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PHAGOCYTIC ACTIVITY OF PERITONEAL EXUDATE CELLS FROM MICE

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ABSTRACT

OELLERMANN, R. A., HOOGENHOUT, JOAN and CARTER, P. Phagocytic activity of peritoneal exudate cells from mice. *Onderstepoort J. vet. Res.* 39(4), 197–204 (1972).

Phagocytic activity of peritoneal exudate cells in mice was stimulated by injection of 0,1 ml mineral oil or varying concentrations of lysolecithin. Optimal lysolecithin concentration was found to be 2 to 5 μg per mouse. The phagocytic activity of peritoneal exudate cells from mice was determined by spectral analysis of dioxane extracts of cells after incubation in the presence of polystyrene latex particles. Maximum uptake of latex particles occurred after an incubation period of 1 h.

Maximum phagocytic activity was observed in cells harvested 3 days after stimulation with mineral oil and approximately 4 days after stimulation with lysolecithin. Electron microscopy of these cells revealed a similar pattern of phagocytic activity.

Latex particles are inert and not metabolized by the cells and their uptake was therefore compared with the phagocytosis of bluetongue virus particles. Electron microscopic studies of the uptake of bluetongue virus showed that although adsorption of the virus on the surface membrane occurred practically no phagocytosis was observed 1 day after stimulation with lysolecithin. Maximum phagocytosis of virus particles occurred 3 to 4 days after stimulation with lysolecithin.

INTRODUCTION

The initiation of the immune response to different antigens results from the interaction of different cells in the body (Wilson & Feldmann, 1972). These interactions appear to be an essential step in the antibody response to most antigens. The role of non-lymphoid cells, particularly macrophages, in the initiation process has been discussed (Humphrey, 1969; Shortman, Diener, Russell & Armstrong, 1970). Macrophages, frequently enhanced the immunogenicity of antigens but the mechanism of stimulation has not yet been resolved. Phagocytic activity of macrophages could therefore be of great significance in the immune ressponse of cells to certain antigens.

Phagocytosis of latex particles by various cells has been investigated qualitatively employing microscopic examination or solvent extraction techniques (Roberts & Quastel, 1963; Rodesch, Néve & Dumont, 1970; Weisman & Korn, 1967). Latex particles are inert and not metabolized by the cells. As their uptake could, therefore, differ from that of biologically active particles it was decided also to study the phagocytosis of bluetongue virus particles. This virus was chosen partly because it was planned to investigate the nature of the immune response to it later.

Although the injection of mineral oil or lysolecithin stimulates both the production of peritoneal exudate and cellular phagocytic activity, only the latter phenomenon was studied in this investigation. The phagocytic activity of peritoneal exudate cells from mice was measured by means of dioxane extraction after phagocytosis of polystyrene latex particles. The results obtained with latex were then correlated with the electron microscopic examination of the uptake of bluetongue virus particles by the same type of cells.

MATERIALS AND METHODS

Animals

Six-week old mice of both sexes from a locally inbred strain were used throughout.

Cells

Peritoneal exudate cells were obtained from mice stimulated by intraperitoneal injections with 0,1 ml mineral oil or varying concentrations of lysolecithin* in physiological saline. On different days after stimulation mice were killed by cervical dislocation and cells harvested by sterile flushing of the peritoneal cavity with 4 to 5 ml cold Eagle's medium. The cells harvested from batches of 10 to 40 mice were pooled and centrifuged at 500 *g* for 7 min in an International, model PR6, centrifuge. The cell pellet was resuspended in fresh Eagle's medium and diluted to the desired concentration after counting in a haemocytometer.

On the average $1,2 \times 10^7$ cells were recovered per mouse. Of these cells 65% morphologically resembled macrophages.

Determination of phagocytic activity

The dioxane extraction technique was similar to that described by Rodesch *et al.* (1970). A total of 1,5 to 2×10^8 peritoneal exudate cells in 10 ml Eagle's medium was seeded per 100 ml Erlenmeyer flask. Incubation was routinely carried out at 37°C for 2 h in the presence of 100 μl of a 2% suspension of polystyrene** particles with a diameter of 109 nm. After incubation the cultures were rapidly cooled to 4°C, the cells removed with a syringe and centrifuged in conical tubes at 4°C for 4 min at 80 *g*. Cells were washed three times with cold Eagle's medium to remove excess latex particles and the tubes drained well after the final centrifugation. The cell sediment was homogenized in 1,5 to 2 ml pure dioxane*** and the extraction continued for 1 h at 25°C. The volume of dioxane used was always adjusted so that the homogenate contained the equivalent of 1×10^8 cells per ml. The extract was clarified by centrifugation at 4°C for 10 min at 4200 *g*. The ultra violet absorption spectrum of the dioxane supernatant was recorded in a Beckman, model DB, spectrophotometer. Pure dioxane or dioxane extracts of cells incubated without latex particles were used as reference.

For comparison, cells were incubated for 1 h in the presence of 100 μl of a purified bluetongue virus suspension (Verwoerd, Louw & Oellermann, 1970) con-

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taining 2×10^{11} plaque-forming units per ml. The phagocytic activity of these cells was examined electron microscopically and compared with the uptake of latex particles.

Electron microscopy

The procedure for electron microscopic examination of cells has been described by Lecatsas & Weiss (1969).

Results

Spectral analysis of cellular extracts

Analysis of the dioxane extracts of peritoneal exudate cells incubated with latex particles (Fig. 1b) showed the characteristic ultra violet absorption spectrum of polystyrene latex particles in dioxane which is illustrated in Fig. 1a. Dioxane extracts of cells incubated without latex particles showed a low absorbance which could be ascribed to residual cellular material.

To determine the efficiency of the cell-washing process before extraction with dioxane, cells were incubated in the absence of latex particles. Cultures were then rapidly cooled to 4°C and latex particles added. Immediately thereafter cells were removed from the flask and the normal washing and extraction procedure followed. The ultra violet absorption spectrum, also shown in Fig. 1b, is not significantly higher than that of the control cell extract. A very efficient removal of unphagocytosed latex particles from the cell culture is thus achieved.

The elevated ultra violet absorption spectrum of dioxane extracts of cells incubated in the presence of latex particles, compared to control extracts, indicates the feasibility of this method to determine the phagocytic activity of peritoneal exudate cells. Similar results were recently also reported by Rodesch *et al.* (1970) for isolated thyroid cells.

Rate of uptake of latex particles

Peritoneal exudate cells were incubated with and without latex particles for 10, 30, 60 and 120 min and dioxane extracts prepared. Absorbance maxima of the latex containing cell extracts at 261 mμ employing the control cultures as reference are presented in Fig. 2.

A marked increase in the uptake of latex particles was observed for incubation periods up to 30 min and

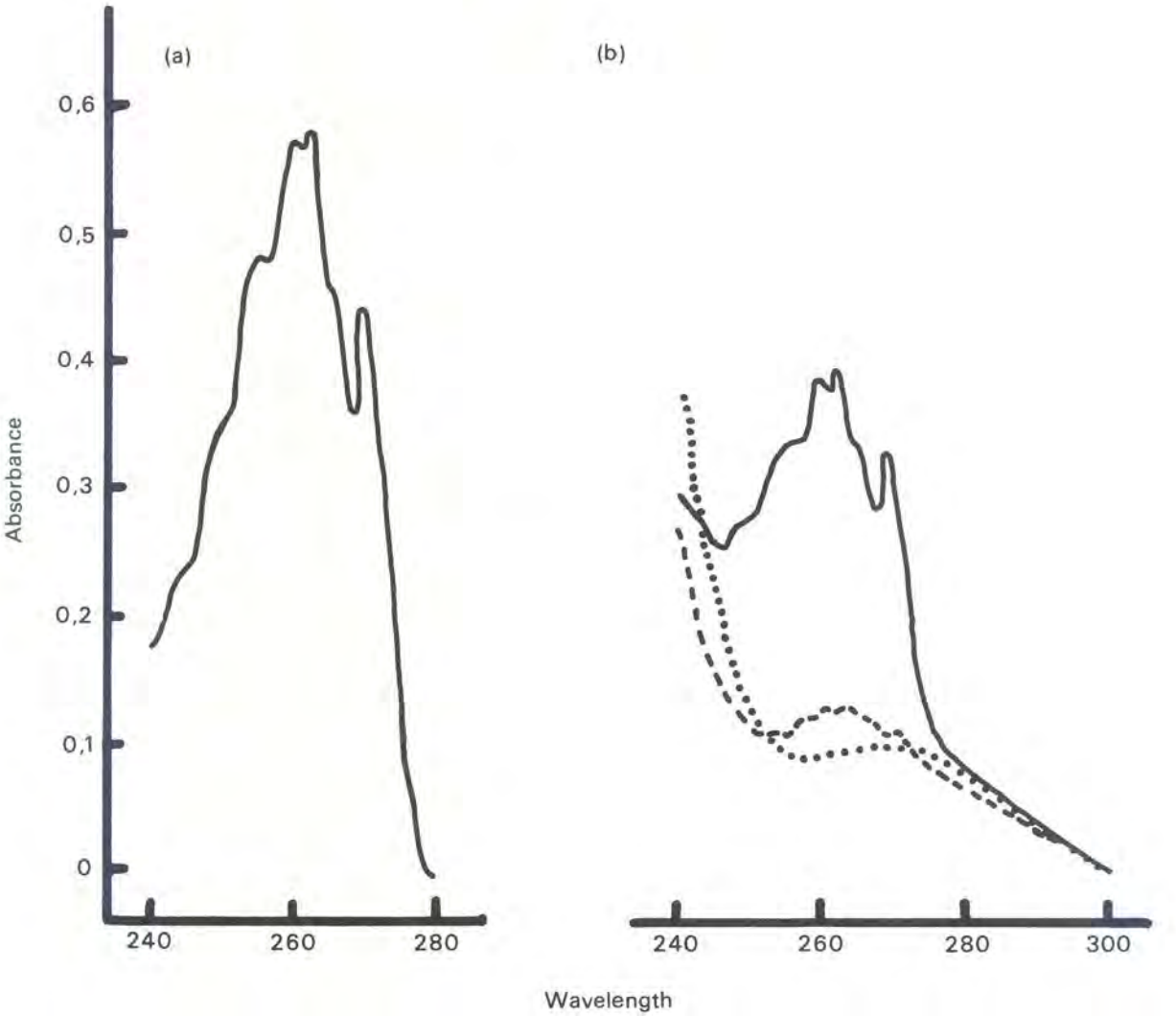

FIG. 1 (a) Ultra violet absorption spectrum of pure latex particles in dioxane, with pure dioxane as reference
(b) Ultra violet absorption spectrum of control cell extract without latex . . . . . ; control cell extract but with latex added after incubaion and immediately washed before dioxane extraction — — — ; and dioxane extract of cells incubated in presence of latex ———. In all cases pure dioxane was used as reference

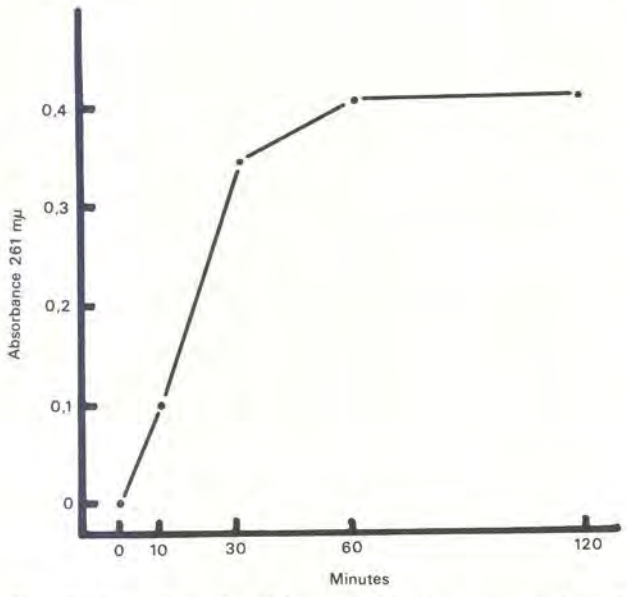

FIG. 2 Rate of uptake of latex particles by mouse peritoneal exudate cells

maximum uptake was achieved after 60 min. For routine purposes an incubation period of 2 h was therefore adopted.

Stimulation with lysolecithin

Mineral oil is frequently used to stimulate the production and phagocytic activity of peritoneal exudate cells (Jacherts, 1966). It is, however, difficult to obtain cell suspensions completely free from oil. An alternative method of stimulation, using lysolecithin, was therefore investigated (O. Westphal, Max Planck Institut für Immunbiologie, Freiburg, personal communication). In this investigation the phagocytic activity of peritoneal exudate cells was considered to be of primary importance. The results of the stimulation by different concentrations of lysolecithin appear in Fig. 3.

A marked increase in the phagocytic activity of cells was observed for concentrations up to 2 μg lysolecithin per mouse. Further increases of lysolecithin concentration up to 1 000 μg per mouse did not change the phagocytic activity of peritoneal exudate cells.

Lysolecithin stimulation versus oil stimulation

From the routine cell counts it was observed that cell recovery decreased in the oil-stimulated mice as the period of stimulation prior to cell harvest was increased from 1 to 4 days. The cell concentrations recovered from the lysolecithin-stimulated mice remained fairly constant over the four-day period investigated. Preliminary experiments also indicated that 7 days after stimulation of mice with either lysolecithin or mineral oil relatively low concentrations of peritoneal exudate cells with a low phagocytic activity were obtained. In order to expand on these observations the production of peritoneal exudate was stimulated with 0,1 ml mineral oil or 2 μg lysolecithin per mouse at 4, 3, 2 and 1 days prior to cell harvest. The phagocytic activity of the different groups of cells was determined by spectral analysis of the dioxane extracts and the results presented in Fig. 4.

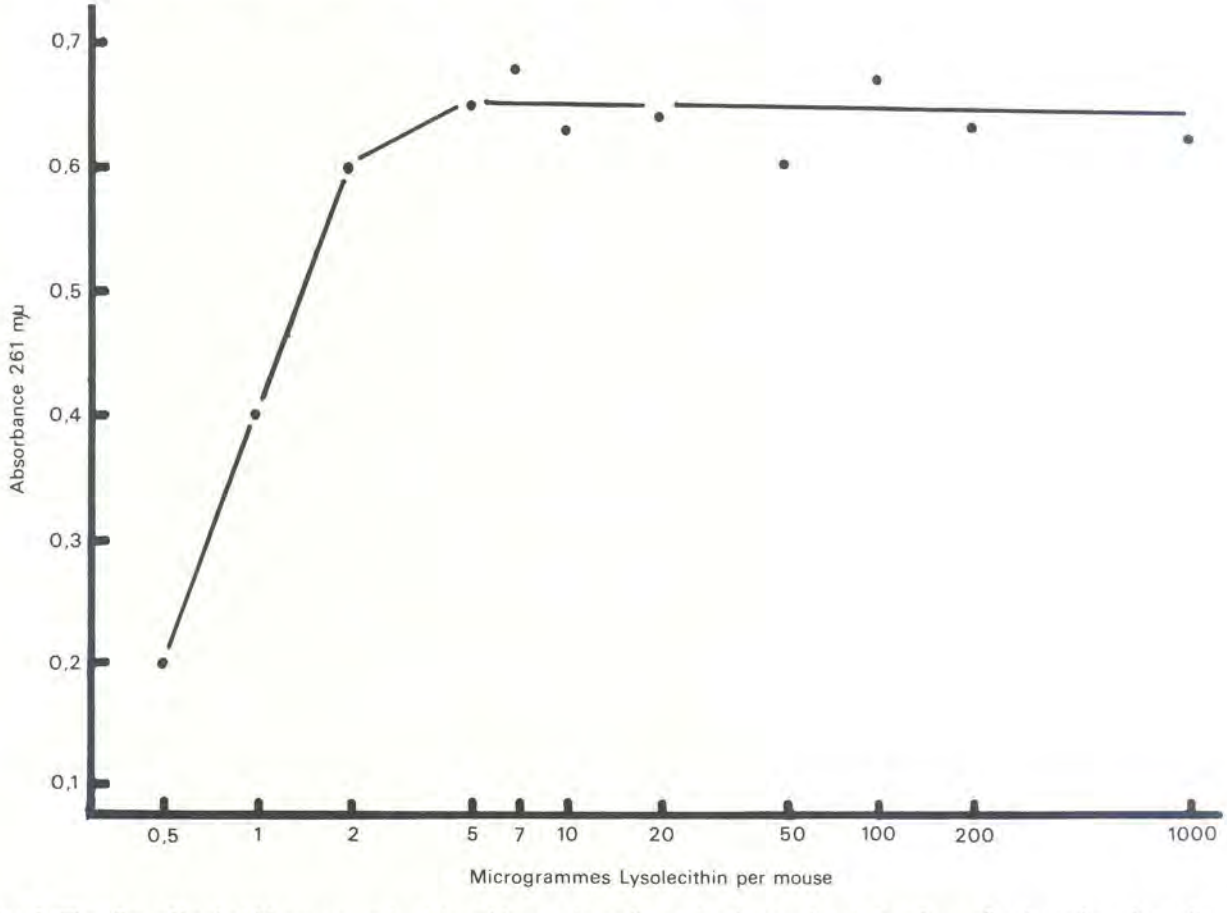

FIG. 3 The effect of lysolecithin concentration injected intraperitoneally on the phagocytic activity of mouse peritoneal exudate cells

A marked increase in the phagocytic activity of mineral oil-stimulated peritoneal exudate cells was found up to 3 days post-stimulation. The lysolecithin-stimulated peritoneal exudate cells showed maximum phagocytic activity when harvested 4 days post-stimulation.

Electron microscopy

(i) *Uptake of polystyrene latex particles*

Large numbers of peritoneal exudate cells obtained at different times after stimulation with either lysolecithin or mineral oil were scanned in the electron microscope. The day after stimulation practically no latex particles were found within the cells. However, with extended periods after stimulation, a dramatic increase in the phagocytosis of latex particles by the cells was observed. Maximum activity was reached about 3 days post-stimulation. These results are in agreement with the spectral analyses reported in Fig. 4.

Electron micrographs of the phagocytosis of latex particles by peritoneal exudate cells appear in Fig. 5 to 8. Different stages of particle engulfment can be seen. Latex particles within the cell occurred separately or within inclusions which varied considerably in size.

(ii) *Uptake of bluetongue virus particles*

The pattern of uptake of bluetongue virus particles was similar to that observed for latex particles. Maximum phagocytosis was observed 3 to 4 days post-stimulation with either lysolecithin or mineral oil.

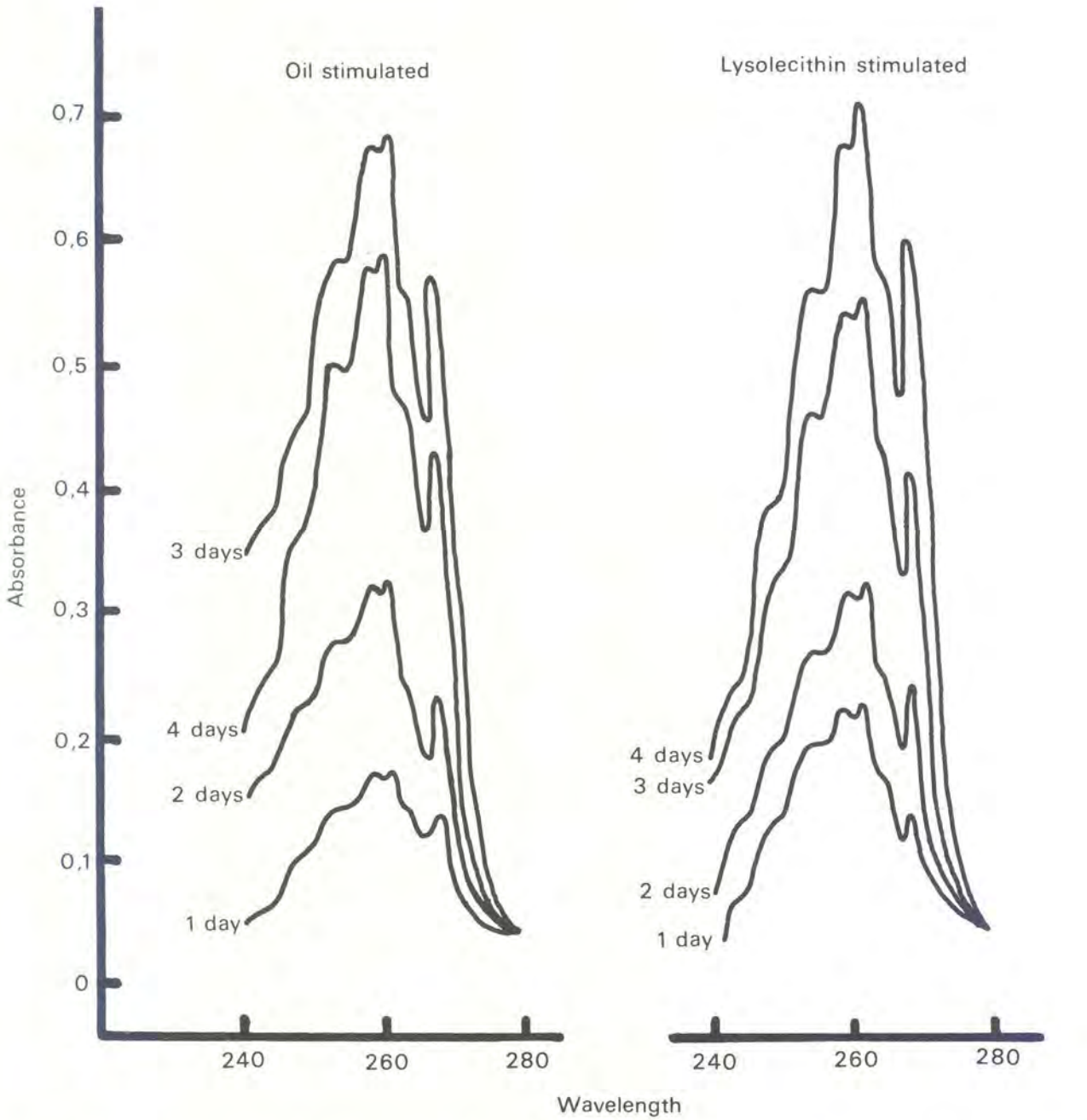

FIG. 4 Comparison of phagocytic activity of oil-stimulated with lysolecithin-stimulated peritoneal exudate cells. Control cell extracts were used as reference

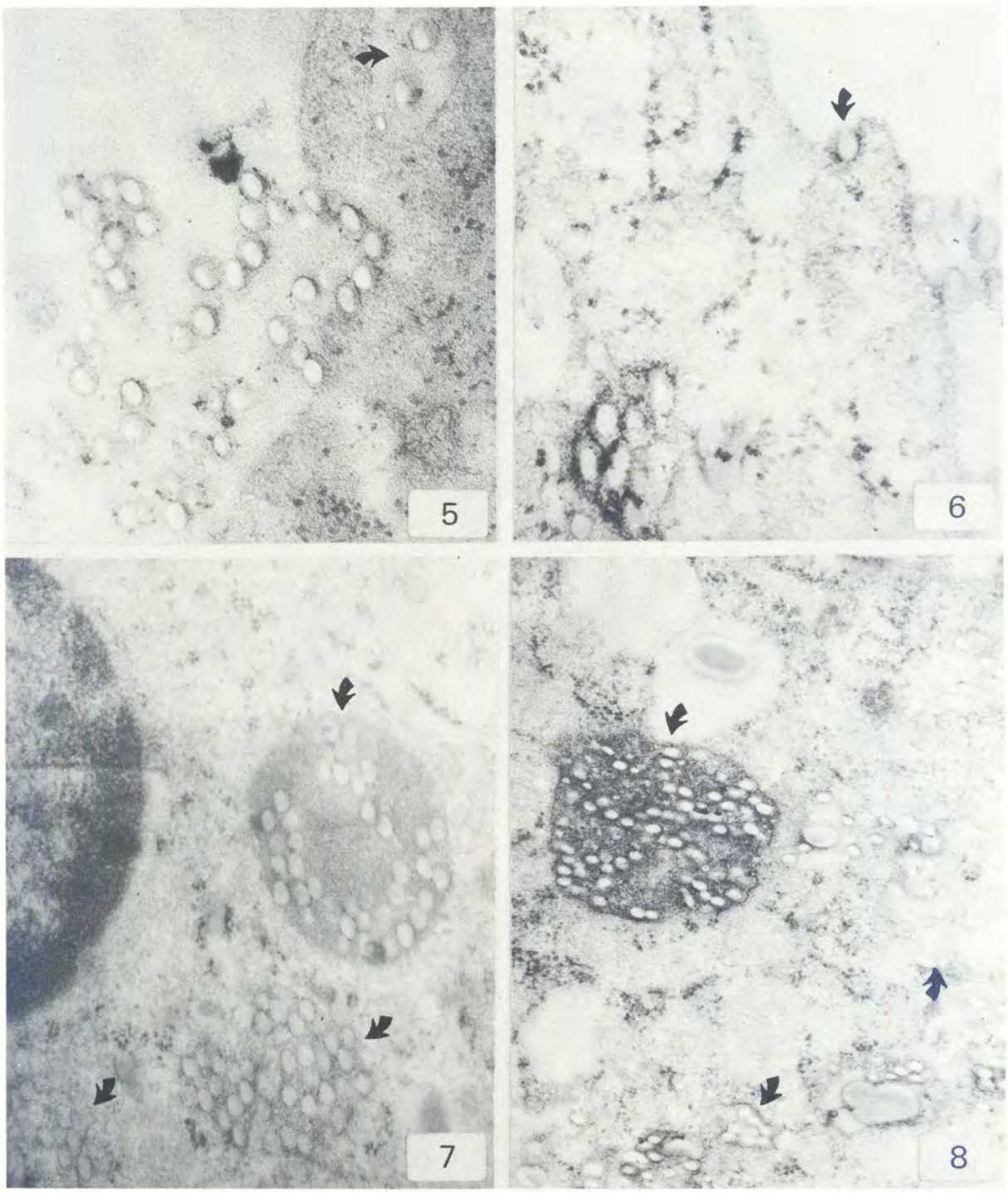

FIG. 5 Electron micrograph illustrating the phagocytosis of latex particles by peritoneal exudate cells 2 days after lysolecithin stimulation. Only few particles (indicated by arrow) occur within the cytoplasm. Magnification: × 80 000

FIG. 6 Illustration of latex particle (indicated by arrow) in the process of uptake by peritoneal exudate cells 2 days after lysolecithin stimulation. Relatively low phagocytic activity of cells was observed. Magnification: × 80 000

FIG. 7 Electron micrograph of large intracytoplasmic inclusions of latex particles (indicated by arrows) in peritoneal exudate cells harvested 3 days after mineral oil stimulation. Magnification: × 40 000

FIG. 8 Electron micrograph of intracytoplasmic latex particles occurring singly or in large inclusions (indicated by arrows) in 4 days post lysolecithin-stimulated peritoneal exudate cells. Magnification: × 40 000

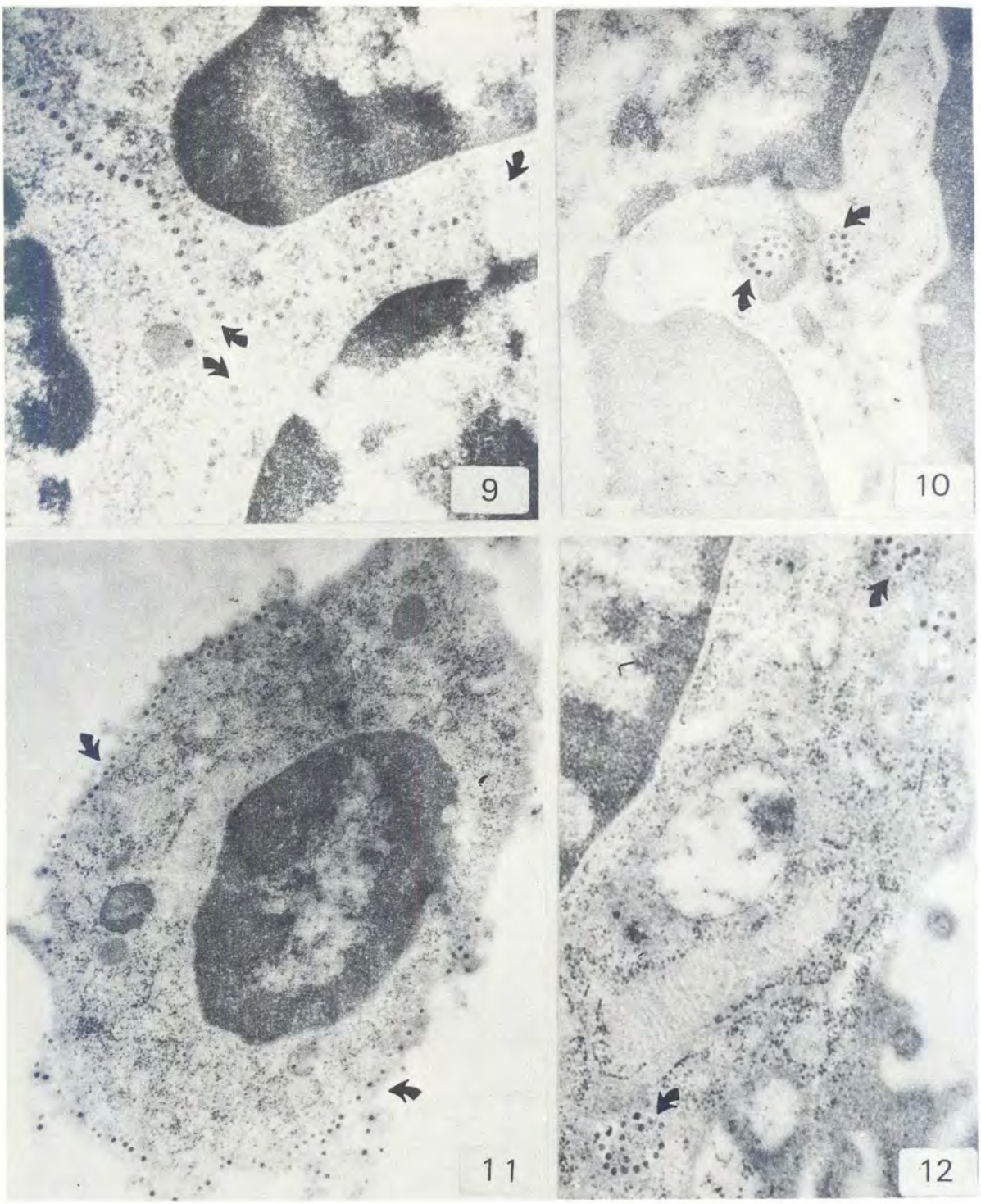

FIG. 9 Electron micrograph of bluetongue virus particles (indicated by arrows) adsorbed on to the surface membranes of 3 closely associated peritoneal exudate cells harvested 1 day after lysolecithin stimulation. Magnification: × 40 000

FIG. 10 Electron micrograph of intracytoplasmic inclusions of bluetongue virus particles (indicated by arrows) in 3 days post lysolecithin-stimulated peritoneal exudate cells. Magnification: × 40 000

FIG. 11 Electron micrograph of 3 days post lysolecithin-stimulated peritoneal exudate cells showing intracytoplasmic bluetongue virus particles shortly after uptake. Magnification: × 30 000

FIG. 12 Electron micrograph of intracytoplasmic inclusions of bluetongue virus particles (indicated by arrows) in 4 days post lysolecithin-stimulated peritoneal exudate cells. Magnification: × 40 000

Electron micrographs reproduced in Fig. 9 to 12 illustrate the phagocytosis of bluetongue virus particles by peritoneal exudate cells. It is of interest to note that 1 day after stimulation with lysolecithin adsorbed bluetongue virus particles were clearly seen on the surface of the cell membrane with only very few intracellular particles. With longer periods after stimulation large numbers of bluetongue virus particles were found within the cytoplasm either singly or within inclusions of varying size.

Discussion

The phagocytic activity of peritoneal exudate cells was investigated by determining the uptake of polystyrene latex particles. This was achieved by spectral analysis of dioxane extracts made from washed cells after incubation in the presence of latex. Considerable variation in the phagocytic activity of cells obtained from different batches of mice was observed. However, good reproducibility was achieved in duplicate determinations on the same pool of cells. Strict adherence to the prescribed experimental conditions was obligatory for success. The general pattern of phagocytic activity of differently stimulated peritoneal exudate cells was supported by the electron microscopic investigation. The dioxane extraction technique could therefore be employed as a quantitative measure of phagocytic activity of peritoneal exudate cells. The convenience of this technique for the direct investigation of phagocytosis by the thyroid follicular cell was also reported recently by Rodesch *et al.* (1970).

Although stimulation of peritoneal exudate cells by mineral oil provided similar results to stimulation by lysolecithin, the former cell preparations could not easily be prepared free from oil. Furthermore, mineral oil is not readily metabolized which could influence results obtained by this method of stimulation. In contrast, lysolecithin is rapidly metabolized and no residual lysolecithin remains in the treated cell population after 24 h (Munder, Ferber, Modolell & Fischer, 1969). Lysolecithin can therefore be employed to considerable advantage in the stimulation of peritoneal exudate cells.

Cells which actively phagocytosed latex particles were also active in the uptake of bluetongue virus particles. Of particular interest, however, was the observation that peritoneal exudate cells harvested 1 day after stimulation seemed to adsorb bluetongue virus actively on the surface membrane. Very little phagocytosis occurred and only isolated particles were observed in the cytoplasm. However, extremely active phagocytosis of virus particles was observed in peritoneal exudate 3 to 4 days after lysolecithin stimulation.

The significance of these observations and the suitability of bluetongue virus as antigen are at present being investigated in a study of the immune response *in vitro*.

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References

Humphrey, J. H., 1969. IV. Antigen handling by macrophages pp. 171-213 in Landy, M. & Braun, W. (Editors), 1969. Immunological tolerance. A reassessment of the immune response. New York: Academic Press.

Jacherts, D., 1966. Antikörper-Synthese *in vitro* I. Spezifische Stimulierung einer Zellkultur durch Phagen-Receptor-Partikel. *Z. med. Mikrobiol. u. Immunol.*, 152, 1-19.

Lecatsas, G. & Weiss, K. E., 1969. Formation of Wesselsbron virus in BHK-21 cells. *Arch. ges. Virusforsch.*, 27, 332-338.

Munder, P. G., Ferber, E., Modolell, M. & Fischer, H., 1969. The influence of various adjuvants on the metabolism of phospholipid in macrophages. *Int. Arch. Allergy*, 36, 117-128.

Roberts, J. & Quastel, J. H., 1963. Particle uptake by polymorphonuclear leucocytes and Ehrlich ascites-carcinoma cells. *Biochem. J.*, 89, 150-156.

Rodesch, F. R., Nève, P. & Dumont, J. E., 1970. Phagocytosis of latex beads by isolated thyroid cells. *Expl Cell Res.*, 60, 354-360.

Shortman, K., Diener, E., Russell, Pamela & Armstrong, W. D., 1970. The role of non-lymphoid accessory cells in the immune response to different antigens. *J. exp. Med.*, 131, 461-482.

Verwoerd, D. W., Louw, Helga & Oellermann, R. A., 1970. Characterization of bluetongue virus ribonucleic acid. *J. Virol.*, 5, 1-7.

Weisman, R. A. & Korn, E. D., 1967. Phagocytosis of latex beads by *Acanthamoeba* I. Biochemical properties. *Biochemistry N.Y.*, 6, 485-497.

Wilson, J. D. & Feldmann, M., 1972. Dynamic aspects of antigen binding to B cells in immune induction. *Nature New Biology*, 237, 3-5.

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