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PERITONEAL MACROPHAGES WHICH PHAGOCYTOSE AUTOLOGOUS POLYMORPHONUCLEAR LEUCOCYTES IN GUINEA-PIGS. I: INDUCTION BY IRRITANTS AND MICROORGANISMS AND INHIBITION BY COLCHICINE

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Summary.—In order to examine macrophages phagocytosing polymorphonuclear leucocytes (PMNs) in detail, we established a new method, whereby a large number of PMN-phagocytosing macrophages (PPMs) were easily induced. PPMs were harvested from the peritoneal cavity after thioglycollate medium, oyster glycogen, phytohaemagglutinin (PHA), *L. monocytogenes* or *S. aureus* had been injected i.p. into guinea-pigs. When thioglycollate medium or oyster glycogen was injected i.p., the number of the PPM reached a peak 48 h later and PPM formed 20% or more of total macrophages. When *L. monocytogenes* or *S. aureus* was injected i.p., the ratio of PPM to total macrophages reached a peak 24 h later. Morphologically, some of the phagocytosed PMNs were not degenerated and the others were at various stages of degeneration. The ability of macrophages to phagocytose PMNs was suppressed when 10^{-6} mol/kg of colchicine was administered i.p. 1 day after the injection of the irritants.

IT HAS LONG been known that macrophages ingest bacteria, fungi, protozoa, viruses, red blood cells, cell debris and autologous dead cells (Movat, 1979). Although many investigators have studied the phagocytosis of autologous erythrocytes in detail (Harris and Kellermeyer, 1970), systematic studies on the phagocytosis of autologous polymorphonuclear leucocytes (PMNs) have not yet been carried out since PMN-phagocytosing macrophages (PPMs) could not be induced easily and in large quantities. In addition, the presence of PPMs was also reported occasionally in clinical cases (Pekin *et al.*, 1967; Spriggs, Boddington and Mowat, 1978). Therefore, detailed investigation of PPMs is warranted.

In the present study, we developed a method by which PPMs could be induced easily and in large quantities. Firstly, we will describe this method; and then report the study of PPMs by electron microscopy and the effect on them of colchicine.

MATERIALS AND METHODS

Animals.—Male and female inbred guinea-pigs of the JY-1 strain or Strain 13, weighing 450–500 g, were purchased from Seiwa Experimental Animal Institute (Ohita, Japan). In most experiments, 2 animals were used and results were expressed as the mean value. In some experiments, only 1 animal was used.

Irritants.—Ten ml of 3% thioglycollate medium (Difco Laboratories, Detroit, MI), 10 ml of 3% oyster glycogen Type 2 (Sigma Chemical Co., St Louis, Mo.) or 5 ml of phosphate-buffered saline (PBS) containing 100 μg PHA-P (Difco Laboratories, Detroit, MI) was injected i.p. into guinea-pigs to induce chemical inflammation. In one experiment, 10 ml of 3% oyster glycogen was injected twice.

Microorganisms.—Ten million *Listeria monocytogenes* or 5×10^8 *Staphylococcus aureus* were

inoculated into the peritoneal cavity of guinea-pigs to induce bacterial inflammation.

Preparation of smears of peritoneal exudate cells.—Peritoneal exudate cells (PECs) were washed out of the peritoneal cavity with 30 ml of Hanks' balanced salt solution (HBSS) containing 0·1% (v/v) heparin at various times after the injection of irritants or PHA, or the inoculation of microorganisms. Total numbers of the harvested live cells were counted by the trypan-blue dye exclusion method. The cells were washed twice and heat-inactivated guniea-pig serum was added to the pellet. Smears of the cells on slides were dried and stained with Wright–Giemsa stain. Polymorphonuclear leucocytes, macrophages and PPMs were counted under an oil immersion objective (× 1000).

Preparation of PECs for electron microscopy.—The pellet of PECs induced by thioglycollate medium was fixed in 1·0% glutaraldehyde in 0·15M sodium cacodylate buffer, pH 7·4, for 1 h in ice, left overnight in the buffer alone at 4°, and postfixed in 1% OsO_4 in 0·15M cacodylate, pH 7·4, for 1 h in ice. After dehydration with alcohol, the cells were embedded in Epon. Sections were examined with an electron microscope.

In vivo effect of colchicine on PPM.—Stock solutions of colchicine (Wako Pure Chemical Industries, Japan) were made up as 10^{-6}M solutions in PBS, pH 7·4, and stored at −20°. Practically 10 ml of 3% thioglycollate medium was injected i.p. on Day 0 and 5 ml of PBS containing 10^{-6} mol/kg colchicine, or PBS only, was injected i.p. on Day 1. PECs were obtained, stained and observed by light microscopy. To determine phagocytic activity of macrophages, 5 ml of PBS containing 0·1% (v/v) latex particles (1·09 μm in diameter, The Dow Chemical Co., Indianapolis, Ind.) was injected i.p. 30 min before harvesting.

RESULTS

PPM induced by irritants

Thioglycollate medium (Fig. 1) or oyster glycogen (Fig. 2) was injected i.p. on Day 0. Numbers of PMNs or macrophages in PECs were counted on Days 1, 2, 3, 4 or 5. The number of PMNs was maximal on Day 1 and rapidly decreased thereafter. On the other hand, the number of macrophages gradually increased. When thioglycollate medium was injected, the ratios of PPMs to total peritoneal macrophages were 23·5% on Day 2 and 18% on Day 3 (Fig. 1). The same ratio for PPMs induced by oyster glycogen was 18·5% on

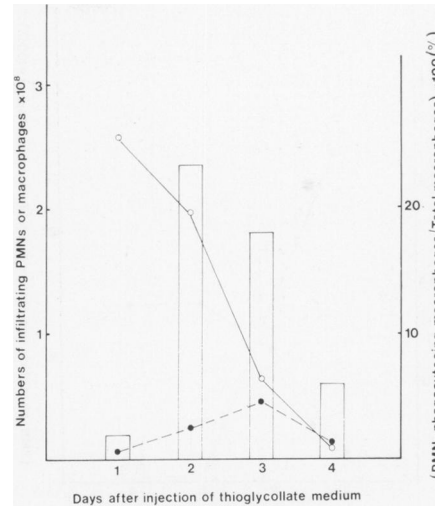

FIG. 1.—PPMs induced by thioglycollate medium. Guinea-pigs of Strain 13 were injected i.p. with 10 ml of 3% thioglycollate medium to induce chemical inflammation. At various times after the injection, PECs were harvested and numbers of PMNs (○——○) and macrophages (●---●) were counted. The percentages of PPMs to total peritoneal macrophages (open column) was calculated.

Day 2 (Fig. 2). PPMs were also induced by PHA 2 days after injection and their percentage was 18% (data not shown).

PPM induced by microorganisms

L. monocytogenes (Fig. 3) or *S. aureus* (Fig. 4) were inoculated on Day 0. The number of PMNs in the peritoneal cavity reached a peak 12 h after the inoculation and rapidly decreased thereafter. The ratios of PPMs to total peritoneal macrophages were 16% on Day 1 and 11% on Day 2 when induced by *L. monocytogenes*, and 26% on Day 1 and 16% on Day 2 when induced by *S. aureus*. The percentage of PPMs reached a peak earlier after inoculation of microorganisms than after injection of irritant. With our methods described above, PPM were examined from various standpoints.

Morphological study of PPM by electron microscopy

Large vacuoles in macrophages con-

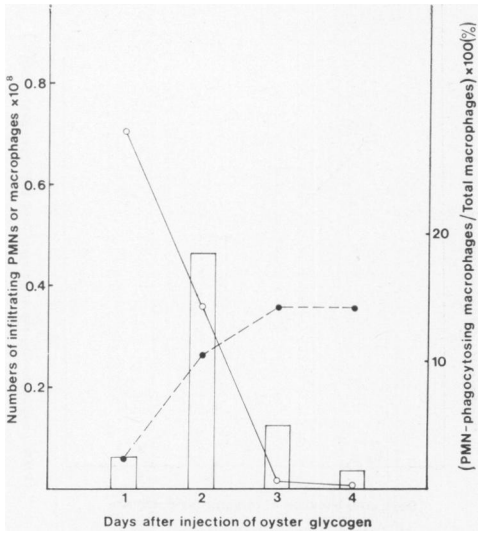

FIG. 2.—PPMs induced by oyster glycogen. Guinea-pigs of Strain 13 were injected i.p. with 10 ml of 3% oyster glycogen to induce chemical inflammation. At various times after the injection, PECs were harvested and numbers of PMNs (○——○) and macrophages (●---●) were counted. The percentage of PPMs to total peritoneal macrophages (open column) was calculated.

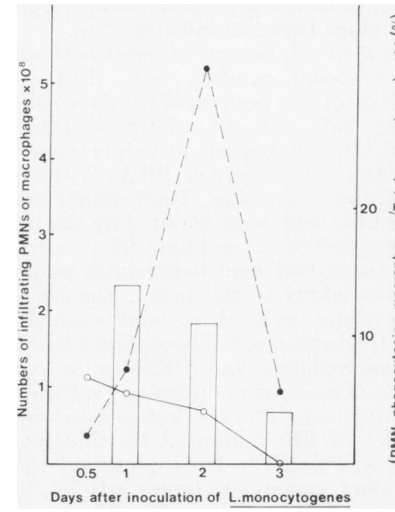

FIG. 3.—PPMs induced by *Listeria monocytogenes*. Guinea-pigs of Strain 13 were inoculated i.p. with 1×10^7 of *L. monocytogenes* to induce bacterial inflammation. At various times after the inoculation, PECs were harvested and numbers of PMNs (○——○) and macrophages (●---●) were counted. The percentage of PPMs to total peritoneal macrophages (open column) was calculated.

tained non-degenerated PMN, characterized by continuous cell membrane, marginated nuclear chromatin and electron-opaque cytoplasm (Fig. 5A), or degenerated PMN, characterized by disappearance of cell membrane, amorphous nucleus and electron-lucent cytoplasm (Fig. 5B). Some of the PPMs contained 2 or more PMNs.

Reinjection of oyster glycogen

Peritoneal exudate cells consisted predominantly of macrophages 3 days after i.p. injection of oyster glycogen. The number of PMNs was very low at that time (Fig. 6A, B). Oyster glycogen was reinjected 3 days after the first injection (Fig. 6B). The number of infiltrating PMNs increased on Day 4 and the percentage of PPMs also increased on Days 4 and 5. Infiltrating PMNs disappeared from the peritoneal cavity on Day 5 (Fig. 6B). These data suggest that PMNs reinfiltrate into the site of inflammation,

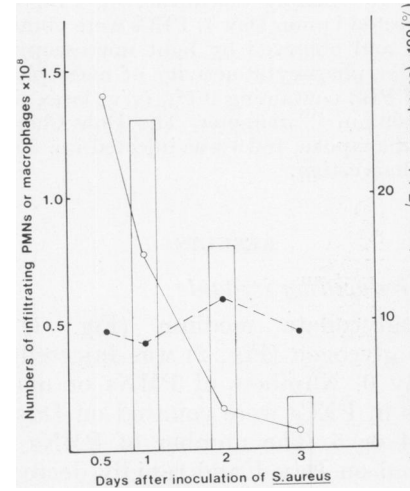

FIG. 4.—PPMs induced by *Staphylococcus aureus*. Guinea-pigs of Strain 13 were inoculated i.p. with 5×10^8 of *S. aureus* to induce bacterial inflammation. At various times after the inoculation, PECs were harvested and numbers of PMNs (○——○) and macrophages (●---●) were counted. The percentage of PPMs to total peritoneal macrophages (open column) was calculated.

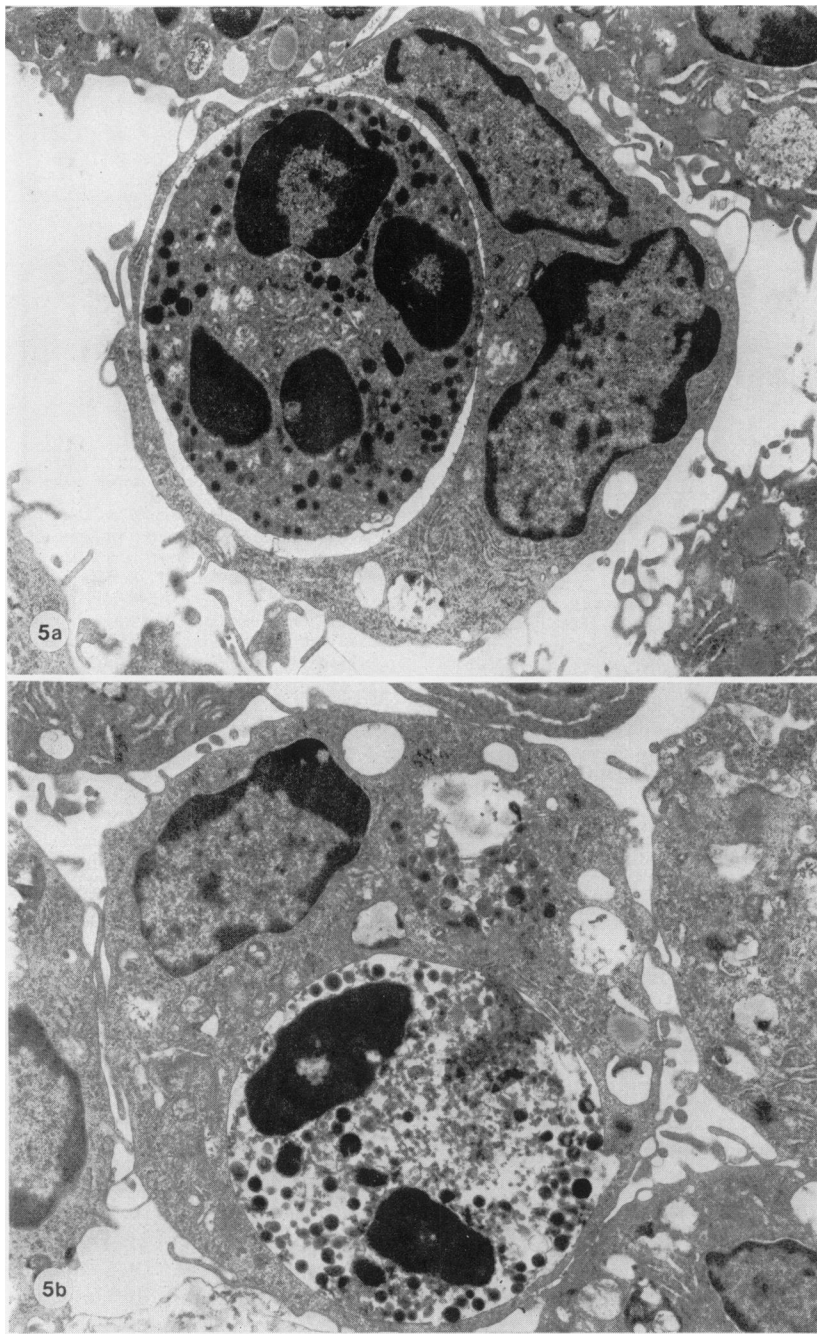

FIG. 5.—Morphology of PPM. Guinea-pigs of the JY-1 strain were injected i.p. with 10 ml of 3% thioglycollate medium to induce chemical inflammation. Two days after the injection, PECs were harvested, fixed and examined in an electron microscope. A macrophage ingests a neutrophil in its large vacuole. a: The ingested neutrophil may be intact, since its cell membrane is intact, nuclear heterochromatin is marginated and cytoplasm is electron-opaque. b: The ingested neutrophil may be degenerated, since its cell membrane has disappeared. The nucleus is amorphous and electron-dense, and the cytoplasm is electron-lucent.

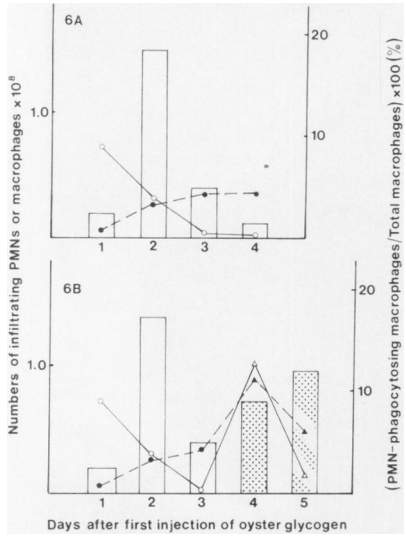

FIG. 6.—Reinjection of oyster glycogen. Guinea-pigs of Strain 13 were injected i.p. with 10 ml of 3% oyster glycogen on Day 0 once (A), or injected twice, on Days 0 and 3 (B). At various times after the injection, PECs were harvested. Numbers of PMNs (○, △) and macrophages (●, ▲) were counted. The percentage of PPMs to total peritoneal macrophages in guinea-pigs injected once (open column) or injected twice (dotted column) were calculated.

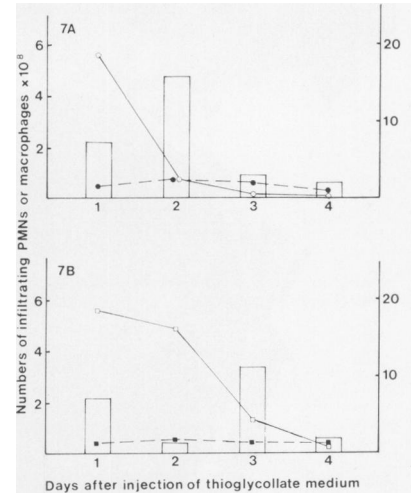

FIG. 7.—Effect of colchicine on the phagocytosis of PMNs by macrophages. Guinea-pigs of the JY-1 strain were injected i.p. with 10 ml of 3% thioglycollate medium on Day 0, with 5 ml of PBS only (A) or PBS containing 1×10^{-6} mol/kg colchicine (B) i.p. on Day 1, and their PECs were harvested at various times. Numbers of PMNs (○, □) and macrophages (●, ■) were counted. The percentage of PPMs to total peritoneal macrophages (open column) was calculated.

where inflammatory macrophages accumulate, and the macrophages can phagocytose reinfiltrating PMNs.

In vivo effect of colchicine

Thioglycollate medium was injected i.p. on Day 0 (Fig. 7B). On Day 2, there was hardly any decrease of PMN in the peritoneal cavity of colchicine-treated guinea-pigs (Fig. 7B). The percentages of PPMs on Day 2 were 16 without colchicine and 1·5 with colchicine. These findings show that macrophages of colchicine-treated guinea-pigs can phagocytose few PMNs in the peritoneal cavity, even when many PMNs are present.

Experiments were repeated in order to compare the effects of colchicine on the rate of ingestion of latex particles with that of PMNs (Fig. 8). Ingestion of latex particles by macrophages was not influenced by colchicine, while ingestion of PMNs by macrophages was inhibited.

DISCUSSION

Macrophages phagocytosing PMN were easily detected in large numbers when thioglicollate medium, oyster glycogen, PHA, *L. monocytogenes* or *S. aureus* was injected into the peritoneal cavity of guinea-pigs.

Several investigators have observed phagocytosis of autologous PMN by macrophages under various conditions. Speirs and Speirs (1963) found PPMs in the peritoneal cavity of mice which had been injected with radiolabelled neutralized tetanus toxin to determine the localization and fate of antigen. Taichman, Uriuhara and Movat (1965) demonstrated PPMs in rabbit skin or hamster cheek pouch after preliminary injection of *E. coli* endotoxin to produce a local Shwartzman reaction. In relation to human diseases, PPMs have been seen in the joint fluid of arthritic patients. Pekin *et al.* (1967) noted that PPMs were found only

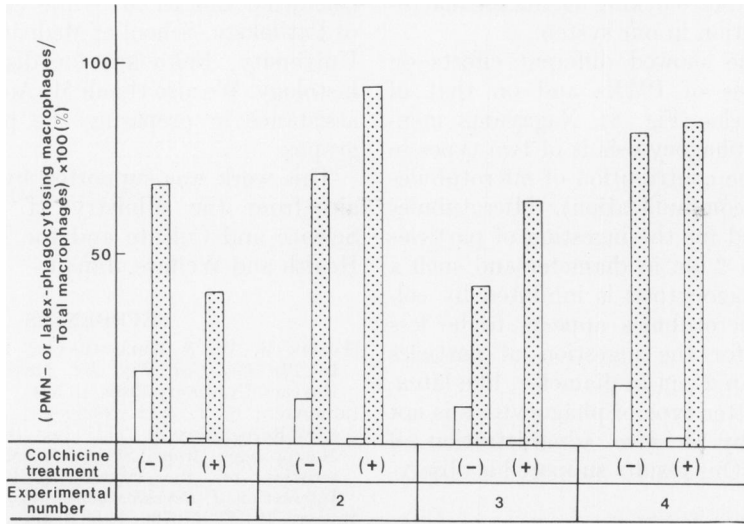

FIG. 8.—Effects of colchicine on the phagocytosis of PMNs and latex particles by macrophages. Guinea-pigs of the JY-1 strain were injected with 10 ml of 3% thioglycollate medium i.p. on Day 0 and 5 ml of PBS containing 1×10^{-6} mol/kg colchicine or PBS only i.p. on Day 1. Peritoneal exudate cells were harvested on Day 2. To investigate macrophage phagocytic activity, 5 ml of PBS containing 0·1% (v/v) latex particles was injected i.p. 30 min before harvesting. Percentages of macrophages phagocytosing PMNs (open column) or latex particles (dotted column) were calculated.

in Reiter's disease and they thought that their presence was valuable for diagnosis. However, further studies have shown that PPMs can be seen in inflammatory joint fluids not only in Reiter's disease but also in other diseases accompanied by arthritis, for example, rheumatoid arthritis or ankylosing spondylitis (Spriggs *et al.*, 1978). PMNs contain many types of enzymes including hydrolases, neutral proteases and myeloperoxidases. Degeneration of PMNs within a confined space in the tissue and release of such enzymes to the extracellular environment may give rise to tissue damage and the spread of inflammation. Our present data imply that macrophages contribute to the regulation of overaccumulation or autolysis of PMN at least to some extent.

As mentioned above, PPMs have occasionally been found in experimental and clinical conditions. But systematic studies of PPMs have not been carried out previously because PPMs could not easily be produced in large quantities.

Our study of PPMs by electron microscopy showed that some of the PMNs ingested by macrophages appeared to be fairly intact (Fig. 5A). If the PMNs were actually intact, it suggests that macrophages actively digest not only degenerated PMNs but also fresh PMNs, and contribute to the regulation of overaccumulation of PMNs to some extent. The results of reinjection of oyster glycogen may support this possibility, since PPMs increased with the appearance of newly infiltrating PMNs. However, we have not yet determined whether ingested PMNs are actually intact or not. We hope to answer this question histologically.

The appearance of PPM was blocked by *in vivo* administration of colchicine (Fig. 7). Colchicine is known to disrupt microtubules (Olmsted and Borisy, 1973) and impair macrophage phagocytic activity (Leibovich and Knyszynski, 1980). Our results imply that macrophages ingest PMNs *via* the microtubules. However, the inhibitory effect of colchicine on the appearance of PPM lasted only for 1 day (Fig. 7B). We need to examine the

effects of other blocking agents for macrophage function in our system.

Colchicine showed different effects on phagocytosis of PMNs and on that of latex particles (Fig. 8). Nagayama mentions that phagocytosis is of two types in terms of the contribution of microtubules (personal communication). Microtubules are required for the ingestion of particles larger than 2 μm in diameter and such a type of phagocytosis is inhibited by colchicine. Microtubules appears to be less necessary for the ingestion of particles smaller than 2 μm in diameter, like latex, and this latter type of phagocytosis is not inhibited by *in vivo* administration of colchicine. Our results support his observation.

When thioglycollate medium was injected i.p. into rats, hamsters and mice, PPMs were rarely seen. The reason for the difference in the appearance of PPMs in guinea-pigs and other species cannot be explained at the present time. The meaning of species difference has to be studied further to understand the role of PPMs clearly.

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REFERENCES

HARRIS, J. W. & KELLERMEYER, R. W. (1970) In *The Red Cell, Rev. Ed.* Mass, Cambridge: Harvard University Press. p. 524.

LEIBOVICH, S. J. & KYNSZYNSKI, A. (1980) *In vitro* Recognition of "old" Red Blood Cells by Macrophages from Syngeneic Mice: Characteristics of the Macrophage-Red-Blood-Cell Interaction. *J. Reticuloendothel. Soc.*, **27**, 411.

MOVAT, H. Z. (1979) The Acute Inflammatory Reaction. In *Inflammation, Immunity and Hypersensitivity: Cellular and Molecular Mechanisms.* Ed. H. Z. Movat. Maryland, Harper & Row. p. 1.

OLMSTED, J. B. & BORISY, G. C. (1973) Microtubules. *Ann. Rev. Biochem.*, **42**, 507.

PEKIN, T. J., MALININ, T. I. & ZVAIFLER, N. J. (1967) Unusual Synovial Fluid Findings in Reiter's Syndrome. *Ann. Int. Med.*, **66**, 677.

SPEIRS, R. S. & SPEIRS, E. E. (1963) Cellular Localization of Radioactive Antigen in Immunized and Nonimmune Mice. *J. Immunol.*, **90**, 561.

SPRIGGS, A. I., BODDINGTON, M. M. & MOWAT, A. G. (1978) Joint Fluid Cytology in Reiter's Disease. *Ann. rheum. Dis.*, **37**, 557.

TAICHMAN, N. S., URIUHARA, T. & MOVAT, H. Z. (1965) Ultrastructural Alterations in the Local Shwartzman Reaction. *Lab. Invest.*, **14**, 2160.