centrifuging at 2000 *g* for 2 min., resuspending in 0·9% NaCl solution and recentrifuging. The washing procedure was repeated two or three times. Subsequently, no polystyrene could be detected in the washings. The cells were extracted with 5 ml. of hot dioxan for 10 min. and then allowed to stand at room temperature for 2 hr. in contact with the dioxan. The cells were removed by centrifuging and the extracted polystyrene was measured with a Beckman DU spectrophotometer in quartz cuvettes (1 cm. light-path) at 253 mμ. The controls were dioxan extracts of cells containing no polystyrene.

Respiration was measured in the conventional Warburg manometric apparatus at 37°. The main compartment of the manometric vessel contained the leucocytes or tumour cells suspended in 3 ml. of Ringer medium. Polystyrene particles suspended usually in 0·1 ml. of water were tipped in from the side arm after temperature equilibration. The centre well contained a roll of filter paper soaked in 0·1 ml. of 7N-potassium hydroxide to absorb CO_2.

The $^{14}CO_2$ produced by the oxidation of ^{14}C-labelled substrates and that trapped as bicarbonate and then liberated by the addition of 0·1 ml. of 2N-sulphuric acid, which also served to terminate the reaction, was absorbed in potassium hydroxide in the centre well of the Warburg vessel. The CO_2 was precipitated as barium carbonate in the presence of carrier, plated on aluminium planchets, and the radioactivity was assayed with a thin-window gas-flow counter. The results were corrected for background and to infinite thinness of the barium carbonate layer.

RESULTS

Iyer *et al.* (1961) showed that the magnitude of respiratory stimulation in leucocytes obtained with polystyrene spherules is comparable with that obtained with killed bacteria. Examination under the phase-contrast microscope revealed rapid uptake of polystyrene spherules by guinea-pig polymorphonuclear leucocytes.

Polystyrene is readily soluble in dioxan and, in this solvent, it has a strong absorption peak at 253 mμ (Fig. 1). A linear relationship exists between the extinction at 253 mμ and the concentration of polystyrene in the range 20–200 μg./ml. The values of the amounts of engulfed particles recorded below have been estimated by means of a calibration curve having a slope of 1·17 (extinction/concentration).

Reproducibility of results. Leucocytes from any one animal give results that are consistent with each other. All experiments were carried out at least in duplicate and the results that are recorded in the Table give the means of these results, each of which has a deviation from the mean not greater than ± 5%. The phagocytic activities of leucocytes derived from one animal are not quantitatively the same as those obtained from another but a variation greater than 25% has not been noticed. Results recorded in the Tables are typical of those

obtained, under our experimental conditions, with leucocytes obtained from any guinea pig.

Use of bovine γ-globulin. Polystyrene particles of very small size tend to clump in the Ringer medium and such clumping introduces experimental difficulties. The addition of certain proteins to the polystyrene suspension greatly diminishes clumping in the medium and facilitates the removal of the extracellular polystyrene spherules adhering to the leucocyte surface. Bovine γ-globulin (fraction II) was found to be the most suitable of the substances tested for the prevention of clumping. Control experiments (Table 1) show that its presence in the medium has no adverse effect on particle uptake by guinea-pig leucocytes.

Recovery of polystyrene from the leucocytes. Results given in Table 2 show the effects of various extraction methods on the recovery of polystyrene from the leucocytes. Nearly all of the polystyrene is recovered from the cells by extracting with dioxan for 10 min. in a boiling-water bath and incubation at room temperature for 2 hr. The engulfed polystyrene spherules are extracted from whole or from ruptured leucocytes equally well.

Variation of uptake of particles with time. Maximum uptake of particles occurs within 20 min. after the addition of polystyrene spherules to the leucocyte suspension (Fig. 2). After this time no further uptake of particles appears to take place.

Respiration of phagocytizing leucocytes. Results given in Fig. 3 show that the stimulation of respiration of the polymorphonuclear leucocytes on the uptake of polystyrene particles varies, within certain limits, with the size of the particles, and that the increased respiratory rate of the phagocytizing leucocytes continues for about 1 hr. after the addition of the particles. After this time the rate of respiration falls to the normal level.

Particle-size requirement for phagocytosis. The results given in Table 3 show that there is both a minimum and maximum limit of particle-size requirement for phagocytosis. Particles 0·264–3·04 μ in diameter are taken up to about the same extent, and the amount of polystyrene uptake is apparently independent of the size of the particles within these limits. The phagocytic cell is able to take up only a limited mass of polystyrene particles of this size range. As the particles increase in size, the number of particles that can be accommodated by a leucocyte proportionately decreases. The 0·088 μ spherules are taken up only to a slight

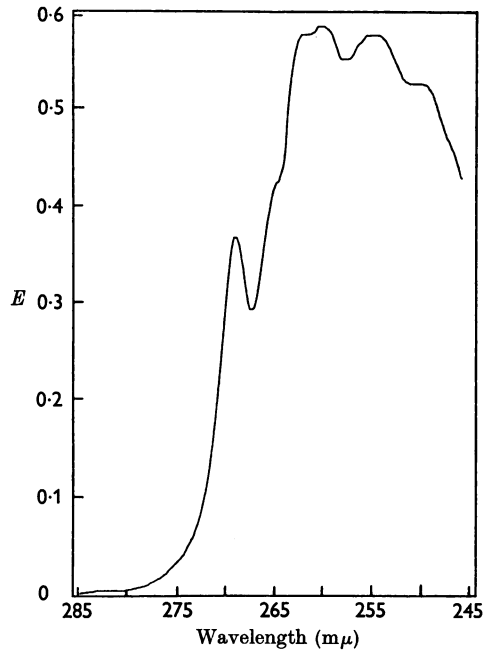

Fig. 1. Ultraviolet-absorption spectrum of polystyrene in dioxan. The absorption-spectrum recording was made with a Bausch and Lomb Spectronic 505 spectrophotometer. Polystyrene particles (0·871 μ in diameter) were dissolved in dioxan to give a concentration of 200 μg. of polystyrene/ml.

Table 1. *Effects of adding various proteins to the medium on the uptake of polystyrene spherules by guinea-pig polymorphonuclear leucocytes*

The phagocytosis experiments were carried out as described in the text. Each flask contained 6·7 mg. of polystyrene spherules, 0·3 μ in diameter.

Protein added	Amount of polystyrene taken up by the leucocytes (μg./mg. wet wt. of leucocytes)
None	21·6
Albumin (5 mg.)	17·5
Sodium caseinate (5 mg.)	14·1
Bovine γ-globulin (5 mg.)	20·8
Bovine γ-globulin (10 mg.)	21·8
Bovine γ-globulin (20 mg.)	19·9

Table 2. *Effects of using various methods of extraction with dioxan on the recovery of polystyrene taken up by leucocytes*

The phagocytosis experiments were carried out as described in the text.

Details of extraction	Amount of polystyrene recovered (μg./mg. wet wt. of leucocytes)
At room temperature for 2 hr.	14·7
At room temperature for 20 hr.	16·6
In a boiling-water bath for 10 min., then at room temperature for 2 hr.	15·8
In a Potter–Elvehjem homogenizer for 2 min., then in a boiling-water bath for 10 min. and then at room temperature for 20 hr.	14·9

degree, and the larger-sized spherules, which are of the same order of magnitude as the leucocytes, are not taken up at all.

Calculations are given in Table 3 of the numbers of particles taken up by the leucocytes. The calculations are made on the following assumptions: the volume of a leucocyte is that of a sphere of radius 7×10^{-3} mm.; the weight of 1 ml. of leucocytes is 1 g. (wet wt.); the volume of a polystyrene spherule is that of a sphere of radius given by the measurements stated in Table 3; the density of polystyrene spherules is 1. These assumptions are obviously approximate and hence the calculation of the number of spherules taken up/leucocyte, i.e.:

$$\frac{\text{wt. of spherules taken up}}{\text{wet wt. of leucocytes}} \times \frac{\text{vol. of each leucocyte}}{\text{vol. of each spherule}}$$

must be considered as approximate. Nevertheless, the calculations give a measure of the ratios of the numbers of spherules taken up/leucocyte as a function of their diameters as well as approximate measures of the absolute numbers of particles taken up.

It is evident from the values quoted in Table 3 that, although a much larger number (24 000) of spherules of diameter 0·088 μ is taken up compared with that (102) of spherules of diameter 0·871 μ, the increase of rate of respiration by the leucocytes is much less in the former case than in that of the latter. The increase of respiration is dependent, in fact, not on the number of particles taken up, but on the total volume of particles taken up. Moreover, it is clear that, just as there is a limit to the volume of particles that can be taken up, this volume being independent of the size of the particles (within the limits quoted), there is a limit to the amount of stimulation of respiration brought about by phagocytosis. The increased rate of respiration is, in fact, a constant and independent of the number of particles taken up and of their

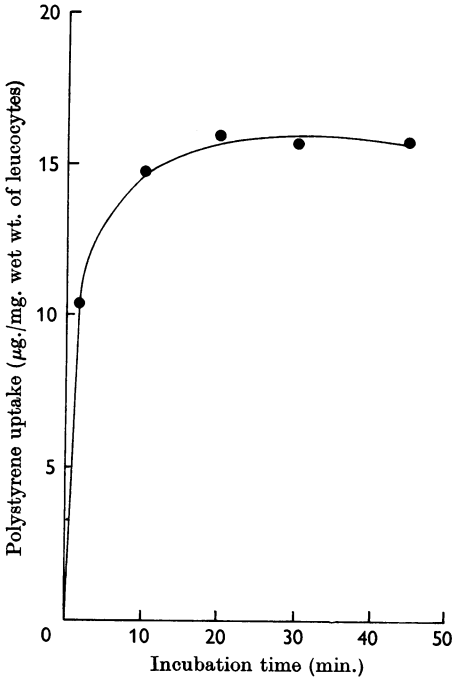

Fig. 2. Rate of uptake of polystyrene by guinea-pig polymorphonuclear leucocytes. Conditions for this experiment were the same as described in Table 1.

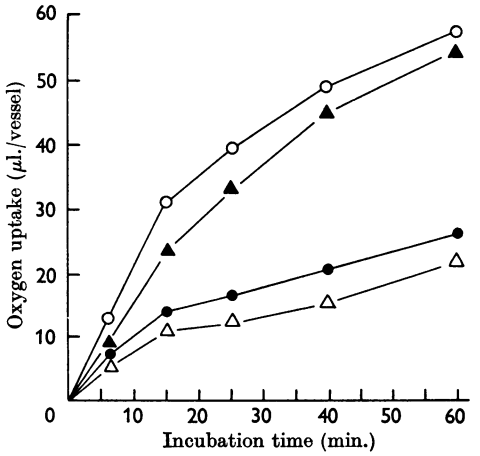

Fig. 3. Effect of polystyrene spherules on the respiration of guinea-pig polymorphonuclear leucocytes. Conditions for this experiment were the same as described in Table 3. △, No polystyrene; ●, 0·088 μ polystyrene particles; ▲, 0·264 μ polystyrene particles; ○, 0·557 μ polystyrene particles.

Table 3. *Effects of polystyrene-particle size on phagocytosis in guinea-pig polymorphonuclear leucocytes*

The phagocytosis experiments were carried out as described in the text. Oxygen uptake was measured in Warburg manometric flasks. Each flask contained 3 ml. of Ringer medium, glucose (10 mM), 6 mg. of bovine γ-globulin, 3 mg. of polystyrene and 30 mg. (wet wt.) of leucocytes.

Diameter of spherules (μ)	Oxygen uptake in 40 min. (μl./vessel)	Polystyrene uptake (μg./mg. wet wt. of leucocytes)	No. of particles/ leucocyte
0·088	17·3	7·4	24 000
0·264	44·7	30·1	3 600
0·557	49·5	28·1	360
0·871	47·6	36·9	102
1·305	51·2	34·3	34
3·04	50·6	35·9	3
7–14	14·1	0	0
12–28	14·6	0	0
No polystyrene	14·9	0	0

individual sizes, within the limits given in Table 3. It seems reasonable to conclude that the phagocyte is able to take up a limited mass of spherules and this limited quantity induces also a limited increase of the rate of respiration.

It is possible that the apparent lack of phagocytosis of freshly precipitated barium sulphate or freshly prepared manganese dioxide (Iyer *et al.* 1961) may be due to limitations of size.

Extent of phagocytosis and concentration of polystyrene. A study of the effects of varying the concentration of polystyrene in the medium on the amount of particles engulfed by leucocytes shows that at concentrations lower than 1 mg./ml. the amount of polystyrene engulfed is directly proportional to the external polystyrene concentration (Fig. 4). Once taken up, the inert polystyrene particles are retained by leucocytes for relatively long periods (Table 4).

Studies with human leucocytes. Studies with leucocytes obtained by differential centrifuging of blood from a patient with leukaemia indicate that the method described above for studying particle uptake by guinea-pig leucocytes can also be employed with the human phagocytes. The results summarized in Table 5 show that the leukaemic leucocytes take up polystyrene particles much less efficiently than do an equal amount of guinea-pig phagocytes.

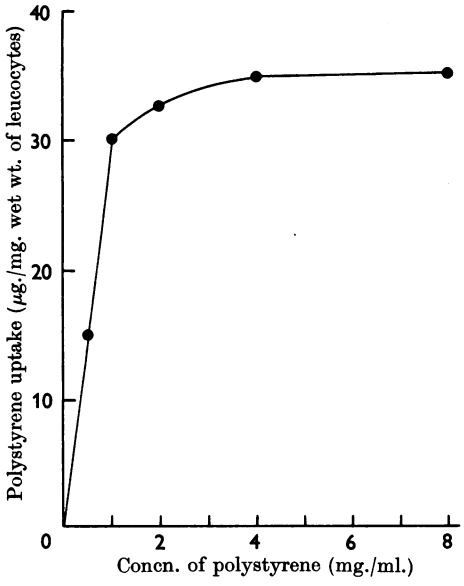

Fig. 4. Effect of variation of polystyrene concentration on the uptake of particles by guinea-pig polymorphonuclear leucocytes. The polystyrene spherules used in this experiment were 0·557 μ in diameter. Incubation was for 20 min. at 37°.

Uptake of polystyrene spherules by Ehrlich ascites-carcinoma cells. Results given in Tables 6 and 7 show that the addition of a suspension of polystyrene spherules to Ehrlich ascites-carcinoma cells brings about an increased rate of oxygen consumption so long as the diameter of the spherule is 0·557 μ or less. Spherules of larger size seem to have no effect on the oxygen consumption. It appears, therefore, that the ascites cells behave in a similar manner to polymorphonuclear leucocytes in their response to the presence of the polystyrene particles, with the exception that the stimulation of cell respiration occurs between different size limits.

Table 4. *Retention of engulfed polystyrene spherules by leucocytes*

Samples (5 mg. wet wt.) of guinea-pig leucocytes, containing polystyrene spherules, 0·871 μ in diameter, were suspended in 5 ml. of Ringer medium and incubated in 25 ml. Erlenmeyer flasks at 37° in a temperature-controlled water-bath shaker.

Incubation time (hr.)	Polystyrene content in leucocytes (μg./mg. wet wt. of leucocytes)
0	39·8
0·33	36·3
2	26·7
4	20·0

Table 5. *Uptake of polystyrene particles by human leucocytes*

The phagocytosis experiments were carried out as described in the text. Each flask contained 5 mg. of polystyrene spherules, 0·557 μ in diameter. The human leucocytes were isolated from the blood of a patient with acute leukaemia.

	Polystyrene uptake (μg./mg. wet wt. of leucocytes)
Guinea-pig leucocytes	27·1
Human leukaemic leucocytes	8·8
Guinea-pig leucocytes, incubated at 0°	3·3

Table 6. *Effects of uptake of polystyrene spherules on the respiration of Ehrlich ascites-carcinoma cells*

Oxygen uptake was measured in Warburg manometric flasks incubated at 37°. Each flask contained 3 ml. of Ringer medium, glucose (10 mM), 6 mg. of bovine γ-globulin, 10 mg. of polystyrene and 50 mg. (wet wt.) of cells.

Diameter of spherules (μ)	Oxygen uptake in 1 hr. (μl./vessel)
0·088	116
0·557	108
0·871	53
1·305	52
No polystyrene	50

A response occurs with the ascites cells with particles of a lower size than takes place with the leucocytes. Part of the stimulated oxygen uptake of Ehrlich ascites-tumour cells in the presence of the polystyrene spherules may be due to constituents of the fluid in which the spherules were originally suspended, but experiment shows that this amounts to not more than one-third of the observed stimulation.

The actual uptake or absorption of polystyrene by the ascites cells is considerably less than that by guinea-pig polymorphonuclear leucocytes for equal weights of the cells.

A preliminary survey of the metabolic requirements for the uptake of polystyrene spherules by Ehrlich ascites-carcinoma cells shows that the uptake is not affected by the absence of glucose from the medium in which the cells are incubated, nor by the presence of 0·1 mM-2,4-dinitrophenol. The presence of 0·2 mM-sodium iodoacetate decreases the uptake of the spherules, but the largest decrease occurs in the presence of a mixture of iodoacetate and 2,4-dinitrophenol (Table 7). It seems likely that the uptake is dependent on cell ATP, and the decrease in the concentration of this by suppression of both glycolytic and respiratory ATP is needed to bring about the maximum suppression of particle uptake.

Effects of particle uptake on the oxidation of glucose and of formate by Ehrlich ascites-carcinoma cells. Uptake of polystyrene spherules by Ehrlich ascites-carcinoma cells in the presence of [1-^{14}C]glucose or of [6-^{14}C]glucose brings about a stimulation of the production of $^{14}CO_2$ which is approximately the same with either labelled glucose (Table 8). The stimulation of CO_2 production is due to an increased rate of turnover of the citric acid cycle, as the stimulation with both [1-^{14}C]glucose and [6-^{14}C]glucose is abolished by the presence of 20 mM-sodium malonate. Particle uptake by the Ehrlich ascites-carcinoma cells does not increase the rate of oxidation of [^{14}C]formate to $^{14}CO_2$ (Table 8). These results show that the biochemical consequences of uptake of polystyrene spherules by Ehrlich ascites-carcinoma cells differ from those found with guinea-pig polymorphonuclear leucocytes. In the latter case there is a stimulation of the hexose monophosphate pathway and a concomitant increased rate of formate oxidation (Iyer *et al.* 1961); in the former case only the rate of operation of the citric acid cycle is stimulated.

Table 7. *Uptake of polystyrene spherules by Ehrlich ascites-carcinoma cells*

The experiments were carried out in 25 ml. Erlenmeyer flasks. Unless otherwise indicated, each flask contained 10 ml. of Ringer medium, glucose (10 mM), 20 mg. of bovine γ-globulin, 100 mg. (wet wt.) of Ehrlich ascites-carcinoma cells and 10 mg. of polystyrene spherules, 0·557 μ in diameter. The flasks were incubated at 37° for 30 min.

	Polystyrene uptake (μg./mg. wet wt. of cells)
Control	5·5
Control, but no glucose added	5·8
Iodoacetate (0·2 mM) added	3·9
2,4-Dinitrophenol (0·1 mM) added	5·4
Iodoacetate (0·2 mM) + 2,4-dinitrophenol (0·1 mM) added	1·8

Table 8. *Effect of particle uptake on the oxidation of glucose and of formate by Ehrlich ascites-carcinoma cells*

Each manometric vessel contained 3 ml. of Ringer medium, 50 mg. (wet wt.) of ascites cells, 6 mg. of γ-globulin, 15 μmoles of glucose, and 10 mg. of polystyrene particles, 0·088 μ in diameter, where indicated. The vessels were incubated for 1 hr. at 37°. The substrate concentrations were: [1-^{14}C]glucose and [6-^{14}C]glucose, $1·5 \times 10^5$ counts/min./vessel; sodium [^{14}C]formate, 2 mM, $1·5 \times 10^5$ counts/min./vessel; sodium malonate, 20 mM.

Substrates	Oxygen uptake (μl./vessel)	$^{14}CO_2$ liberated (counts/min./vessel)
[1-^{14}C]Glucose	49	1157
[1-^{14}C]Glucose + particles	127	2933
[1-^{14}C]Glucose + malonate	43	1655
[1-^{14}C]Glucose + malonate + particles	45	1274
[6-^{14}C]Glucose	50	97
[6-^{14}C]Glucose + particles	125	1956
[6-^{14}C]Glucose + malonate	38	56
[6-^{14}C]Glucose + malonate + particles	42	37
[^{14}C]Formate	49	227
[^{14}C]Formate + particles	121	231

DISCUSSION

The results obtained in the present study reveal that the uptake of polystyrene spherules by polymorphonuclear leucocytes is confined to particles within certain limits of size, the amount taken up with particles of diameter 0·088 μ being considerably less than that with particles of diameter 0·264 μ. Particles whose diameters lie between 0·264 μ and 3·04 μ are taken up to approximately the same extent, the weight taken up being about constant. Above a diameter of 7 μ polystyrene spherules are not taken up. The number of particles taken up varies very greatly, calculations showing a hundred times more particles of diameter 0·264 μ being taken up than particles of diameter 1·3 μ. In spite of the large differences in numbers of particles taken up, the stimulated rates of oxygen uptake are approximately constant for a given mass of particles taken up. It would appear that there is a limit to the weight, or volume, of particles taken up, and that this either imposes, or is associated with, a

limit to the increased rate of respiration. As glycolysis is considered to be responsible for the energy needed for particle uptake in the leucocytes (Sbarra & Karnovsky, 1959; Iyer *et al.* 1961), it is unlikely that a limit to the rate of oxygen consumption sets the limit to the amount of particles taken up. If the increased rate of oxygen consumption is due to the release of an enzyme accomplishing the oxidation of NADPH (Iyer & Quastel, 1963), it seems reasonable to conclude that maximum uptake of the particles brings about the release of the maximum quantity of the NADPH oxidase whose amount presumably is rate-limiting. The factors that determine the size limits for uptake are as yet unknown.

Uptake or absorption of polystyrene spherules occurs with Ehrlich ascites cells, though the amounts taken up by these cells are considerably smaller than those taken up by guinea-pig polymorphonuclear leucocytes. Moreover, the size limits for uptake by Ehrlich ascites-carcinoma cells differ from those found for the leucocytes, the upper limit being much less for the former cells than for the latter. Stimulation of the rate of oxygen consumption occurs with ascites cells on particle uptake as with the leucocytes. The uptake of polystyrene spherules by Ehrlich ascites-carcinoma cells is not determined solely by glycolytic energy, as it is unaffected by the absence of glucose. The fact that only a combination of a glycolytic inhibitor (iodoacetate) and an uncoupler of oxidative phosphorylation (2,4-dinitrophenol) greatly diminishes particle uptake by the ascites cells makes it evident that the energy for uptake in these cells comes from ATP derived from either glycolysis or respiration. A suppression of energy from one metabolic process is compensated by increased energy from the other (Quastel & Bickis, 1959).

Stimulation of respiration in Ehrlich ascites-carcinoma cells brought about by particle uptake consists of an increased rate of turnover of the citric acid cycle, as demonstrated by the abolition of this stimulation on the addition of sodium malonate. The respiration of Ehrlich ascites-carcinoma cells in the absence of glucose is much more sensitive to malonate than the lowered rate of respiration taking place in the presence of glucose (Quastel & Bickis, 1959). The process of particle uptake by the tumour cells appears to overcome the depressing action of glucose on the tumour-cell respiration and thus counteracts the Crabtree effect. The oxidation of formate is not stimulated by particle uptake in the tumour cells. This is in contrast with the stimulation that occurs with guinea-pig polymorphonuclear leucocytes on particle uptake. It seems reasonable to conclude that particle uptake by the tumour cells involves energy derived from ATP, as a result of which ADP is formed, and this, in turn, stimulates the tumour-cell respiration (Chance & Hess, 1959). With guinea-pig polymorphonuclear leucocytes only glycolytic energy is involved in particle uptake (Sbarra & Karnovsky, 1959; Iyer *et al.* 1961), and the stimulated respiration, occurring as a consequence of particle uptake, is due to the increased rate of oxidation of glucose through the hexose monophosphate pathway.

SUMMARY

1. The uptake of polystyrene particles by polymorphonuclear leucocytes and Ehrlich ascites-carcinoma cells has been investigated by a new method based on extraction of the polystyrene with dioxan and the determination of the polystyrene spectrophotometrically.

2. At concentrations lower than 1 mg./ml., the amount of particles engulfed by leucocytes is directly proportional to the polystyrene concentration in the medium.

3. The uptake of the polystyrene spherules is confined to particles within certain size limits which differ with the ascites cells from those with leucocytes. Particles whose diameters lie within these limits are taken up by leucocytes to approximately the same extent.

4. The energy for particle uptake in the ascites cells apparently comes from either glycolysis or respiration.

5. Particle uptake by Ehrlich ascites-carcinoma cells is apparently accompanied by a stimulation of the citric acid cycle, in contrast with the stimulation of the hexose monophosphate pathway that occurs with leucocytes on particle uptake.

6. Stimulation of formate oxidation that occurs on particle uptake with leucocytes does not occur with the ascites cells.

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