Polykaryocytes and Rheumatoid Disease

D. G. PALMER, MD, MRCP, FRACP, Senior Lecturer in Rheumatic Diseases, Department of Medicine, University of Otago Medical School, Dunedin, New Zealand

The enigma of rheumatoid disease is sufficient reason for examining any aspect of this condition that might have some bearing upon aetiology. This article draws attention to the presence of polykaryocytes within tissues involved by the rheumatoid process and among cells grown in culture from both articular synovium and from synovial fluid.

The terms 'polykaryocyte', 'giant cell', and 'multinucleated cell' are used synonymously. No well-defined distinction between large polykaryocytes and syncytial masses has been made, but the latter are very large, contain 100 to 300 nuclei, and are of irregular outline.

HISTOLOGICAL FINDINGS

Polykaryocytes have been recognised in synovium involved by rheumatoid disease, in the effusion that may accompany pleural involvement, and in subcutaneous rheumatoid nodules.

In the synovium, Grimley and Sokoloff (1966) described a 'rheumatoid' multinucleated giant cell characterised by peripherally located nuclei, an abundant eosinophilic central cytoplasmic zone, marginal PAS-positive granules and strong acid-phosphatase activity. These cells were thought to have been derived from Type A (phagocytic) synovial surface cells and could be distinguished from other forms of multinucleated cells that might be present, particularly foreign-body giant cells. They were usually found within a zone lying 25 to 100 microns below the synovial surface (Fig. 1). Such 'rheumatoid' multinucleated cells were associated with a positive test for rheumatoid factor. Donald and Kerr (1968) and Muirden (1970) have confirmed these findings.

Nosanchuk and Naylor (1968) found multinucleated cells to be a unique feature of pleural fluid smears obtained from patients with rheumatoid pleurisy. Such cells were found in pleural fluid samples obtained from five of ten patients with rheumatoid disease, but were absent from specimens obtained from over 1,200 patients with pleural effusions from other causes. From illustrations in their paper, these cells appear similar to the 'rheumatoid' giant cells of the synovium.

In the subcutaneous nodules of rheumatoid disease, multinucleated cells may be found at the periphery of the necrotic zone. It is doubtful if these cells are of specific significance in this situation since similar cells have been described in the nodules of rheumatic fever (Dawson, 1933).

Multinucleated cells may rarely be present in stained smears of effusion

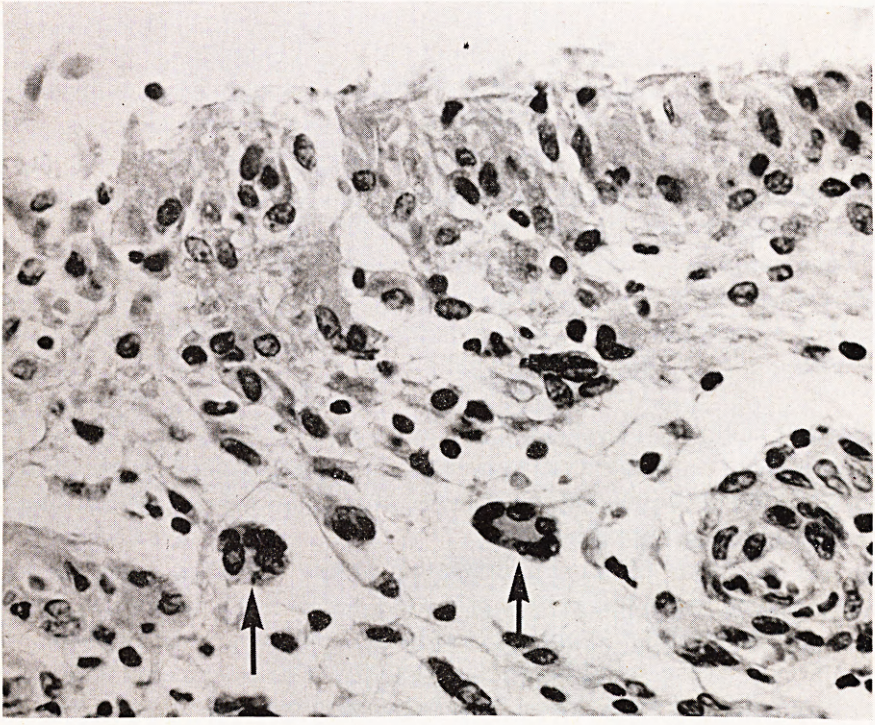

Fig. 1. Multinucleated cells (arrows) in synovium involved by rheumatoid disease (H & E × 620).

fluid aspirated from joints involved by osteoarthrosis and among the superficial synovial cells that are shed when trypsin is instilled into the knee-joint at post mortem (Williamson *et al.*, 1966). They have been described in the inflamed synovium of the joints of laboratory animals subjected to various forms of experimental synovitis (Gaunt and Thomas, 1966; Muirden and Peace, 1969). Their appearance under such circumstances indicates that occasionally some form of synovial giant cell may be present in the normal human synovium, and that multinucleation may, in the presence of a synovitis, merely reflect a non-specific cellular response.

CELL CULTURE FINDINGS

Polykaryocytes appear in explants of rheumatoid synovial tissue within the first few days of culture, but tend to be of late and irregular appearance in explants of non-rheumatoid synovium (Stanfield and Stephens, 1963; Bartfeld, 1965). Stanfield (personal communication) considers this difference

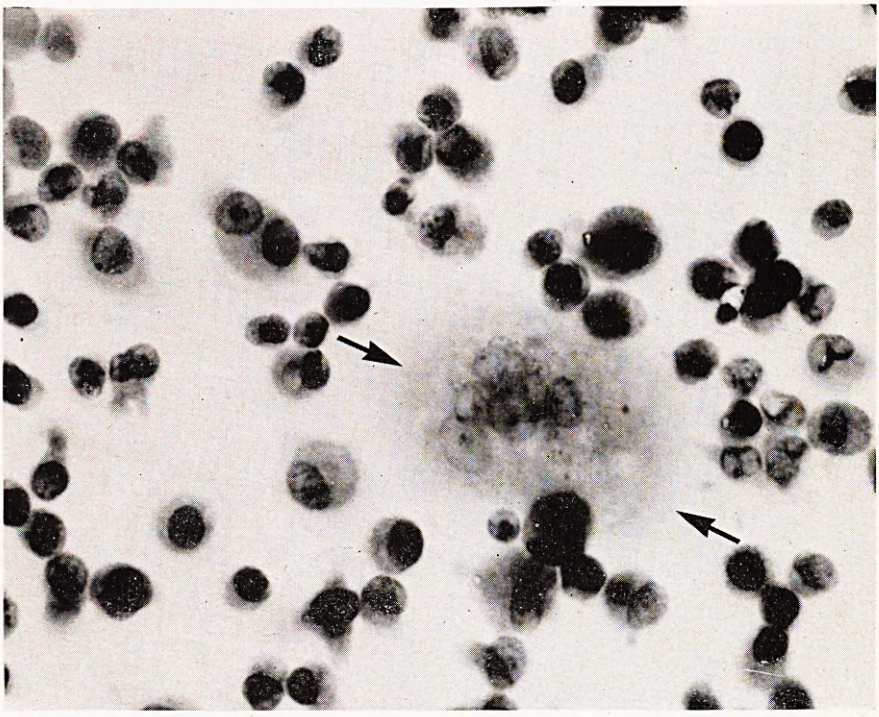

Fig. 2. A polykaryocyte (arrows) present in a 16-hour trypsin-dispersed culture of a rheumatoid synovium (H & E × 620).

in culture is closely related to the degree of inflammation in the explanted tissues.

In trypsin-dispersed cultures of rheumatoid synovium, polykaryocytes containing numerous nuclei may be present within the first few hours of culture (Fig. 2). These multinucleated cells appear to form by fusion of macrophage-type cells (occasionally by the fusion of fibroblast-type cells), for nuclei are occasionally seen within cytoplasmic bridges linking adjacent cells, and mitotic figures among the incorporated nuclei have never been observed (Palmer, 1970). This process is very rapid when contrasted with the develop-

ment of polykaryocytes from normal blood macrophages which occurs only after 10 and 25 days of culture (Berman and Stulberg, 1962).

When dispersion cultures of rheumatoid synovium are grown for two or three days in the presence of heat-inactivated rheumatoid serum, the formation of extensive syncytial masses is encouraged. These masses may be close to 1 mm in diameter, are of irregular outline, and contain very large numbers of

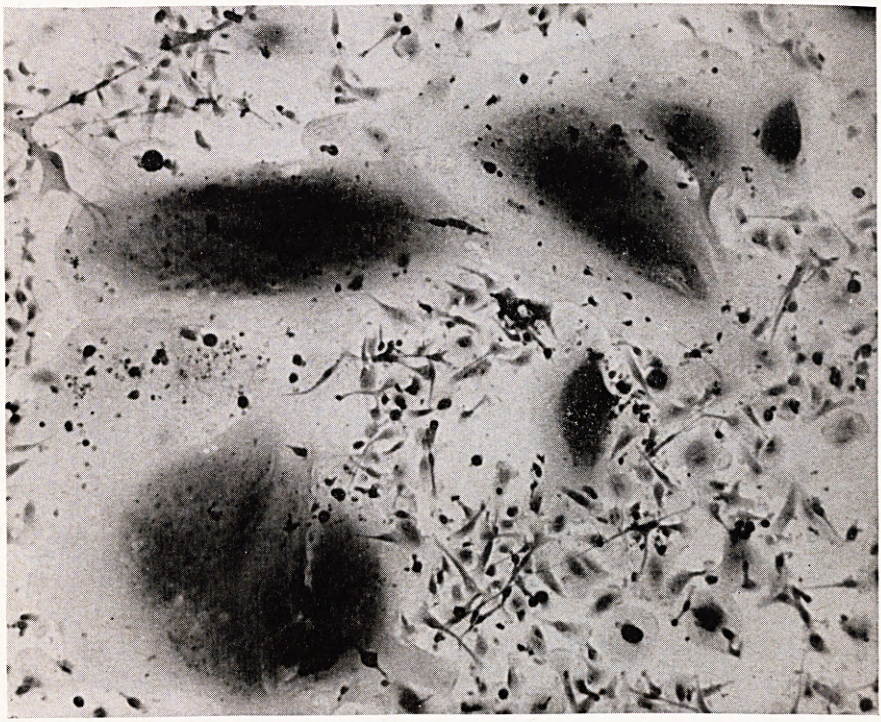

Fig. 3. Syncytial masses formed when cells from a trypsin-dispersed culture of a rheumatoid synovium were grown in the presence of heat inactivated rheumatoid serum. The magnification is only 25 per cent of that used in Figs 1 and 2 (H & E × 155).

nuclei (Fig. 3). They closely resemble the cytopathic effects that a number of viruses produce in cultures of particular cell lines. Because normal synovial tissues do not contain the infiltration of inflammatory cells seen in rheumatoid tissues, comparable dispersion cultures of normal tissues have not been successfully established and cultured under similar conditions. Multinucleated cells, however, were not described by Fraser and Catt (1961), nor by Williamson *et al.* (1966) in dispersion cultures of synovial cells collected from normal human knee-joints shortly after death.

Probably the best available control tissue for the purposes of comparison with rheumatoid synovium is normal lung, in that it contains not only fibroblasts, but also significant numbers of macrophages. Trypsin-dispersed cultures of normal human lung (obtained at thoracotomy) develop macrophages containing as many as 12 nuclei after three days of culture in the presence of heat-inactivated rheumatoid serum (Fig. 4), but large syncytial

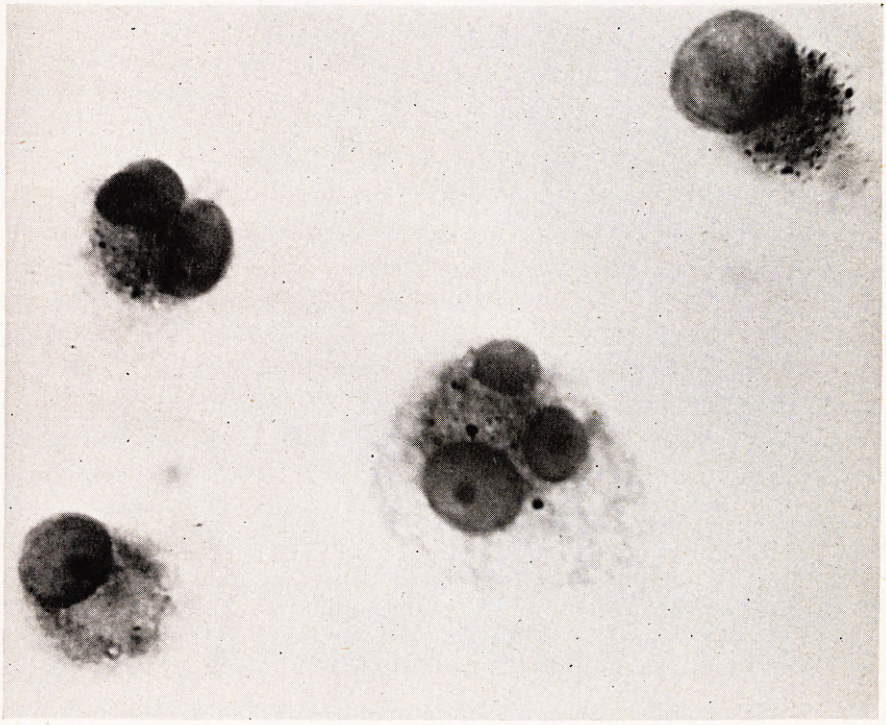

Fig. 4. Multinucleated macrophages, without the formation of syncytial masses, in a trypsin-dispersed culture of normal lung tissue. Culture continued for three days in the presence of heat-inactivated rheumatoid serum. Note the presence of intracellular carbon granules (H & E × 1080).

cell masses have not been seen to form even after ten days of culture. Thus, the formation of syncytia seems to be a rather special property of the macrophages of rheumatoid synovium.

The macrophage-polykaryocytes of rheumatoid tissues appear to affect the proliferation of fibroblasts in culture. It is possible, for example, to establish cultures from the cell population present in rheumatoid synovial effusions and from that in the effusions that may accompany osteoarthrosis. Polykaryocytes form from the macrophages that develop in culture from both

sources, but whereas no further change develops over a period of several weeks in those cells developed from rheumatoid fluids, those of osteoarthritic origin become overgrown by fibroblasts (Fig. 5) (Palmer, 1971). Furthermore, if numerous macrophages and polykaryocytes migrate from and encircle a cultured explant of rheumatoid synovium, a fibroblastic sheet does not form

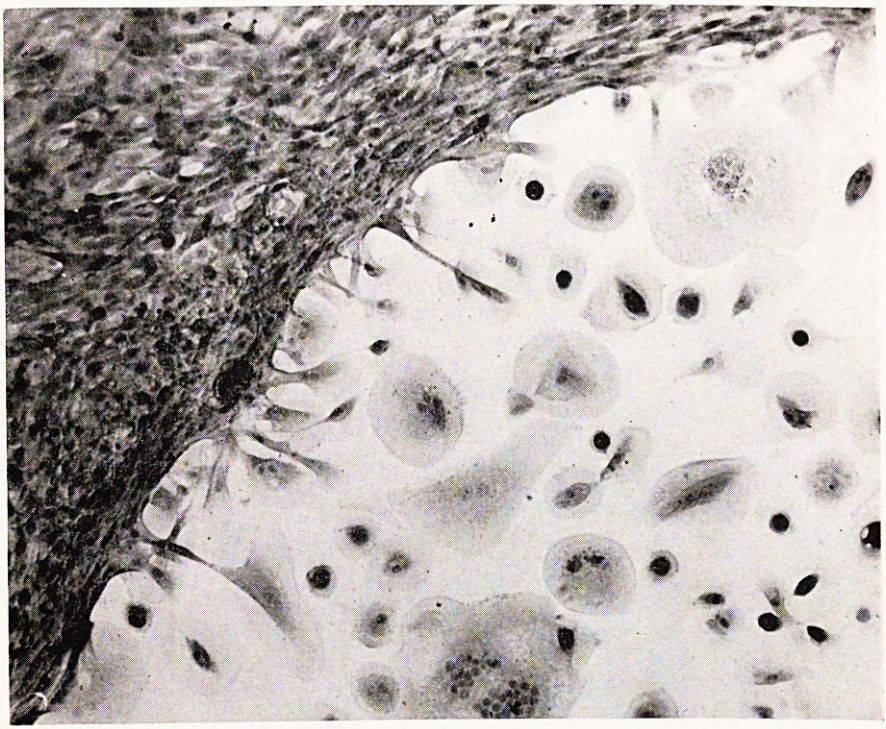

Fig. 5. A 14-day culture established from the synovial fluid of a joint involved by osteoarthrosis. A fibroblastic sheet has formed which has fortuitously retracted to expose underlying macrophages and polykaryocytes (H & E × 155).

about that particular explant. At the same time, dense fibroblastic outgrowths appear about other explants excised from the same fragment of tissue and cultured in the same vessel. These observations suggest that the macrophage-polykaryocytes developed from rheumatoid synovial tissue *in vitro* have some suppressive effect on autologous fibroblast-type cells. The mononuclear cells of rheumatoid synovial effusions also display cytotoxicity towards homologous fibroblast-type cells (Hedberg and Källen, 1964), although Maclennan and Loewi (1970) have implicated the lymphocytes in this.

Of the conditions known to promote polykaryocyte formation *in vitro*, that

which could be of the greatest significance *in vivo* is the possible presence of a virus. The phenomenon of polykaryocytosis as a cytopathic effect of a virus infection, both in host tissues and in cultured cells infected in the laboratory, has been reviewed by Roizman (1962a) and Poste (1970). The numerous reported failures to cultivate a virus from rheumatoid tissues do not necessarily invalidate this possibility. Roizman (1962b) found that a small, rather than a large inoculum of virus induced polykaryocytosis when added to a cell culture, and that anti-viral antibody might actually enhance the phenomenon. The virus need not be living (Henle *et al.*, 1954).

There have been many attempts to show that rheumatoid disease is an infective process, particularly a viral infection. It is well known that certain viruses may cause a polyarthritis (Walton, 1968; Ford, 1968). Hamerman and Smith (1968) reported that rheumatoid synovial cells in culture show partial resistance to infection by Newcastle disease virus, while Grayzel and Beck (1970) have reported a most significant resistance of similar cell lines to the virus of rubella. This resistance can be transferred to rabbit synovial cells (Smith *et al.*, 1970). Warren *et al.* (1969) have claimed the demonstration of an agent that causes a vasculitis in mice injected with rheumatoid synovial membrane homogenates, and which is transmitted to subsequent generations.

If direct evidence of a viral origin of this disease were to be found, such well-documented phenomena as auto-antibody formation, lysosomal activation (Goldfischer *et al.*, 1968), and the inherent metabolic changes of the rheumatoid cell so carefully documented by Castor and Dorstewitz (1966) would be readily explained. The polykaryocyte of rheumatoid disease may well be the cell towards which such a search might most profitably be directed.

SUMMARY

In vivo, multinucleated cells are a rather characteristic feature of the rheumatoid inflammatory response. *In vitro*, normal tissues that contain macrophages —including synovium, lung and blood—develop polykaryocytes when cultured. In dispersion cultures of rheumatoid synovial membrane this phenomenon is greatly accelerated and can be further exaggerated by supplementing the culture medium with heat-inactivated rheumatoid serum, suggesting the possibility of a viral cytopathic effect. When macrophages and polykaryocytes from rheumatoid synovium or fluid become established in culture, concurrent fibroblastic proliferation is suppressed.

Acknowledgement

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Marriage Guidance

No person who labours under any incurable malady ought to marry. He thereby not only shortens his own life, but transmits misery to others: but, when both parties are deeply tainted with the scrofula, the scurvy or the like, the effects must be still worse. If such have any issue they must be miserable indeed. Want of attention to these things, in forming connections for life, has rooted out more families than plague, famine, or the sword; and as long as these connections are formed from mercenary views the evil will continue.

In our matrimonial contracts, it is amazing so little regard is had to the health and form of the object. Our sportsmen know that the generous courser cannot be bred out of the foundered jade, nor the sagacious spaniel out of the snarling cur. This is settled upon immutable laws. The man who marries a woman of sickly constitution and descended of unhealthy parents, whatever his views may be, cannot be said to act a prudent part. A diseased woman may prove fertile; should this be the case the family must become an infirmary; what prospect of happiness the father of such a family has we shall leave any one to judge.

(From William Buchan's *Domestic Medicine*, fifth edition, 1776.)