Lymphoedema, Macrophages and Benzopyrones

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Summary

The role of macrophages in lymphoedema is discussed, with particular reference to post mastectomy lymphoedema. In the latter, the normal course of events is detailed using clinical and experimental evidence. Particular importance is placed on the events of the latent phase since it is during this time that important changes are occurring in the functioning of the blood-tissue-lymph system. These changes are not usually demonstrable clinically until the end of the latent phase when lymphoedema becomes manifest.

Evidence suggests that the majority of these changes can be linked with changes in the functioning of the members of the mononuclear phagocytic system. Of particular importance is the disruption to the normal tissue remodelling processes as we know in lymphoedema the delicate balance between the deposition and lysis of collagenous fibres is shifted in favour of deposition — thus fibrosis occurs. The basic mechanisms behind such changes are discussed.

A group of drugs, called the benzopyrones have been shown both clinically and experimentally to be of benefit in reducing most forms of high protein oedemas including lymphoedema. It is shown that they can do this by stimulating the rather depressed functioning of the members of the mononuclear phagotic system. The exact mechanism of action of these drugs is discussed with particular emphasis on coumarin which is one of the components of Venalot.

Introduction

While little is known about the changes in the functioning of the blood-tissue-lymph system during the progression of lymphoedema, nothing is known about the reactions of the cell populations which constitute the affected tissues. Based on previous evidence from other forms of high protein oedemas it has become evident that the members of the phagocytic system (both fixed and free circulating) play an important role in maintaining the delicate balance between the maintenance of a healthy, normal functioning blood tissue lymph system and a diseased, inadequately functioning one.

In this light I will report on macrophage function in lymphoedemas, what determines their particular functional capacity and what changes to this capacity can mean in terms of the balance between health and disease.

1. Macrophage Activation

Before the importance of macrophages in untreated lymphoedema and the effect of benzopyrones is dealt with some background information is necessary.

There are a large variety of stimuli which can give rise to the appearance of specific functions relating to the expression of what is called the "activated state" in macrophages. These stimuli can be divided into two main classes

1: Those where activation results from an immunologically specific interaction between sensitized lymphocytes and antigen, this is termed "specific activation".
2: Those were activation does not have an immunological basis termed "non specific activation".

Let me now give you some more details about specific activation. It occurs as a consequence of an interaction between an antigen (microbial or otherwise) and specifically sensitised lineage cells (1, 2). The stimulated lymphocytes then release lymphokines which in turn may activate the macrophages. These lymphokines are non specific soluble factors (3).

This it can be generally claimed that some degree of macrophage activation follows every cell mediated reaction, although quite often it may not be recognised as a consequence of the apparent absence of a functional role for such an event.

What of non specific activation? there are many stimuli which will induce non specific macrophage stimulation although the end result of this activation depends somewhat one the stimulus. These stimulants are extremely well reviewed and I will go no further with them here (4, 5, 6, 7).

I will however mention that the benzopyrones are capable of causing non specific activation, but before I go into detail about this action on macrophages, some clarification of terminology must be made.

The activity of any cell varies from one time to the next as the demands for its function by the body change. Macrophages are complex cells and have multiple functions, thus it is important to remember that changes in one function need not occur in synchrony with changes in other functions.

For this reason when the term "activation" or "stimulation" is referred to, it is imprecise unless the function which is activated or stimulated is carefully defined.

Activation refers to the state in which the cell is endowed with the capacity to destroy or restrict the growth of in an immunologically non specific way, microbial and or non microbial targets (8). Activation in the above sense is accompanied by modifications in macrophage physiology (9), such as increased metabolic activity, production of enzymes, migration and motility, phagocytic capacity as well as the ability to mature and undergo mitosis. However, in the absence of formal proof of any increased cytotoxic activity, it is better to use the term "stimulation" when reference is made to any of the above biochemical or morphological alterations (8, 9a).

2 Role of macrophages in inflammation and lymphoedema

While lymphoedema could hardly be called "chronic inflammation", it is a progressive pathophysiological process in which inflammation may play an important role at various times. The stimulus for this instance is the excess accumulation of protein and fluid as a consequence of the failure of the lymphatic system to remove the awaiting load (10). The infiltration of macrophages and of other cells into the affected areas is intended to be of benefit to the host. Phylogenetically, one can follow the progression of the power of these cells in their attempt either to degrade, remove or sequestrate the offending stimulus. Often external environmental factors may influence the functional capability and state of well being of the infiltrating cell. The offending stimulus remains; frustrated, the cells pour out everything they have in an attempt to win the battle but often it is lost, the excessive (but well meant) and often inappropriate production and release of not only enzymes but also of mediators designed to attract more help for the purpose of removing the offending stimulus then leads to, either damage to the nearby host tissues or on a lesser scale to changes in the normal functional and remodelling processes of the tissues and of the cells which they are composed.

The work of *Cohn* (11, 12, 12a) makes it a well established fact that macrophages contain a wide variety of lysosomal enzymes and that their synthesis and secretion is strongly linked to extracellular stimuli. It is noteworthy that only potentially digestable material will cause significant increases in the intracellular lysosomal levels and result in their selective release. Inter particulate matter has no effect (13).

While acid hydrolases have been the star players involved in the digestion and removal of extracellular stimuli, neutral proteinases have only provided a supporting role. Recently the secretion of a number of neutral proteinases has been reported (14) two of which, were characterised in terms of the natural substrates of collagen and elastin. *Davies* (15) has claimed that while acid hydrolases are released rapidly in response to an external stimuli, neutral proteinase release is slower, albeit continues for a much longer duration. However, while acid hydrolases can easily be released even from unstimulated macrophages (9, 15).

Neutral proteinases seem to require some form of stimulus, either inflammatory or phagocytic (15).

Now I will examine possible roles of macrophages in untreated lymphoedema and then the effect of benzopyrones on them.

Firstly, just how important are macrophages in lymphoedema? Thermal and other inflammatory stimuli, particularly of the subacute or chronic nature are characterised by an accumulation of mononuclear cells (16). In lymphoedema the situation is somewhat similar (17), although as yet there is no accurate indication of the numbers of mononuclear cells, their rate of entry or of their longevity.

Olzewski (18) has recorded the presence of focal accumulations of macrophages around dilated lymphatic vessels in the period of latent and chronic lymphoedema. While more direct evidence of their presence is needed there is accumulating a large body of indirect evidence.

3. The normal course of events in post mastectomy lymphoedema

The radical excision of mastectomy and (often) subsequent irradiation results in a destruction of many lymph collectors, the extent of which depends on the anatomical variation of the lymph collecting system of the upper arm of the individual patient. A higher number of collectors generally means a greater chance that some may be preserved.

With the excision, there often follows a transient oedema. In some patients this persists while in others it resolves, only to become manifest at some later time as a consequence of a failure of the mechanisms which normally allow adequate lymph drainage from the arm.

The resulting mechanical insufficiency of the lymph circulation (low-lymph flow failure) results in secondary functional and organic alterations as described by Földi (10). The subsequent metabolic disturbance causes the mesenchymal tissue to react, resulting first in the formation of fibrils followed by a complex interdigitating network of fibrosclerotic tissue (Földi-Börscök and Piller, pers. obs.). Initial deposition is in the regions immediately surrounding the affected lymphatic vessels. The thickened sclerosed fibrils cannot function to pull apart the lymph capillary endothelial cells, thus more protein accumulates and the condition worsens, spreading radially out from the affected vessels.

At this time the arm is still in the latent phase.

Lymphangiograms reveal growing lymph stasis despite the absence of clinical lymphoedema. Olszewski (19) has shown electron micrographs of lymph capillaries which show incompetence on interendothelial junctions. Casley-Smith (20) has shown the presence of permanently open junctions leading to force pump insufficiency.

The continual spreading of the fibrosclerotic tissues results in a constant change in the transport capacity of the remaining system (the latter of which is the product of the total cross section of the functioning lymph vessel system and the lymphokinetic forces responsible for the propulsion of the lymph).

Any sudden increase in the lymph load awaiting transport (due to exercise, local heating, infection a cut or a scratch) can result in a sudden failure of the system to carry the load. The protein which cannot be removed remains in the tissues resulting in a raised colloid osmotic pressure and accumulation of the fluid. The latent phase thus ends: lymphoedema is now clinically demonstrable by plethysmographic or circumference measurements.

Lymphoedema, broadly taken is a consequence of the failure of the lymphatic system to remove all of the awaiting lymphatic load; protein rich fluid thus accumulates forming poorly oxygenated stagnating areas. There is the subsequent accumulation of metabolic products and conditions approach those of metabolic acidosis. Although there is no direct evidence that I know of in lymphoedema, in an analogous condition of thermal injury where there is stagnation of fluid and metabolic products, the phagocytic activity of the fixed phagocytic members is depressed (21, 22, 23).

The peripheral (circulating) mononuclear population is little effected. Thus in lymphoedema we may have the accumulation of mononuclear phagocytic cells, some of which may have been stimulated when they entered, but which have now a phagocytic activity which is depressed. Even though further cells are always entering, they soon loose their functional capabilities. So protein, cellular metabolic products and cellular debris accumulate, since firstly it cannot be removed by the lymphatic system and secondly because the cells which also normally play an important role in its removal are essentially nullified. It seems that upon entry into such tissues the phagocytic cells in the majority become engorged with protein, enter a quiescent stage during which time they may or may not complete digestion of the engulfed material (24). Since in lymphoedema in the regions of fluid accumulation, the intercapillary distances are increased, both the chance of the laden cell or of the products of digestion to escape the area is reduced.

4. Fibrosis — the balance between deposition and lysis

A protein rich environment is one of the prerequisites for the formation and deposition of fibrosclerotic tissue (25); basically 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 are some different notions as to the cells responsible for its reabsorption.

There is, however, some very substantial evidence which shows that the macrophages can play an important role. Salthouse and Matlaga (26) have shown that collagen lysis is maximal when histological examination reveals macrophage proliferation, while Parakkal (27) confirmed the collagen lysing ability of macrophages by E/M examination. Földi-Börscök and I have also observed macrophages attached in large numbers to collagenous fibrils in the early phase of lymphoedema although there was not always the presence of phago-

lysosomal vacuoles nor of pseudopodic extensions. Possibly these cells were stimulated at the time of their migration to the site but this has since ceased.

There are two pathways by which the existing fibrous tissue has been shown to be degraded (28). The first involves the breaking off of fragments of collagen from the fibres and possibly involves the action of a collagenase. By very close juxtaposition of the cell surface to the fibre surface, this process can be readily controlled and would eliminate the loss of enzyme, since it would be taken up again by the cell along with the fragments of collagen. The second pathway does not involve phagocytosis but only the extracellular release of enzymes. The fibrotic tissue is digested extracellularly until the fragments are small enough to diffuse into the vascular system.

In lymphoedemas, since macrophage activity may be depressed in varying degrees, these processes will be slowed or halted, further turning the balance in favour of collagen deposition. The end result is the continuing fibrotic induration progressing towards the hard late phase of lymphoedema if not treated.

5. Collagenases: origins and actions

Collagenases are responsible for the degradation of native collagen (which is the major structural protein of connective tissue). The result is its fragmentation, usually at specific points of the helical region of the collagen chain. Collagenase is certainly responsible for the first phase of collagen lysis, however, the exact mechanism by which the activity is controlled is still clouded with doubt. Reynolds et al. (29) have suggested that the connective tissues are able to synthesize specific collagenase inhibitors which will control the activity of the enzyme locally. They believe that the balance between the active collagenase and its inhibitor determine whether collagen will be formed or removed (this is the balance between its lysis and deposition).

Collagenase can exist in both latent and active forms in the various tissues. For the latent form to become active a proteinase is required (29) or alternatively, the active enzyme may

be bound to an inhibitor in a reversible manner (30); alpha-2-macroglobulin is one such potent inhibitor (31).

There are many proteinases which have been recorded to activate the latent collagenase (reviewed *Reynolds* et al. (29). Thus any changes in these enzymes will result in corresponding changes in the delicate balance between collagen deposition and lysis. As I have dealt with earlier, it is well known that in chronic lymphoedema there is a progressive fibrotic induration into the tissues, the prime reason being a shift in the balance towards collagen deposition, not necessarily because of an increased rate of deposition but because the normal mechanisms responsible for its lysis are suppressed.

The immediately obvious reason for this would seem to be either a reduction in the availability of the proteinases necessary for activation of the pro-enzyme or an increase in inhibitory substances like alpha-2-macroglobulin (31, 32).

Although the exact importance of the macroglobulines in lymphoedemas has not been ascertained, there are some indirect observations which are extremely interesting. Firstly, in acute lymphoedema the extracellular acid and neutral proteinases activity levels in both the skin and the serum are depressed compared to the normal (33).

Evidence suggests this is a consequence of their deactivation (34) possibly due to their trapping in the alpha-2-macroglobulin molecules (35).

6. Effect of benzopyrones on macrophage activity and function

The "activation" of cytotoxic activity in macrophages requires days or even weeks". However, their "stimulation" occurs immediately on contact with the appropriate signal from outside of the cell. These immediately stimulated events include the extracellular release of enzymes, the enhancement of locomotion and chemotaxis, phagocytosis, lysosomal-phagosomal fusion and digestion (36). What types of external signal will cause this immediate stimulation? *Wilkinson* (36) has sug-

gested substances which have an affinity for the hydrophobic interior of the bilaminar layer of the cell membrane. Some forms of mild protein denaturation which increase its hydrophobicity have been demonstrated to cause such stimulation (37, 38).

In fact the binding of any substance with a nonpolar side group to the protein performed a stimulatory function (39, 39a).

Goldberg et al. (40) have shown that the charge of the protein is very important in the determination of its half life. Those most rapidly degraded are those which are least charged in the lysosomal interior. The aviditiy with which a protein binds to the cellular membrane receptors is also a function of its denaturation (41) being related as mentioned above to the extent of exposure of hydrophobic residue.

I will now examine the effect of benzopyrones on proteins which the macrophages are to phagocytose and their effect on the cells themselves.

The benzopyrones can certainly bind to serum (41, 43, 44) and tissue proteins (45), the extent of which is dependant and closely related with the number of phenolic groups (43). Their avidity for binding is dependant upon the ability to form hydrogen and hydrophobic bonds with the proteins (46). The benzopyrones, by binding to the proteins in this way may help in making them more "attractive" for the phagocyte.

Electrophoretic determinations (47) have shown that benzopyrones bind to complement factors and in doing so possibly alter the "attractiveness' of the latter with respect to phagocytosis and subsequent fragmentation by the resident tissue or circulating phagocytes. The fragments may possess chemotactic activity and be responsible for stimulation.

Alternatively, there is the possibility as described earlier, that the binding of either a drug protein complex or the benzopyrones directly to the macrophage membrane results in stimulation.

If so, then this event would most likely occure at the level of the circulating mature

monocytes before they actually enter the affected area. In this respect it may mean that not only will a higher proportion of these cells become stimulated but also that they are chemotactively more sensitive. Some evidence shows the accumulation of more macrophages in benzopyrone treated lymphoedematous limbs compared with untreated ones.

Once in the stimulated state, the extracellular release of enzymes may result in further enhanced complement fragmentation.

Hill and Ward (48) have shown the presence of proteases which can cleave the third component of complement into active fragments. Suggesting a possible importance for the role of complement in a non immunological way. Schorlemmer et al. (49, 50) have shown that macrophages in their stimulated state can cleave the third component of complement giving rise to C3b which can further stimulate macrophages. The benzopyrones by binding to complement and altering its "attractiveness" may thus start off this cascade of events.

While we still have not solved the exact importance of the various ways in which the benzopyrones stimulate the phagocytic activities of the macrophages we have plenty of concrete evidence that the benzopyrones do in fact stimulate macrophages. This evidence comes in the form of all the cardinal signs of stimulation. They increase the rate of phagocytosis and subsequent rapidity and completeness of digestion of protein, they increase the migration and motility as well as the extracellular release of enzymes (Reviewed Piller, 51).

In lymphoedema it is well evidenced that benzopyrone administration enhances the removal of the accumulated protein (52, 53) by causing its lysis (51).

7. Drug induced proteolysis

The lysis of the accumulated protein, means the fragments can now leave via the blood vascular system and are no longer dependant on the lymphatic system for their removal. Because the protein leaves, so too does the accumulated stagnating fluid which was previously held by virtue of the osmotic action of the former.

Oxygenation improves, and normal functional activities are restored.

This form of proteolysis has been termed drug induced proteolysis. Its importance has been emphasized by the very strong correlations between the extra-cellular protease activity levels and the oedema reducing ability of various anti-inflammatory drugs (54, 55).

The importance of the macrophages is shown by the events which occur following their destruction by silica (56). Under these conditions the oedema was not reduced.

However, often when talking of induced proteolysis, the idea of causing further injury or disruption arises. This form of proteolysis is very much different from that used by pharmacologists as a measure of inflammation. Normal proteolysis of inflammation represents a measure of the spillage of cell contents and serves as a non specific indicator of tissue and cellular derangement, but that induced by drug action such as the benzopyrones is a limited or controlled proteolysis aimed specifically at reducing some of the more injurious effects of this derangement and to help in the restoration of normal functioning of the blood tissue lymph system. An explanation of those events is given in Piller (51).

8. The action of coumarin (a representative benzopyrone) on macrophages

What evidence either direct or indirect do we have for the action of the benzopyrones on macrophages?

Firstly, Kovach et al. (57) have shown that the administration of melilotus compounds enhance the clearance of carbon from the blood. This ability is held to be due to a stimulation of the mononuclear phagocytic system of which the macrophages, both free circulating and fixed tissue are the principle components. The liver is one of the most dense concentrations of potentially phagocytic cells of the body. Daoust (58) in fast showed that about 30% of its weight consisted of these cells. Thus the effect of the benzo-

pyrones in these cells can be used (albeit with reservations) as a magnifying glass to gain an indication of their possible effect on cellular components elsewhere in the mono-nuclear phagocytic system (M.P.S.).

Stimulators of the M.P.S. can be divided into two broad groups. Those which increase the numbers of phagocytic cells (consequently resulting in an increase in the number of pha-gocytic sites) and those which have little or no effect on cell numbers but which increase the probability of partial engulfment (59, 60).

Those of the former group are known to in-crease liver and spleen weights (59).

Coumarin (a benzopyrone) has been shown to increase liver weights after 48 hours. In one experiment the increase was 13% (.01 < P < .05). Taking into account the data and calculations of Wish et al. (61), Daoust (58), and St. George (62) about stimulators of the mononuclear system, this result represents a possible increase of 60% in phagocytic cell numbers in the liver alone. Since the resident cells of the mononuclear system are known not to divide this must in the majority be the consequence of entry of large numbers of cir-culating mononuclears.

Coumarin has also been shown to enhance the removal of radio labelled homologous protein as well as polyvinylpyrrolidine from the blood after thermic injury lasting for six hours (63).

The benefit of coumarin in lymphoedemas resulting from total lymphatic occlusion has also been shown. At this point it is necessary to make a small regression to emphasize its importance. It is well known that from half to all of the total circulating protein enters the extracellular spaces every 24 hours. Most of this protein does not re-enter the vascular system unless delivered by the ductus thora-cous to the venous system. Generally, lymph-oedema is associated with a greatly reduced protein clearance (52).

Such a situation occurs after radical mastec-tomy where often lymph nodes are either re-sected or irradiated thus destroying their func-tional capabilities. The latter also prevents re-generation of these structures. While there have been reports which have shown improve-ment of protein outflow from the affected limb following microvascular surgery, the buried dermal flap operation and the staged subcutaneous excision, most of the procedures are tedious, not always available and anatomi-cally unexciting. In addition, there must be considered the patients' desire to go through with the operation as wells as the inevitable re-occurrence of the lymphoedema.

Studies with the benzopyrones have shown that they remove the need for a functioning lymphatic system (25). Thus an experiment was designed which involved a total occlusion of all lymph vessels leading from the limb (52).

The rate of removal of subcutaneously inject-ed ^{51}Cr homologous protein and ^{125}I labelled PVP was followed over 48 hours. The dis-appearance rate of the protein and PVP were represented by

$$C_t = C_o \exp(-kt)$$

while these results are very interesting I be-lieve that the ratio of protein/PVP is most sig-nificant, since the PVP behaves as a similar sized but non metabolizable control for the protein, thus accurately controlling the vari-ous conditions present in the individual legs. The ratio Protein: PVP could be approximated to

$$(c_p/C_{PVP})_t = (C_P/C_{PVP})_o \exp - (k-k)$$
$$= (C_P/C_{PVP} \exp - K)$$

considering only the group with lymphoedema K_p was 1/2 that of the normal group, but treatment with coumarin significantly improved it to a level which was not significantly differ-ent from the untreated control. K_{PVP} was not significantly affected by coumarin treatment of the lymphoedema group.

More importantly, the ratio Protein/PVP. This showed that in the lymphoedema group treat-ed with coumarin, it is very significantly im-proved.

Thus using a model of the acute phase of lymphoedema, which involves a total occlusion of all lymphatic vessels leading from the limb,

it has been shown that coumarin treatment can restore the clearance of protein to near normal levels.

This removal without the aid of a functioning lymphatic system suggested that the drugs action was one of causing lysis of the accumulated protein the fragments of which could then leave the tissues the way the protein originally entered. The predominant cells involved in this lysis seem to be the macrophages and as has been shown elsewhere in this paper, they are quite a common cell of lymphoedematous lesions.

Before I go into the complexities of the action of coumarin under in vivo conditions I would first like to detail some results of in vitro experiments which help to confirm the important role that macrophages play in the lysis of protein.

Firstly, coumarin alone does not cause protein lysis by a direct action even following incubation with protein for 50 hours (53).

Thus in order to function it needs the tissues and the cells within.

Using cell culture techniques involving macrophages it was shown that while there is some normal lysis of protein by these cells, it is dramatically enhanced when coumarin is added to the medium. An increase to 220% has been reported (64). These findings are similar to those demonstrated by *Houck* and *Sharma* (65) for other antiinflammatory drugs on fibroblasts. Since fibroblasts are also an important constituent in lymphoedema — they are responsible for the formation and deposition of the collagenous tissues — attempts have been made to determine the effect of coumarin on these cells, to date without reproducable success due to difficulties in maintaining stable cultures.

Dunn et al. (66), have investigated the effect of coumarin on macrophage phagocytosis and have found an increase in their phagocytosis of latex particles of 38% following 2 hours of incubation. Using subcutaneously implanted coverslips to assess macrophage numbers and function in an *in vivo* model *Piller* (67), has shown that coumarin administration not

only results in more macrophages migrating and attaching to the coverslip, but that a higher proportion are stimulated compared with the untreated animals. This applied only for the skin side, why this should be so is not known. A repetition of the same experiment with Venalot (containing coumarin as well as sodium rutin sulphate salts) showed that as well as a significantly higher number of macrophages there was a significantly higher percentage which was stimulated. This applied for the coverslips facing the skin and muscle sides.

Previous studies of lymphoedema by *Olszewski* (19), have shown focal accumulations of mononuclear cells around the affect lymph collectors. *Casley-Smith* et al. (68), have found macrophage accumulations in the rabbit ear model. *Piller* and *Clodius* (69) using a similar model have shown that while more macrophages may be accumulating, fewer of them are in the stimulated or active state. Other experiments involving the migration of monocytes onto subcutaneously implanted coverslip in the limbs of dogs with chronic lymphoedema have also shown some interesting results. *Piller* and *Clodius* (24) have found there was no significant difference in the total mononuclears migrating onto the coverslips in the lymphoedematous or normal limbs. However, on examination of the percentage distribution of the macrophages and of their morphological characteristics some interesting differences arose. Irrespective of whether the skin facing or the muscle facing side of the coverslip was examined the total number of macrophages in the lymphoedematous limbs was about 50% of the corresponding normal limbs.

Similarly, macrophages with pseudopods were only about 40% the number in the normal limb. Since pseudopods are one of the major morphological characteristics used to assess whether the macrophage is stimulated or not, it would seem that in lymphoedema not only is the monocyte macrophage ratio reduced but fewer of the macrophages are in an active state.

Another point from this work was that when the various morphological characteristics of the macrophages (pseudopods and numbers

of vacuoles) were expressed as a percentage of the total numbers of these cells there was little change in the percent with pseudopods but generally those of the lymphoedema limb had a larger number of vacuoles, indicating either an additional uptake of particulate matter fluids and cell debris or a reduced turn-over of vacuoles. Much more work needs to be performed in this field before this can be described with any degree of certainty.

Conclusions

The study of the lymphatic system and the problems associated with its disfunction have always lagged far behind the blood vascular system. The interrelationships between the cell populations of the tissues (in particular the phagocytic components) in health and disease and the functioning of the lymphatic system is even less well known. In the foregoing presentation I have attempted to present a little of what is known about macrophage function, how it alters in lymphoedema to the detriment of the functioning of the blood-tissue-lymph system and how the majority of these changes in function can be re-modified by the appropriate drug therapy.

It would seem from the scanty number of experiments which have been performed that the possession of an adequately functioning phagocytic system is of cardinal importance in high protein oedemas, particularly in lymph-oedema. Normally under these conditions macrophage and general phagocytic function is depressed.

Restoration of the functional capabilities of the phagocytic system should be high on the list of priorities in the therapy of probable lymphoedema cases (i.e., all mastectomy patients). Evidence suggests that this mode if therapy may prevent or at least considerably slow the progressive histopathological state we know as lymphoedema.

Clinical trials with the benzopyrones which are believed to restore the functional capabilities of the macroöhages, have certainly produced some very startling results to date. These trials have already been well documented (70, 71, 72).

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