Macrophage and Tissue Changes in the Developmental Phases of Secondary Lymphoedema and During Conservative Therapy with Benzopyrone

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Received October 26, 1989

Summary. The normal role that the macrophage plays in tissue homeostasis is presented along with the morphological and functional changes that occur to the macrophage population as the lymphoedema progresses from the latent to the chronic phase and then with the treatment with a representative benzopyrone called coumarin.

Underlying the lymphoedema, there is a chronic inflammation. It is this, in association with the accumulating protein and the subsequent alterations it produces in the tissues that attract monocytes and macrophages to the affected area. Despite the fact that macrophages are facultative anerobes, and that larger numbers than normal accumulate, the tissue conditions result in a depression in their activity levels. Apart from these tissue conditions there is the possible production of deactivating proteins such as transforming growth factor beta 1 and 2. Evidence for this deactivation comes from enzymatic studies in which levels of typical macrophage enzymes are reduced and from morphological work which has shown a reduction in pseudopods and a tendency to accumulate large amounts of lipid in their vacuoles.

As a consequence of this deactivation further protein accumulation occurs thereby osmotically attracting fluid. Also there is a tendency for the tissues to become fibrotic as the balance between collagen lysis and deposition shifts towards the latter since it has been shown that macrophages have an important role in collagen lysis.

The administration of coumarin stimulates the macrophages resulting in their return to normal or supranormal activity levels within the lymphoedematous tissues. As well as this there is an increase in macrophage numbers. The reasons for stimulation are uncertain, however, alterations in the fine structure of the proteins and complement which make these more attractive for phagocytosis seem the most likely. The end result is an rapid enhanced breakup of the excess interstitial protein and the removal of the osmotically attracted fluid together with a more gradual removal of the deposits of fibrotic tissue by the non-stimulated macrophage. Clinically this manifests itself as a softening of the tissues, a reduction in circumference of the lymphoedematous extremity, a return to normal tissue remodelling processes and a range of subjective improvements for the patient.

To date none of the experimental studies into the development of primary and secondary lymphoedema have been specifically directed at the macrophage-monocyte cell lineage. However, there have been many studies which have dealt with them as a secondary objective of other major investigations. This report attempts to bring together the findings relating to the underlying role of the mononuclear phagocytic system, and in particular the macrophage, in the development of secondary lymphoedema and in its treatment. Where information is lacking about macrophages in secondary lymphoedema, relevant findings in studies of other forms of oedema, including thermal wounding and primary lymphoedema will be used.

In order to appreciate the importance of the macrophage in lymphoedema it is first necessary to deal with a definition of lymphoedema.

In 1985 a consensus was reached by members of the International Society for Lymphology that a standard reference must be used for the future classification of lymphoedema. Accordingly that of Foeldi (1983) was accepted as being the most appropriate. Thus, lymphoedema is defined as "a high protein oedema caused by the combined lymph transport capacity and tissue proteolytic activity being less than normal while the lymphatic load remains normal" (Casley-Smith, 1985).
However, this is low output failure of the lymphatics and of the tissue proteolytic systems (in which the macrophages play a major role) which must be distinguished from high output failure when the lymphatic load is greater than normal but the lymphatic system and tissue proteolytic system capacities are normal or near normal.

Most often the situation is not so clear cut and the lymphoedema is associated with inflammation, the latter being the consequence of higher than normal levels of interstitial proteins (Casley-Smith and Casley-Smith, 1986). Under these conditions there is an increased lymphatic load, with reduced transport capacities leading to safety-valve oedema (Foeldi, 1983).

Lymphoedema is progressive; it has four underlying components; these being excess protein in the tissues and the concomitant oedema, chronic inflammation and associated excess fibrosis. In the early stages oedema predominates while in the latter stages it is excess fibrosis. The accumulated interstitial protein is the cause of the tissue oedema initially while in the latter stages it acts as a mediator of chronic inflammation. With time the fibrous tissues contract, interfering with the transport capacities of the remaining lymphatics, thereby worsening the condition. Simultaneously, the macrophages descend into a quiescent stage resulting in a reduced phagocytic function.

Macrophages can be specifically activated as a consequence of some type of immunologically specific stimulus or non specifically activated when there is no immunological involvement. While this area has been well covered (Nelson, 1972; Mauel, 1976; Piller, 1980) there are still some controversies. Most of these stem from the fact that macrophages are complex cells with multiple functions and that a change in one function may not be mirrored by a change in another. Here I will confine my discussions to the role of the action of the macrophage on the high protein oedema and the influence of the benzopyrones on these cells.

The normal course of events in secondary lymphoedema

These are summarised in the figure and have been dealt with in detail by Piller and Clodius (1978a, b, 1985). Briefly, the sequence of events is: A destruction through excision or irradiation of many of the lymph collectors. There may be a transient oedema. The situation thus induced is one of mechanical insufficiency (low lymph flow failure) of the lymph circulation. Associated with this are secondary functional and organic alterations (Foeldi, 1969). The resulting metabolic disturbance initiates changes in the functioning of the mesenchymal tissues, initially resulting in a network of collagenous fibrils followed later by a complex interdigitating network of fibrous tissue.

The initial deposition is in the areas surrounding the affected lymph vessels. The thickened fibrils cannot function to pull apart the lymph capillary endothelial cells and thus the condition worsens with a radial spreading out from the affected vessels. Even at this stage the arm usually is still in the latent phase (Piller and Clodius, 1978b). However, lymphangiograms taken at this time reveal growing problems of lymph stasis while electron micrographs show incompetent interendothelial lymph capillary junctions (Olszewski, 1977). At a similar time permanent open junctions can also be detected which lead to force pump insufficiency (Casley-Smith, 1964).

Due to the progressive spread of fibrotic tissues there is a constant change in the transport capacity of the remaining system. This is not so much of a problem except under conditions of a suddenly increased lymphatic load. There are many events and conditions which predispose this (Clodius, 1977b) ranging from local heating, a scratch, exercise and infection. As a consequence there is a sudden failure of the lymphatic system to carry the increased load. The protein which normally is removed by the lymphatics accumulates in the tissues and the subsequent raised tissue colloid osmotic pressure attracts fluid. The latent phase has now ended and lymphoedema is clinically manifest and can be measured by plethysmography and circumference changes.

The latent phase: Macrophage numbers

There are no clinical studies dealing with actual tissue macrophage numbers, however, a large number of experimental studies have provided detailed evidence in this phase. In a model of obstructive lymphoedema in dogs, Olszewski (1977) showed focal accumulations of mononuclear cells (a high proportion of which appear to be macrophages) around dilated lymph vessels in the first few months after the surgical intervention. In a similar model, in the first month after surgical intervention, Altorfer et al. (1977) showed marked phagocytosis by endothelial cells (which was not seen in forms of chronic lymphoedema) as well as large numbers of phagocytes in what they describe as "fibrinoid material within the lymphatics" as well as wide-spread accu-
mulations of PAS positive phagocytes distributed throughout the connective tissues of the epi- and subfascial compartments. This increase has also been confirmed in a model of lymphoedema of the rabbit ear six weeks into the latent phase (CASLEY-SMITH et al., 1977a), and at 3 months into the latent phase for a dog model of chronic secondary lymphoedema similar to that of CLODIUS and ALTORFER (1977) and CLODIUS (1977a).

So while it is well documented that in the latent phase of chronic lymphoedema there are increases in macrophage numbers, there has only been one unpublished study which has dealt with the quantitative aspects of tissue macrophage numbers. This study by MATISONS (unpublished), is summarised in CASLEY-SMITH (1983). In normal tissue of the skin and fascia there were $11 \pm 1.1 \times 10^6$ macrophages, while 24 h after the creation of acute lymphoedema the numbers significantly increased to $19.5 \pm 1.4 \times 10^6$ macrophages per cm$^3$. The numbers slightly, but not significantly increased in the muscle tissue from $5.2 \pm 0.5 \times 10^6$ to $8.5 \pm 1.3 \times 10^6$. It has been emphasised that if one was performing tissue culture work, this number would be quite substantial, however, in vivo when one considers all the other entities that must fit into 1 cm$^3$, the number of macrophages is enormous. Obviously then from an evolutionary point of view, such large numbers would not be found unless they had an important role to play in lymphostasis.

The clinically manifest phase: Macrophage numbers

As I indicated in the previous section, from a morphological point of view there is much evidence indicating a distinct boundary between the latent phase and the clinically manifest phase of lymphoedema (Fig. 1). However, clinically the tendency is to use circumference or volume differences between the normal and lymphoedematous extremities when this is possible or the presence or absence of pitting. There are a whole range of other means of detecting the clinical status of a lymphostatic disorder but most have drawbacks. There is direct and indirect lymphography (which may exacerbate the lymphoedema), computed tomography and nuclear magnetic resonance imaging (both of which are very expensive), quantitative lymphoscintigraphy (which currently lacks standardisation procedures) and direct fluid protein concentration sampling by needle (which also may exacerbate the lymphoedema). These and other methods are briefly reviewed in CASLEY-SMITH (1985). There is however, one remaining technique which is non invasive, easily standardised and very cheap, and that is tonometry. The instrument was first described by CLODIUS, DEAK and PILLER in 1976, and has been used in a wide range of clinical trials since to provide an accurate indication of the extent of fluid and fibrotic tissue accumulation. (CLODIUS and PILLER, 1976, 1978; PILLER and CASLEY-SMITH, 1989).

But what of the macrophages in this clinically manifest phase? Again human studies are sadly lacking, but a wide range of experimental models closely approximating clinical manifestations of lymphostasis have been investigated with often apparently contradictory results. ALTORFER et al. (1977) reported "phagocytes, frequently seen during the latent phase, are rare in chronic lymphoedema" while in 1980 CASLEY-SMITH, CLODIUS and PILLER reported a 50 fold increase in their numbers in the skin of a similar dog model of chronic lymphoedema. As well as this there have also been reports of increases in macrophages in the epifascial compartment of a model of chronic inflammation (CASLEY-SMITH and GAFFNEY, 1981).

In support of ALTORFER et al. (1977), PILLER and CLODIUS (1979) found only half the numbers of macrophages attaching to the skin side of subcutaneously implanted coverslips in conditions of chronic lymphoedema compared to the corresponding normal limb. Of great interest, however, was the fact that there were significant reductions in the lymphoedematous limbs of the ratio of macrophages to total mononuclear cell counts.

There are two possible explanations for these contradictory findings. It may be that in the dog model of PILLER and CLODIUS (1979) and ALTORFER et al. (1977), the level of oedema meant that macrophages were separated by large areas of fluid and were not as frequently seen and that their migration was slowed due to the greater distances they would have to travel through such tissues (CASLEY-SMITH and CASLEY-SMITH, 1986). Or, as in thermally injured tissues, phagocytic activity simply may have been depressed due to low tissue oxygenation, stagnating oedema fluids and acidic tissue conditions (PILLER, 1976b). In fact closer examination of the morphological characteristics of the coverslip macrophages has shown supporting evidence for this depression or inactivity in the form of a 66% reduction in macrophages with 2 or more pseudopods and a higher percentage with more than ten cytoplasmic vacuoles (PILLER and CLODIUS, 1979). In further support of this inactivity has been the evidence of CASLEY-SMITH and GAFFNEY (1981), and CASLEY-
SMITH, CLODIUS and PILLER (1980) which has shown macrophages containing large lipid deposits appearing like adipocytes, as if the macrophage had ingested too much protein initially and had converted it to fat.

In another model of chronic lymphoedema involving the rabbit ear model the findings also appear inconsistent. CASLEY-SMITH et al. (1977a) in a light microscopy study found an increase in macrophages 8 months after the creation of lymphoedema. They also mention a reduction in tissue protein and oedema levels. In a similar model with a similar duration of lymphoedema, PILLER and CLODIUS (1978b), in a study of tissue enzyme levels, found significant reductions in neutral proteinase and B glucuronidase and a reduction of acid protease. B glucuronidase is a typical lysosomal hydrolase of macrophages, acid protease activity levels closely approximate those of cathepsin D another well known macrophage hydrolase while neutral proteinases have been found to be secreted from macrophages (PILLER and CLODIUS, 1978b). What this suggests is either a reduction in the number of macrophages present in these tissues or perhaps a depression in their activity levels. Certainly there is support for both, in that there may be an increase in migration of macrophages into the lymphoedematous area but that soon after arrival their activity level is depressed and they become quiescent. It is noteworthy that it is unlikely that non stimulated macro-
phages will attach themselves to implanted cover-slips.

The normal role of the macrophages

As I mentioned earlier, lymphoedema is a progressive condition characterised by four main components namely, excess protein and fluid in the tissue, chronic inflammation and a tendency towards excessive fibrosis. But before I deal with the role of these cells under the conditions of lymphoedema, some additional background information is necessary.

Firstly, there are a large variety of stimuli which can give rise to the appearance of specific functions which when observed are usually referred to as an 'activated state' with respect to the macrophage. The stimuli can be divided into two main classes, that where the activation results from some immunologically specific interaction between sensitised lymphocytes and an antigen termed 'specific activation' and that where activation does not have an immunological basis called 'non specific activation'. Whether the stimulus for the activation is specific or non specific it must be remembered that the activity of any cell varies from one time to the next as its demands for its function by the body change. Also macrophages are complex cells with multiple functions and that a change in one need not occur in synchrony with other functions (PILLER, 1980).

Since inflammation would seem to be the primary reason for the alteration in the cellular population of the lymphoedematous limbs (CASLEY-SMITH and CASLEY-SMITH, 1986), I will summarise our current understanding of the role of the macrophage using the inflammatory reaction as the basis.

Normally the resident macrophages in inflamed tissues derive from circulating monocytes, however, a proportion of them originate from immature mononuclear phagocytes. During inflammation, not only does the number of circulating monocytes increase, but many of these migrate to the site of inflammation where they differentiate into macrophages. Monocyte production under these conditions is controlled by the humoral factor FIM, a protein synthesised and secreted by inflammatory macrophages (VAN FURTH, 1985).

It has been shown that these inflammatory and resident macrophages, in contrast to specifically activated macrophages, are not able to inhibit the growth of intracellular pathogens, nor are they able to kill tumor cells effectively. Perhaps more importantly for the point of our macrophage migration studies in lymphoedema (PILLER and CLODIUS, 1979), is the suggestion that they do not spread as readily in culture (JOHNSON et al., 1986) although the above comments should be taken with caution, in view of the use of poor descriptive terminology. What this means is that the majority of resident macrophages as well as most of those which enter inflammed tissues may not be functioning optimally for the duration of their stay. In other words their activity level and their effectiveness is depressed (PILLER, 1980).

Let's now look into the reasons why macrophages accumulate, why their functional activities become depressed and its consequences.

In 1970 WILLOUGHBY and DI ROSA proposed that one of mediators of chronic inflammation was the accumulation of plasma proteins which were in some way altered by their stagnation in oedematous tissues. It now seems fairly certain, that chronic lymphoedema is or has as an underlying component, chronic inflammation, since many of the changes observed in the tissues as well as vascular changes are also observed in inflammatory reactions (CASLEY-SMITH and GAFFNEY, 1981; CASLEY-SMITH, 1986). In terms of numbers, a $40 \times$ increase in macrophages superficial to the deep fascia was recorded along with the observation that the macrophages contain large accumulations of lipid, at times giving the appearance of adipocytes (CASLEY-SMITH and GAFFNEY, 1981). While COHN and BENSON (1965) have shown that excess proteins enhance the transformation of monocytes to macrophages and that protein alone will cause them to lyse more protein more effectively (EHRENREICH and COHN, 1967), there would seem to be some switching mechanism either associated with the inflammatory process or with some aspect of lymphoedema that causes a decline in their functional abilities (PILLER and CLODIUS, 1979; CASLEY-SMITH and CASLEY-SMITH, 1986).

With respect to the former it seems that products from activated macrophages can cause damage to the endothelium, fibroblasts and the parenchyma (TSUNAWAKI et al., 1988), pointing to the need for macrophage deactivators. TSUNAWAKI et al. (1988) has found two such deactivating proteins, transforming growth factor beta 1 and beta 2. Whether levels of these are elevated during the latter stages of thermal oedema and in lymphostasis yet remains to be seen. Another alternative explanation stems from reports in thermal oedema where by patients and animals who died through thermal injury had a depressed phagocytic capacity of their mononuclear phagocytic system, while those who survived showed normal or enhanced activity (RITENBURG and HANBACK, 1967).
Even though the macrophages are facultative anaerobes (Hunt et al., 1975), their activity is depressed, perhaps due to build up of lactate and hydrogen ions or other metabolic products. This form of deactivation may be separate from or in association with the deactivating proteins described by Tsunawaki et al. (1988).

While the details of the causes of this are beyond the scope of the current article, it must be mentioned that there are some similarities to be found between thermally induced oedema and lymphostatic (lymph) oedema in terms of tissue and cellular changes. The main similarities relate to the accumulation of a protein rich fluid and macrophages in the affected area together with significant vascular changes, and in later stages, a tendency to interference with tissue remodelling processes associated primarily with changes in the functional capabilities of the macrophages and fibroblasts. For detail about this I refer you to Casley-Smith and Casley-Smith (1986).

In lymphostasis as in thermal wounding, the foundation stimulus for the accumulation of the macrophages is the accumulation of protein and fluid in the interstitium (Foeldi, 1969, 1983).

Why are the macrophages present?

Phylogenetically, we can follow the progression in power of these cells in their attempt to degrade, remove or sequestrate the offending stimulus. Often however, as seems to occur in thermal wounding and lymphostasis, certain parameters of the extracellular environment influence the functional capabilities of the infiltrating cells. The end point is that the stimulus remains, and in the short term these cells pour out everything they have enzymatically in an attempt to remove the stimulus.

If this stimulus (the accumulated protein) remains, depending on the tissue recruitment rates of the macrophage and its proliferation, there will be an excessive (but well intentioned) outpouring and inappropriate production and release of enzymes and mediators. As has been shown earlier (Tsunawaki et al., 1988) this will cause tissue damage if not curtailed by the production of macrophage deactivating factors. So eventually the resident macrophage population is depressed but population levels are still greater than in normal tissues (Casley-Smith and Casley-Smith, 1986) even though this might not appear so when migration study results are examined (Piller and Cldius, 1979). These depressed macrophages are generally filled with larger numbers of protein or lipid containing vacuoles (Piller and Cldius, 1979), and may even take on the appearance of adipocytes (Casley-Smith and Casley-Smith, 1986).

But how do the changes in the extracellular environment lead to such processes as progressive fibrotic induration of the lymphoematous tissues as has been documented both clinically (Cldius and Piller, 1978) and experimentally (Casley-Smith, Cldius and Piller, 1980; Piller and Cldius, 1985).

A protein rich environment is one of the prerequisites for the formation and deposition of fibrosclerotic tissue and this means that the balance between the deposition and lysis of collagen is shifted in favour of the former.

While all of the available evidence indicates that fibroblasts synthesise collagen, there is not yet consensus as to the cells responsible for its reabsorption.

Some studies, indicate that the fibroblasts can participate in this reabsorption (Houck and Sharma, 1969; Deporter and Ten Cate, 1980), however, there is substantial evidence (Woessner, 1976; Keiser, 1980; Vaes et al., 1981), indicating that macrophages play an important role. SaltHouse and Matlaga (1972) found collagen lysis is maximal when histological examination indicated macrophage proliferation while Parakkal (1969), confirmed this collagen lysing ability by electron microscopical examination. Piller and Foeldi-Borscok (unpublished) have also observed macrophages attached in large numbers to collagenous fibrils in the early phase of lymphoedema. There are two pathways by which existing fibrous tissue is degraded (Woessner, 1976). The first involves the breaking off of fragments of collagen from the fibres and possibly a collagenase. Certainly, collagenases are produced by macrophages (Werb and Gordon, 1974). It is suggested that by close juxtaposition of the cell to the fibre surface, the process can be readily controlled. This would also mean that the micro environment between the cell and the fibre could be maintained at a pH near or at what is optimal for the more common macrophage enzymes (Woessner, 1976). The fragments are then phagocytosed where the breakdown process is completed within the macrophage.

The second pathway does not involve phagocytosis but only the extracellular release of enzymes (Cohn, 1975). The macrophage lysosomal enzymes mainly have their optimal activity in the pH range of 3-5, however, the main enzymes display a moderate activity even at a physiological pH (Woessner, 1976), thus indicating the possibility of extracellular breakdown of the collagen. The resulting fragments then enter the vascular system. But as we have seen, even
though the tissue macrophage numbers are increased in lymphoedema, their activity level is reduced. Part of the consequence of this is a shift in the balance between collagen deposition and lysis towards the former.

This alteration may be superimposed on another altered mechanism which will also tip the balance in favour of collagen deposition. As I indicated above, collagenase is responsible for the first phase of collagen lysis but its control mechanism seems clouded with uncertainty. Reynolds et al. (1977), suggested that connective tissues are able to synthesise specific collagenase inhibitors which exert local control on the enzyme. They believe that the balance between the active collagenase and its inhibitor will determine whether collagen is formed or removed. It is known that collagenase can exist in both latent and active forms, however, for the latent form to become active, a proteinase is required (Reynolds et al., 1977). Alternatively, the active enzyme may be bound to an inhibitor such as alpha-2-macroglobulin (Werb and Gordon, 1974) in a reversible manner (Shinkai et al., 1977). There seem to be many proteinases which have been recorded to activate the latent collagenase, some of the former of which originate from macrophages (Reynolds et al., 1977). Support for this mechanism comes from studies which have shown significant reductions in tissue levels of acid proteinase, neutral proteinase and B glucuronidase in chronic lymphoedema in which the lymphatics were partially blocked (Piller and Cloodi, 1979). However, this could be explained by the increased migratory distances that macrophages had to travel to attach to the coverslip and due to the fact that inactive or non-stimulated cells are unlikely to attach (Casley-Smith and Casley-Smith, 1986). In normal subcutaneous tissues, coumarin (a representative benzopyrone) has not only been shown to significantly increase macrophage numbers but also the percentage showing characteristics of stimulation (Piller, 1978).

The importance of the macrophage in oedema resolution

Silica is a selective macrophage poison (Pearsall and Weiser, 1970; Piller, 1976b). When silica is administered for a week prior to the creation of the oedema, coumarin no longer reduces the fluid content of the tissues (Piller, 1976b; Casley-Smith et al., 1977b, 1978). The reason for this, is that the excess protein is no longer lysed as has been demonstrated by quantitative electron microscopy using mass densitometry of protein concentrations (Casley-Smith et al., 1977b, 1978). A range of other benzopyrones have been shown to act similarly (Casley-Smith and Casley-Smith, 1986). So macrophages are crucial for the reduction of oedema by the benzopyrones.

The initial and continuing stimulation of the macrophage

The indications from experimental work are that benzopyrones very rapidly result in stimulation of the macrophage, which is evidenced by the extracellular
release of enzymes, enhancement of locomotion and chemotaxis, phagocytosis, lysosomal fusion and digestion (Piller, 1977, 1980; Casley-Smith and Casley-Smith, 1986). But what is the external signal which causes this rapid stimulation? While we have no direct evidence from studies with the benzo-pyrone, some other evidence provides a clue. Some forms of mild protein denaturation, which change its hydrophobicity can cause stimulation as can the binding of any substance with a nonpolar side group (Wilkinson and McKay, 1971; Wilkinson, 1974, 1976). Goldberg et al. (1976) has shown also that the charge on the protein is very important in that those which are most rapidly degraded are those which are least charge in the lysosomal interior. The avidity with which a protein binds to cellular membrane receptors of the phagocyte is also a function of its denaturation (Lloyd, 1976).

The benzo-pyrone binds to serum (Bauer-Staeb and Niebes, 1976; Piller, 1977d) and tissue proteins (Piller, 1977e), the extent of which is related to the number of phenolic groups (Bauer-Staeb and Niebes, 1976). The avidity for binding is dependant on the ability to form hydrogen and hydrophobic bonds with the proteins (O’Reilly, 1967). These structural changes make the proteins more attractive to the macrophage.

In addition, electrophoretic determinations (Piller and Schmidt, 1977) have shown that benzo-pyrone binds to complement factors and in so doing possibly alter the attractiveness of the latter with respect to phagocytosis, the end result being stimulation of the resident tissue or circulating phagocytes. The complement fragments released may also possess chemotactic activity and be responsible for further macrophage stimulation although this has not yet been proven.

The other alternative, as I suggested above is that the drug-protein or the drug alone, binds to the macrophage resulting in direct stimulation. If this is so, then stimulation is most likely to occur at the level of the circulating mature monocytes, which being chemotactically more sensitive, migrate into the lymphoedematous area where in a stimulated state, they then mature, differentiate and proliferate.

Once in this stimulated state, the extracellular release of enzymes may result in further enhanced complement fragmentation. That proteases can do this has been shown by Hill and Ward (1969), suggesting the important role for complement in a non immunological way. Schorlemmer et al. (1976, 1977) have supported the role of macrophages in such fragmentation by finding that stimulated macrophages can cleave the third component of comple-


---------: The influence of various benzopyrones on acid and neutral protease activity levels, the cells from which they may arise and their importance in the resolution of lymphoedema. Res. Exp. Med. 170: 115-124 (1977a).


