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'Science is always wrong: it never solves a problem without creating ten more.'

G. B. SHAW

Chemotaxis of Leucocytes*

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The term chemotaxis (chymitaxis) has been introduced by PFEFFER[1] to describe the activity of chemical substances which determine the direction of migratory cells. In plants and animals chemotaxis occurs in a wide variety of cell types and organisms. It may be positive or negative, i.e. towards or away from the stimulating substance. It serves many different functions, such as in reproduction[1,2], nutrition[1,3], cellular organization[4], inflammation[5–7], and avoidance of harmful substances[3]. The biologically important effect of chemotactic substances is to attract cells towards a specific area where they can perform a particular activity. For example, in the process of fertilization, the eggs of certain plants release substances which direct the sperm towards it[1,2]. Or in the myxamoeba *Dyctiostelium discoideum* the initiator cell attracts other single cells of this species to aggregate around it and thereby form a multicellular organism[4]. Obviously cells which exhibit a chemotactic response differ in various respects as e.g. in type of cell and in their function. One may therefore expect that in order to transmit the necessary information to the right cell type, the chemotactic stimulus must have a considerable degree of specificity. Evidence for cell specific chemotaxis has indeed been presented in plant sperm[1] and in inflammatory cells[8]. Thus chemotaxis may be regarded as a recognition mechanism for mobile cells, linked to their migratory activity.

Leucocytes take part in the inflammatory response. The leucocytes accumulating in the extravascular tissues are apparently guided to the inflammatory site. Numerous workers have assumed that chemotaxis plays a major role in this process. LEBER[5] and many subsequent workers have therefore studied chemotaxis in leucocytes[9,10]. However, its role in the accumulation of leucocytes in the tissues is not clarified. Progress has been hampered mainly by lack of reliable and adequate techniques. In 1962 BOYDEN[7] developed a new in vitro technique for measuring chemotaxis which proved to be more efficient than those used previously. This technique has resulted in considerable progress in the analysis of the mechanism leading to chemotaxis. As a consequence new concepts have been developed on the relationship between chemotaxis and leucocyte accumulation in inflammatory sites. It is the purpose of this review to discuss recent work in this field.

Measurement of Chemotaxis

Chemotaxis in vivo has not yet been convincingly demonstrated. Chemotaxis can, however, be demonstrated in vitro by various techniques. With the exception of BOYDEN's method[7] these different in vitro techniques have been extensively reviewed by HARRIS[10,11] and by MCCUTCHEON[9]. The method developed by BOYDEN[7] consists of a chamber with 2 compartments separated by a filter membrane, which is permeable for migrating cells (Figure 1).

If a chemotactic agent is placed in the lower compartment (A), then the cells present in the upper compartment (B) will migrate through the filter (F). Chemotaxis is evaluated by counting the number of cells which have reached the lower side of the filter (Figure 2).

This technique allows (a) demonstration of both random and directional migration, (b) easy quantitation of chemotaxis, (c) distinction between the response of different cell types, and (d) analysis of the mechanisms involved in induction of chemotaxis. None of the earlier techniques is of comparable versatility and

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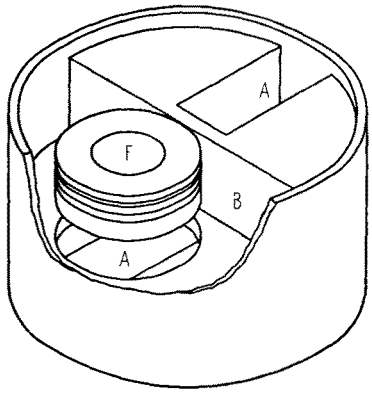

Fig. 1. Chamber for estimating chemotaxis. The chemotactic agent is placed in the lower compartment A. The cells present in the upper compartment B will migrate through the filter. Pore size of filter F: 3 μ or more for granulocytes, 8 μ for macrophages. Chemotaxis is evaluated by counting the number of cells which have reached the lower side of the filter (BOYDEN[7]).

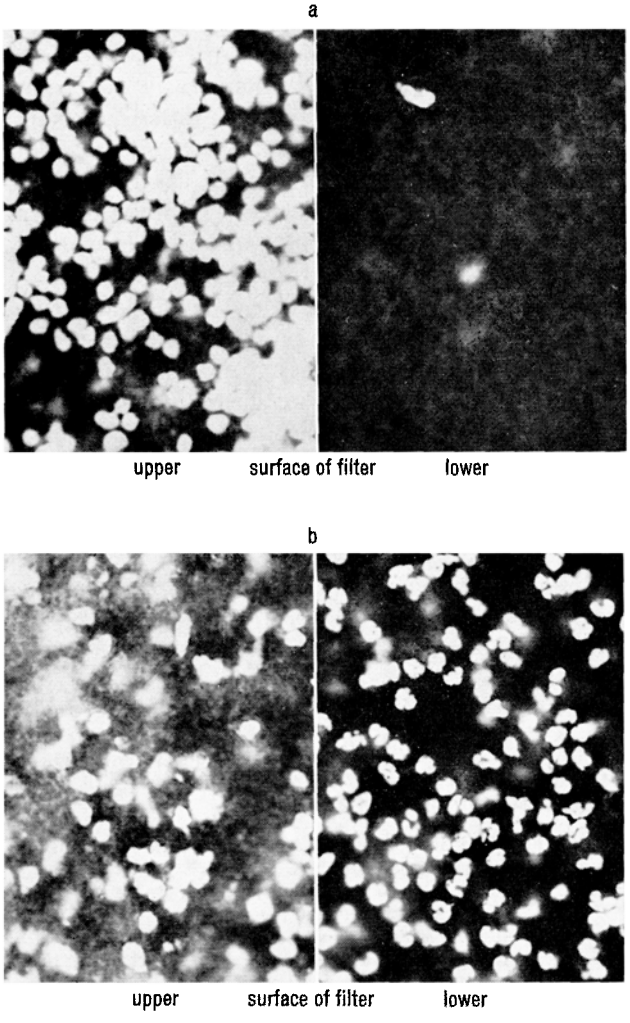

Fig. 2. Result of a chemotaxis experiment with granulocytes: (a) Control: Antigen-antibody complex and heated serum in lower compartment; few cells have migrated to the lower surface of the filter. (b) Experimental: Antigen-antibody complex in the presence of fresh normal serum in lower compartment; many granulocytes have migrated from the upper to the lower surface of the filter.

efficiency. Some of the methods, such as the capillary tube techniques described by WRIGHT and COLEBROOK[12], the slide cell technique as described by MARTIN et al.[13], and the tissue culture method[14,15] permit neither demonstration of directional migration nor distinction between the different cell types. With still others, such as the slide cover slip method[16–18] or the capillary tube method of LEBER[5] quantitation is difficult. Finally, none of these methods have been successfully used to elucidate the mechanisms involved in the formation of chemotactic mediators.

Leucocytes Showing Chemotaxis

Chemotaxis has been demonstrated for leucocytes of many vertebrates. The following cell types have been shown to exhibit a chemotactic response in vitro: neutrophil granulocytes, eosinophil granulocytes, monocytes, macrophages from embryos, spleen (see review by HARRIS[10]), peritoneal exudates[8], and alveolar washings (unpublished results). No tests have yet been carried out on basophil leucocytes. Chemotaxis in cells resembling medium or large lymphocytes from peritoneal exudates have, however, been described[8], but further data are necessary to substantiate that these cells are indeed lymphocytes. Attempts to demonstrate chemotaxis in small lymphocytes from blood or lymph nodes have failed so far[8,19]. These cells did not respond to test agents inducing chemotaxis in granulocytes or macrophages. There are several reasons which could explain the failure to demonstrate chemotaxis in small lymphocytes. (a) It has recently been shown that chemotaxis of leucocytes is a cell specific process involving different mediators[8]. The unresponsiveness of small lymphocytes to substances inducing chemotaxis in other cell types does not therefore mean that lymphocytes are not capable of exhibiting a chemotactic response. It may be that these cells have not yet been subjected to the right stimulus. (b) Small lymphocytes do not stick to the filters used in the BOYDEN technique as well as do granulocytes or macrophages. The filter may not therefore be suitable for migration of lymphocytes. (c) Lymphocytes may not be subject to chemotaxis.

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Nature of Chemotaxis

The following definition has been given by McCUTCHEON[9]: 'Chemotaxis is a reaction by which the direction of locomotion is determined by chemical substances in the environment. If the direction is towards the stimulating substance, chemotaxis is said to be positive, if away from the stimulating substance the reaction is said to be negative, if the direction of movement is not definitely towards or away from the substance in question, chemotaxis is indifferent or absent.'

Most studies in chemotaxis of leucocytes are concerned with positive chemotaxis. Negative chemotaxis has been reported in plant sperm[1] but there is no convincing evidence for it in leucocytes.

Many workers have shown that chemotactic stimulation can result in directional migration of cells[7,16–18,20]. They assumed that the direction of the cells was determined by a concentration gradient of the chemotactic agents. The significance of a gradient for the chemotactic response has, however, only recently been studied in detail using BOYDEN's technique[20]. The behaviour of rabbit granulocytes[20] and mononuclear cells[8] from peritoneal exudates in the presence and absence of a chemotactic gradient was found to be similar. The results of these experiments are schematically summarized in Figure 3.

This Figure shows that the cells can migrate from a low to a higher concentration of chemotactic substances (positive gradient). Thus chemotactic attraction can lead to leucocyte accumulation in vitro. On the other hand, these cells are trapped in a chemotactic medium provided the test solution has little or nor chemotactic activity (negative gradient). Thus leucocyte accumulation due to a chemotactic gradient can be the result of chemotactic trapping as well as of directional attraction or both.

Furthermore, at variance with the results of DIXON and McCUTCHEON[21] chemotactic agents were also found to enhance random migration[8,20]. Enhanced random migration is observed if (a) both the cell suspending medium and the test solution contain similar concentrations of chemotactic agents (Figure 3, 'no gradient'), or (b) the suspending medium already contains a relatively high concentration of chemotactic agent, even if the concentration in the test solution is much higher than in the cell suspending medium. It is possible that under the latter conditions the cells are no longer able to sense a chemotactic gradient and they migrate therefore at random[20,22].

Thus chemotactic stimulation can induce increased locomotion of leucocytes as well as changes in direction. Evidence has been presented that cells can undergo repeated stimulation[22] and that the direction of migration can be reversed[23]. This suggests that chemotactic stimulation of cells is a reversible process allowing reorientation of the direction of migration.

Substances Inducing Chemotaxis

Many different substances including polysaccharides[24,25], polypeptides and proteins[26–30] have been found to exert a chemotactic activity[10]. Although their mode of action was not really clarified, most earlier workers tended to believe that they act directly on the leucocytes[9,11,24]. On the other hand, evidence for an indirect action has been presented[26,31]. Furthermore, HARRIS[32] and other authors[33] believed that these substances have a similar effect on all types of leucocytes showing chemotaxis. If differences in the response of different inflammatory cells were observed they were interpreted to be of a quantitative nature[33]. In view of the recent work, these concepts need to be reevaluated.

BOYDEN[7] showed that antigen-antibody complexes are not chemotactic per se, but exert their effect by inducing formation of chemotactic mediators in fresh serum. This was the first convincing evidence that

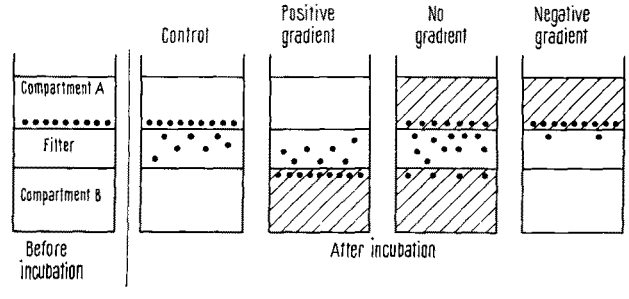

Fig. 3. Schematic representation of the significance of a gradient for the chemotactic response of leucocytes in vitro. The figure shows that cells can migrate from a low to a higher concentration of cytotaxin (positive gradient) and that the cells cannot leave a highly chemotactic medium (negative gradient) which leads to chemotactic trapping. If the cell suspending medium and the test solution contain chemotactic agents in similar concentration (no gradient) they can no longer sense a gradient and move at random with increased motility (KELLER and SORKIN[8,20]).

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chemotactic agents do not necessarily have a direct effect on leucocytes. Evaluating the mode of action of various other substances, KELLER and SORKIN[34] have shown that indeed many agents act in a way apparently similar to antigen-antibody complexes, that is by inducing formation or possibly unmasking of chemotactic mediators in fresh serum (Table I). Unlike the aforementioned agents other substances, however, were found to be chemotactic without serum, probably because they exert a direct effect on the cells just as do the mediators formed in fresh serum (Table II)[34,35]. These studies thus showed that the conventional term 'chemotactic substance' included chemotactic mediators and at the same time the agents which induced the formation of these mediators. It seemed indicated to find more precise and adequate terms for these different groups of agents and it has therefore been suggested to classify chemotaxis inducing substances by their mode of action. Those with a direct effect on cells were termed *cytotaxins* and those which induce formation of cytotaxins as *cytotaxigens*[35].

The term leucotaxin which has been introduced by MENKIN[26] although useful at the time seemed inappropriate for the following reasons: (1) it was coined for one particular polypeptide occurring in inflammatory exudates which was claimed to have vasoactive as well as chemotactic properties. Recent studies have, however, indicated that there exist a variety of different chemotactic mediators and at least one of them is a high molecular weight protein complex. (2) The term leucotaxin reflects the view that all types of leucocytes react to the same chemotactic mediator. It has been shown, however, that cytotaxins can be specific for a particular type of leucocyte and on the other hand it is not known whether cells other than leucocytes can respond to these mediators.

Until now only a few cytotaxins have been evaluated for their cell specificity. Several of them were found to be specific for granulocytes, such as those present in antigen-antibody treated serum, peptone, culture filtrates from *Escherichia coli* and 'bactocasiton'[8]. The specificity of those cytotaxins which attract mononuclear cells has not yet been investigated. The data available at the present time nevertheless indicate that cytotaxins can be further classified by their cell specificity. It may well be that there exist different specific mediators for each cell type such as neutrophil cytotaxins, eosinophil cytotaxins, macrophage cytotaxins, basophil and lymphocyte cytotaxins.

(*A*) *Cytotaxigens.* Such substances which exert their chemotactic activity by inducing formation of cytotaxins can derive from exogenous as well as endogenous sources. Many agents can act as cytotaxigens when incubated in fresh serum; they are inactive when incubated in heated serum or in absence of serum. This has been demonstrated with certain antigen-antibody mixtures, heat-aggregated γ-globulins, endotoxin preparations, washed bacterial cells of *Staphylococcus albus* or *E. coli*, tuberculoprotein[34,35], zymosan[23], liver cells[36], granulocytes or macrophages[37], as well as broken lysosomes from these cells (BOREL, KELLER and SORKIN, to be published), and subcellular fractions from liver cells[38] and plasminogen-streptokinase mixtures[42,45]. Some representative results are shown in Table I.

These various cytotaxigens have in common that they induce formation of heat stable (56 °C) cytotaxins when incubated in fresh normal serum. It has been shown that the effect of subcellular fractions from liver cells[38] is due to formation of immune complexes with natural antibodies present in the normal serum and it is probable that also other agents such as bacteria or endotoxins can act in a similar way[35]. But it would be premature to conclude that all substances which are cytotaxigenic when incubated in fresh serum act in this manner and lead to the formation of the same mediators. It has been shown that at least plasminogen-streptokinase mixtures act in a way different from antigen-antibody complexes[42,45].

Also damaged tissues such as severely burned skin, minced fragments of liver or rat cardiac muscle, following incubation with fresh homologous serum were found to be chemotactic when tested in fresh heparinized human plasma. No such effect was observed when they were incubated in fresh serum[39]. The difference between serum and plasma in this reaction has not been clarified.

The incubation of antigen-antibody complexes[8] or frozen and thawed granulocytes or macrophages[37] in

Table I. Chemotactic effect of various agents on granulocytes in the presence of fresh or heat-inactivated normal rabbit serum

Agents incubated in normal rabbit serum	Granulocytes/Field		
	Control, no agent	Fresh serum + agent	Inactivated serum + agent
HSA-rabbit-anti-HSA	3	381	6
Heat-aggregated human γ-globulin	1	369	1
PPD	3	106	3
Glycogen	1	60	5
Proteus endotoxin	2	327	3
S. albus (heat-killed)	2	71	3
Lysosomal extracts of granulocytes	15	216	44

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fresh serum leads to formation of cytotaxins, which were found to be specific for granulocytes. Evidence has been presented that also cytotaxins acting on macrophages may occur in normal serum[40]. It is possible that some cytotaxigens can also and even exclusively so, induce formation of macrophage cytotaxins.

(*B*) *Cytotaxins.* (*1*) *Exogenous cytotaxins.* Chemotactic activity specific for polymorphs has been found in Witte's peptone and in bactocaseine. Caseine (Hammersten) contains active material for both polymorphs and macrophages. Furthermore, bacteria such as *E. coli* and *Staph. albus* release cytotaxins into their culture medium which are specifically chemotactic for polymorphs[8]. Evidence has been obtained that polymorph cytotaxins released by *E. coli* have a lower molecular weight than those formed in fresh serum on incubation with antigen-antibody mixtures[35]. Also a culture filtrate of tubercle bacilli attracts granulocytes (KELLER and SORKIN, unpublished results) and has little if any effect on mononuclear cells. LASFARGUES and DELAUNAY[33] have observed that out of several microorganisms tested only one particular coccus was capable of inducing chemotaxis in macrophages. The authors suggested that the apparently inactive microorganisms release products toxic for macrophages and are therefore not chemotactic for these cells. On the basis of our recent observations on cell specific cytotaxins released by bacteria however, it would be of interest to evaluate whether their results could be explained by formation of a macrophage cytotaxin in one microorganism and its absence in the other.

(*2*) *Endogenous cytotaxins.* (*a*) *Serum cytotaxins.* It has already been shown above that interaction of cytotaxigens with serum can lead to the formation of cytotaxins. Several cytotaxins have been found in the serum[40–42]. Those formed on interaction with antigen-antibody mixtures have been found to be specific for polymorphs[8]. They are not dialysable[35]. There is evidence that hemolytic complement plays a role in the generation of cytotaxins[43]. Purified C'5,6,7 complexes (molecular weight $> 300{,}000$) were claimed to act as cytotaxin[41,44,45]. Also various other findings seem to support the view that complement plays a considerable role in generating chemotactic activity. Thus cytotaxin formation induced by antigen-antibody complexes or zymosan is reported to be significantly reduced in C'6 deficient rabbit serum and in C'5 deficient mouse serum[44]. Antibodies which fix no or little complement such as duck antibody or $7S\gamma_1$ from the guinea-pig have little if any activity in inducing chemotaxis if compared with complement fixing antibodies ($7S\gamma_2$ from the guinea-pig or precipitating rabbit antibodies)[44,46]. In contrast STECHER and SORKIN (unpublished results) have found that antigen-antibody treated C'6-deficient rabbit serum was as a highly chemotactic for granulocytes as similarly treated normal rabbit serum.

These findings and others showing lack of correlation between the complement-fixing activity of heat-aggregated γ-globulin as measured in terms of hemolysis and their capacity to induce cytotaxin formation[47] indicate that alternative pathways may lead to cytotaxin formation following incubation of these agents in serum. In addition there is evidence for the presence of other cytotaxin(s) in antigen-antibody treated serum[40]. Furthermore density gradient studies reveal a broad distribution of chemotactic activity in antigen-antibody treated serum which is quite distinct from the sharp peak observed with the purified activated C'5,6,7 complex[45].

A further cytotaxin attracting polymorphs has been found in serum treated with mixtures of streptokinase and plasminogen[42]. This cytotaxin which is not formed following treatment of serum with antigen-antibody mixtures is a dialysable split product of C'3 with an approximate molecular weight of 6000[42,45]. It appears to be different from anaphylatoxin which has no chemotactic activity.

In addition to these cytotaxins acting on polymorphs there is evidence for yet another cytotaxin in serum capable of attracting mononuclear cells[40]. The mechanism of its formation and its chemical properties have not yet been studied. It is, however, not formed on interaction of fresh serum with antigen-antibody complexes[8], or by frozen and thawed granulocytes and macrophages[37].

(b) *Cell derived cytotaxins.* Many authors have demonstrated that tissues or tissue products are chemotactic[9,23,36,39]. It has recently been shown that cells can exert this activity both by means of cytotaxigens or/and by releasing cytotaxins (BOREL, KELLER and SORKIN, to be published). Polymorph cytotaxins are released from frozen and thawed granulocytes or macrophages but not from lymphocytes. Also granulocytes and alveolar macrophages cultured in vitro release cytotaxins acting on granulocytes. Granulocytes and macrophages but not lymphocytes were also found to release some chemotactic activity for macrophages. When subcellular fractions of rabbit granulocytes, peritoneal or alveolar macrophages were prepared, polymorph cytotaxins were found in the postnuclear but not or only to a slight extent in the lysosomal fraction (BOREL, KELLER and SORKIN, to be published).

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Lymph node permeability factor[48], has not yet been tested for its chemotactic activity in vitro. With regard to its possible role in delayed hypersensitivity it would be of particular interest to evaluate its effect on mononuclear cells.

Mode of Action of Cytotaxins

We have to ask next how a chemotactic stimulus is translated into cellular movement? Very little is known on the biochemical reactions at the cellular level which are involved in the chemotactic response. There is evidence that the action of cytotaxins on cells is reversible[22,23]. Furthermore, it has been found that various cytotaxins differ in their cell specificity. This suggests that each cell type is characterized by a particular type of chemotactic receptor[8]. Whether one cell type carried more than one receptor and whether some receptors are shared by the different cell types is unknown.

WARD and BECKER[50] have found that in granulocytes a proesterase becomes activated on their interaction with antigen-antibody treated rabbit serum or purified C'5,6,7. It is conceivable that this proenzyme is a chemotactic receptor in granulocytes. The enzyme has been termed 'activatable esterase' and was found to be a serine esterase which differs from various other esterases by its inhibition profiles with phosphonate esters. BECKER and WARD[51] have presented evidence that at a later stage of the reaction another serine-esterase which has been termed 'activated esterase' is necessary for the development of the chemotactic response.

The following hypotheses have been put forward to explain amoeboid motion in leucocytes: (a) CARTER[52] has suggested that chemotaxis is a special case of haptotaxis (cell movement on a gradient of adhesion). No experimental evidence for this hypothesis has been presented so far. It has been reported that in vivo cells need a suitable surface such as connective tissue fibres[53], but it may well be that haptotaxis and chemotaxis are independent phenomena, each of them being an important factor in cell migration. (b) The surface theory regarding the leucocyte as a drop of liquid which moves by physical forces[49]. Leucocyte migration has, however, been found to be highly dependent on ambient temperature and metabolic processes[54]. (c) Amoeboid motion is an active process based on a mechanism effecting the contractile system and gel-sol transformation in the cell. These mechanisms have been studied extensively in amoeba[55], but not yet in leucocytes.

Inhibition of Chemotaxis

It has been shown above that there exist different cytotaxin forming mechanisms leading to cytotaxins differing in their chemical and biological properties. This complicates any attempts to achieve inhibition of chemotaxis in vivo because it seems very unlikely that a single inhibitor could specifically block all these different mechanisms unless it acts at the cellular level. The diagram shows schematically the different levels at which chemotaxis can be inhibited. (a) Inhibition of cytotaxin formation, (b) inactivation of cytotaxins, and (c) inhibition at the cellular level (Figure 4).

(*1*) *Inhibition of cytotaxin formation.* Until now all inhibition studies have been performed with granulocytes and most of them in one particular system, namely chemotaxis induced by incubation of antigen-antibody complexes or heat-aggregated γ-globulin in fresh serum. Anti-inflammatory agents such as 3:5:6-benzyl-ethyl-D-glucofuranosid, 1:2-diphenyl-3:5-dioxo-4*n*-butyl-pyrazolidine did not inhibit cytotaxin formation following incubation of aggregated γ-globulin in fresh serum[56]. Cytotaxin formation in antigen-antibody treated serum was inhibited by EDTA[44] and N-CBZ-glycyl-phenylalanine[22,41], whereas ε-aminocaproic acid had no such effect[22]. ε-aminocaproic acid was, however, found to inhibit chemotaxis of granulocytes induced by incubation of streptokinase and plasminogen in serum[42]. No studies have yet been performed on other cytotaxin forming systems as e.g. the release of cytotaxins from cells.

(*2*) *Inactivation of cytotaxins.* Substantial loss of chemotactic activity in antigen-antibody treated serum has been observed following incubation with N-CBZ-

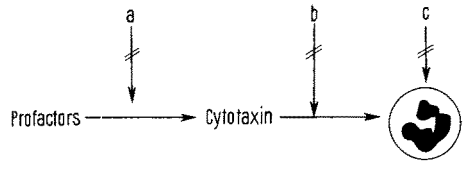

Fig. 4. Diagram presenting schematically the various levels at which inhibition of chemotaxis can occur. (a) Inhibition of cytotaxin formation. (b) Inactivation of cytotaxins. (c) Inhibition of action of cytotaxins on cells.

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α-glutamyl-L-tyrosin. This agent was shown to dissociate the chemotactic C'5,6,7 complex[41]. Our own experiments performed under similar conditions provided no evidence for an inhibitory action of this compound[22]. In vivo the chemotactic activity produced by zymosan in circulating blood has a half life of less than 25 min[41], but its mode of inactivation is unknown.

(*3*) *Inhibition at the cellular level.* Several inhibitors were found to act at the cellular level. Migration of granulocytes is inhibited by hydrocortisone and by prednisolon in concentrations of 100 γ/ml and higher[57–60]. These concentrations seem rather high for therapeutic use. Chloroquine inhibits at a concentration of $10^{-5}M$[57].

WARD and BECKER[50,51] have found 2 esterases involved in the chemotactic response of rabbit granulocytes which can be distinguished by their inhibition profiles with phosphonate esters. The so-called 'cell dependent' inhibition occurs as a result of pretreatment with phosphonates interacting with the 'activated esterase' of the granulocytes. 'Chemotactic factor dependent' inhibition can only be observed with cells subjected to chemotactic stimulation in the presence of the phosphonates. It is due to inhibition of the 'activatable esterase'. Various acetate esters can specifically protect against cell dependent inhibition.

Endogenous substances also can inhibit chemotaxis. Thus evidence has been presented that cytotaxins can inhibit chemotaxis by blocking the activatable esterase of polymorphs[61], although this effect is not always observed[22]. PAGE et al.[62] have presented evidence that a heat labile endogenous inhibitor of chemotaxis in granulocytes is present in sera of nephritic patients. This inhibitor disappears from the sera of these patients after removal of the affected kidney. The detailed mode of action of this inhibitor has not been elucidated. Indications for endogenous rabbit serum inhibitors of chemotaxis of rabbit granulocytes have recently been obtained using chromatographic methods (BOREL, WILKINSON and SORKIN, to be published).

No inhibition studies have yet been performed with regard to cytotaxins from other sources or with cell types other than granulocytes. As many inhibitors are evaluated in vitro with regard to their possible anti-inflammatory action in vivo, the in vitro model should take into account which cell type(s) are involved in the inflammatory response in vivo.

Chemotaxis and Recognition of Foreign Matter

Already LEBER[5] suggested that leucocytes recognize injured tissue sites by chemotaxis and METCHNIKOFF thought that chemotaxis plays an essential role in guiding phagocytes to the foreign matter to be engulfed[63]. Leucocytes apparently differ in their ability to recognize a particular type of inflammatory agent; for some experiments indicate that cell specific chemotactic mediators can selectively transmit the chemotactic information to a particular cell type[8,37]. Recognition of foreign matter can have its basis in specific immunological reactions, but there is also evidence that in chemotaxis non-immunological mechanisms have to be considered as well.

(a) *Chemotaxis mediated by specific immune reactions.* BOYDEN[64] has suggested the discrimination between foreign and indigenous substances is not dependent on the leucocytes but appears to be a function of humoral factors, most likely globulins.

Immune complexes can induce chemotaxis following incubation in fresh serum. They are most effective when formed in the equivalence zone[7]. Furthermore, the cytotaxigenic activity varies with the species from which the antibody is derived and with the type of antibody. $7S\gamma_2$ antibody of the guinea-pig on incubation with the antigen in fresh serum induces pronounced chemotactic activity for polymorphs. $7S\gamma_1$ antibody of the guinea-pig has little or no such effect under similar conditions[46]. In the rabbit the chemotactic activity of serum induced by precipitating antigen-antibody complexes was found to be confined to polymorphs (Figure 5). Mononuclear cells did not respond[8]. It will be of interest to evaluate whether different types of antibodies can induce chemotaxis of different cell types.

BOYDEN[65] found that amongst different agents incubated in normal serum those which were phylogenetically most distant proved to be most effective in inducing chemotaxis, probably because the sera had more natural antibodies against these agents. He found that extracts containing macromolecules from plants and bacterial products tend to be strongly chemotactic in such a medium, while fish and insect material occupy an intermediate position. Recently ELSON and WEIR[38] have presented evidence that 'natural' antibodies are responsible for the chemotactic response observed following incubation of subcellular fractions of rat liver in fresh serum. The finding that cells and subcellular fractions can induce chemotaxis when incubated in nor-

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[61] E. L. BECKER and P. A. WARD, *Immunopathology*, V. Int. Symp. (Ed. P. A. MIESCHER and P. GRABAR, B. Schwabe & Co., Basel 1968), p. 189.
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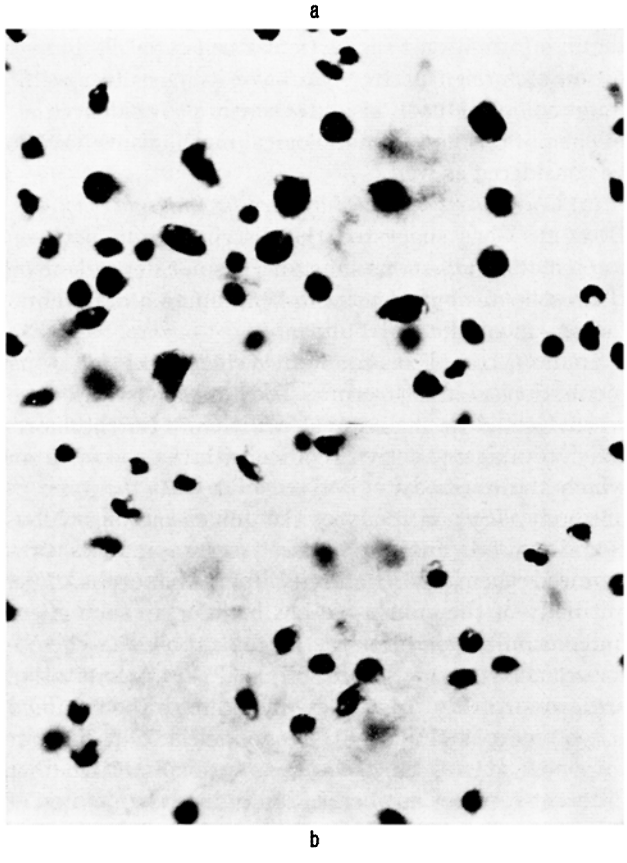

Fig. 5. Demonstration of differential chemotaxis: different chemotactic effect of antigen-antibody treated rabbit serum and casein on the same suspension of peritoneal exudate cells. (a) Polymorphs and mononuclear cells attracted by casein. Weigert-congo red. × 390. (b) Polymorphs attracted by antigen-antibody treated rabbit serum. Weigert-congo red. × 390. (KELLER and SORKIN[8]).

mal serum may however indicate that the phylogenetic relationship is of limited importance[38] (BOREL, KELLER and SORKIN, to be published).

(b) *Chemotaxis without apparent immune reaction.* It has been shown that antigen-antibody complexes need to be incubated in fresh serum to induce chemotaxis[7,34]. They are not chemotactic when incubated in absence of serum[34]. Unlike these immune complexes a number of agents however are chemotactic in absence of serum and their chemotactic activity cannot be increased by addition of normal serum[34]. Such substances are present in bacterial culture filtrates[35], casein, peptone, bactocasitone[34] and in postnuclear fractions of polymorphs or macrophages (BOREL, KELLER and SORKIN, to be published). These findings indicate that the effect of these other agents is not mediated by antibody and that they act directly on the leucocytes. This view is in agreement with findings of PAGE et al.[62] showing that in patients with agammaglobulinaemia neutrophil exudation is not dependent on the ability to produce antibodies. The presence of cell-bound antibodies against these apparently directly acting agents has not been excluded but recent data on macrophage migration from capillary tubes suggest that cytophilic antibodies have an inhibitory rather than an enhancing effect on cell migration[66].

The data presented above indicate that chemotaxis of leucocytes can be induced following specific immune reactions as well as without apparent immunological reactions. It has been shown that some cytotaxins are specific for a certain cell type of a given species[8] although these same cytotaxins cross the species barrier[34]. This is in line with the view that chemotaxis plays a significant role in cellular perception of foreign matter. Many exogenous substances elicit this response. But, also endogenous materials may induce chemotaxis which is then probably part of the discrimination mechanism between normal healthy and modified tissue cells.

Chemotaxis in Inflammation

There is convincing evidence for chemotaxis in vitro. The same cannot be said for chemotaxis in vivo, as there are no reliable criteria for its demonstration in the body. It has nevertheless been claimed that chemotaxis plays a major role in inflammation. Based on recent in vitro experiments this concept is reevaluated below.

Chemotaxis and vascular permeability. Various vasoactive mediators have been tested for their chemotactic activity in vitro. Histamine, serotonin, bradykinin, kallidin, and cationic proteins from lysosomes of rabbit granulocytes were found to be without activity on rabbit granulocytes[34,41,67]. Furthermore, histamine failed to induce chemotaxis in guinea-pig eosinophils (KELLER and SORKIN, unpublished results). These data indicate that the vasoactive mediators are different from those inducing chemotaxis. This is in line with in vivo observations by several workers which showed lack of correlation between the capacity of various substances to induce leucocyte emigration in vivo and to increase vascular permeability[29,68,69]. Although the mediators for the vascular events and for chemotaxis seem to be different, both phenomena may nevertheless influence each other. On one hand substances affecting the blood flow and vascular permeability such as adrenalin can affect the degree of leucocyte accumulation[70]. On the other hand, the leucocytes which have

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accumulated under chemotactic stimulation may release vasoactive substances such as cationic proteins from neutrophils[71].

Relation of chemotaxis to stickiness and emigration of leucocytes. Stickiness of leucocytes to the endothelium is one of the early steps in inflammation and it is a prerequisite for the emigration of these cells. There is evidence that stickiness results from chemical products diffusing from the site of injury[72–75]. It appears unlikely that these substances are identical with cytotaxins because in vivo observations have shown that stickiness is not necessarily followed by emigration of leucocytes into the extravascular tissues[76]. It is, however, conceivable that cytotaxins can induce diapedesis once the leucocytes adhere to the endothelium. SPECTOR[77] has suggested that some factors can favour the diapedesis of mononuclear cells relative to that of polymorphs. It is conceivable that these factors are cell specific cytotaxins.

Chemotaxis and accumulation of emigrated leucocytes. Whereas the significance of chemotaxis for stickiness and emigration of leucocytes is not clear, there are good grounds for the belief that it plays a role in attracting and trapping the emigrated leucocytes. Since chemotaxis cannot be directly demonstrated in the tissues, the evidence for its significance in vivo is indirect.

Arguments for the view that chemotaxis is involved in inflammation. (a) *The migratory response in vitro and in vivo.* Earlier workers have postulated that cells responding to a chemotactic stimulus in vivo must show directional migration[9,32]. GRANT[78], however, has pointed out that experimental pathologists are thereby trapped by their own definition of chemotaxis. It has indeed been shown in vitro that in absence of a gradient or in areas with high cytotaxin concentrations the cells migrate at random[8,20]. These are most likely the conditions present near the centre of an inflammatory site. The cytotaxin concentration diminishes with increasing distance from the centre of damage and the leucocytes which now can sense a gradient will respond by directional movement into the region of injury. BUCKLEY[79] studying migration in vivo observed directional migration in the marginal area of the inflammatory site and random migration in its centre. These findings are in line with the in vitro studies mentioned above. The failure of various other workers[32,75,76] to observe directional migration in vivo is presumably due to the fact that in well vascularized tissues most leucocytes leave the blood vessels in or near the centre of the lesion and reach therefore immediately the area of maximal chemotactic activity. In vivo observation of leucocyte locomotion thus allows no clear conclusion at all as to whether chemotaxis is involved in this process or not. The recent in vitro studies, however, clearly exclude the observation of random migration in vivo as evidence against chemotaxis.

(b) *The chemotactic effect of many substances measured in vitro is similar to their leucotactic activity in vivo.* A great number of agents which are leucotactic in vivo such as bacteria, endotoxins, glycogen starch, peptone are also chemotactic when tested in vitro[10]. HURLEY[36] has performed a comparative study on the chemotactic activity of liver tissue and of granulocytes under various conditions in vitro and in vivo. He found a good correlation between the capacity of these test solutions to cause early leucocyte emigration in vivo and to induce chemotaxis of polymorphs in vitro. Furthermore, guinea-pig $7S\gamma_2$ antibody with its antigen induced pronounced chemotaxis in vitro and massive granulocyte infiltration in vivo, whereas $7S\gamma_1$ antibody has little effect in vitro as well as in vivo when tested under similar conditions[46,80]. Complement can be involved in chemotaxis of polymorphs in vitro[44] and its presence in lesions of Arthus type reaction has been demonstrated[81]. On the other hand pronounced polymorph infiltration has been observed in C'6-deficient rabbits in vivo[82]. These in vivo findings agree with our results in vitro, which demonstrate that following interaction of antigen-antibody complex with normal or C'6-deficient rabbit sera a similar chemotactic activity for granulocytes can be observed. (STECHER and SORKIN, unpublished results.)

A patient with C'2-deficiency showed depressed neutrophil exudation and his serum showed lack of ability to generate chemotactic activity. Nephritis patients who have in their plasma a substance which inhibits chemotaxis of polymorphs in vitro, show a depression of neutrophil exudation in vivo[62].

(c) *Cytotaxins are formed in vivo.* It has recently[20] been suggested that in order to obtain at least indirect evidence for chemotaxis in vivo, it will be necessary to show that substances which are chemotactic in vitro have been formed in vivo and are present in the inflammatory site. Formation of cytotaxins in vivo has been demonstrated in circulating blood following i.v. injection of zymosan or aggregated γ-globulin[41] and in peritoneal exudates of the rabbit following injection of

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glycogen[22]. Since it has been shown that these agents are chemotactic in vitro when incubated in fresh serum these facts seem to indicate that the same mediators have been formed in vivo and that they have provoked the accumulation of leucocytes.

Washed exudate cells, however, when incubated in vitro can release cytotaxins (unpublished results). This indicates that cytotaxins found in the exudate fluid may not necessarily be due to glycogen interacting with serum components. They may as well have derived from the emigrated cells themselves. Thus the presence of cytotaxins in inflammatory exudates is by itself no proof that the cells present have accumulated following chemotactic stimulation. The interpretation of the finding that cytotaxins are formed in the circulating blood is subject to similar reservations. Thus in order to establish a more precise relationship between chemotaxis and leucocyte accumulation by indirect evidence it will be necessary to determine each cytotaxin separately and relate its presence in the inflammatory site to leucocyte accumulation.

Arguments against the view that chemotaxis is involved in inflammation. (a) *Discrepancies between in vitro and in vivo experiments.* Several authors have reported discrepancies between in vitro and in vivo experiments[9–11,24]. The recent data mentioned above show that cytotaxins are formed by various mechanisms and that they differ in their chemical and biological properties. Until now most experiments have, however, been performed with granulocytes as target cells. Furthermore, in vitro tests are limited to show one or a few out of many different mechanisms leading to cytotaxin formation. If for example chemotaxis is measured in absence of serum only few agents will induce leucocyte migration (Table II).

Many of these agents which are inactive in the absence of serum, can, however, act as cytotaxigens when incubated in fresh serum (see Table I). Other agents may presumably lead to the release of cytotaxins or cytotaxigens from cells. This clearly shows that whether or not discrepancies between in vitro and in vivo experiments are observed can depend on the test conditions. In order to prove that there are really fundamental discrepancies between in vivo and in vitro experiments, it will be necessary to set up a variety of in vitro tests representing all the different mechanisms which can lead to cytotaxin formation in vivo.

Differences between in vivo and in vitro experiments have also been observed with regard to the responding cell type. Thus staphylococci produce lesions with predominance of polymorphonuclear leucocytes whereas with Mycobacteria tuberculosis mononuclear cells prevail, although both types of microorganisms were found to exert a similar chemotactic effect on polymorphs and monocytes when tested in vitro[11,83]. These differences may have their basis in the complex host-parasite relationship involved in leucocyte accumulation in vivo. It will be argued in detail below how depending on the intra- or extracellular localization of the microorganisms different cytotaxins may be released.

(b) *Chemotaxis of lymphocytes in vitro has not been reported.* There is no definite proof that lymphocytes are not subjected to chemotaxis. The possible reasons for this have been discussed above.

In summary these considerations indicate that at the present stage of our knowledge the seeming lack of correlation between chemotaxis in vitro and leucocyte accumulation in vivo may be more apparent than real. The arguments suggest that chemotaxis may be regarded as a reasonable though still hypothetical explanation for the accumulation of leucocytes in vivo.

Selective chemotaxis as an explanation for leucocyte accumulation in vivo. There is convincing evidence that at least in acute inflammation leucocyte accumulation is due to emigration from the blood rather than to proliferation of local cells[84–86]. HARRIS[32] has discussed 3 possible explanations for selective leucocyte accumulation in vivo. (a) Selective adherence of leucocytes to the endothelium of small vessels; (b) selective chemotaxis; (c) the leucocytes emigrate and accumulate together but some cell types are gradually removed while others persist.

There is no evidence for selective adherence. Gradual removal of one cell type but not the other does not explain why in some hypersensitivity reactions of the immediate type the eosinophils prevail, whereas in others neutrophils are predominant. Furthermore, it does not help to understand why in delayed type

Table II. The chemotactic effect of various agents on rabbit granulocytes in the absence of serum

Agent in GEY's solution	Cells/field
None	0
HSA-rabbit-anti-HSA	0
Heat-aggregated human γ-globulin (0.2 mg/ml)	0
Heat-aggregated bovine γ-globulin (0.2 mg/ml)	0
PPD (0.066 mg/ml)	2
Glycogen (3.6 mg/ml)	0
Proteus endotoxin (2 μg/ml)	0
S. albus (3.2×10^6/ml) (heat-killed)	4
Witte's peptone (220 μg/ml)	156
Bactocasitone (2 mg/ml)	126
Casein (Hammersten) 2 mg/ml)	240
Postnuclear fraction from granulocytes	81
Culture filtrate *E. coli*	105
Heated ultrafiltrate of tubercle bacilli culture (0.1 mg/ml)	95

[83] H. HARRIS, Br. J. exp. Path. *34*, 276 (1953).
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hypersensitivity lesions the polymorphonuclear response is relatively weak and the mononuclear response relatively strong if compared with Arthus type reactions. Recent work of SPECTOR[77] suggests the existence of factors favouring emigration of one cell type relative to that of another cell type. Such factors could cause that different cell types are present in characteristic and varying proportions in different types of lesions and at different stages of a particular lesion. As it has recently been shown that cytotaxins differ in their cell specificity they could be the factors responsible for this effect. Cell specific chemotaxis seems to be an appropriate explanation for differential emigration and accumulation of leucocytes in the tissues. We have therefore suggested that the histological pattern of an inflammatory lesion reflects within certain limits the time sequence of formation, the amount and the cell specificity of the cytotaxins present in the inflammatory site[8]. One prerequisite is that the particular target cells are present in sufficient numbers and are within the range of the chemotactic field. This may for example play a role in the alveolar space where plenty of macrophages are present which can respond to chemotactic stimulation, whereas the granulocytes will first have to emigrate from the blood. Certainly many other factors come into play which influence the composition of inflammatory exudates such as the survival time of each particular cell type, local cellular proliferation, particularly in chronic inflammation[77], the relative number present in the blood[70], or factors which can trap cells by inhibiting their migration[87,88].

The significance of the different cytotaxins in inflammation. The obvious role for the different cell specific cytotaxins would be to determine the cell type(s) to be attracted. In addition to cytotaxins differing in their cell specificity, a variety of cytotaxins with similar cell specificity has been found. Thus cytotaxins with a considerable degree of specificity for polymorphs can derive from serum, from culture filtrates of bacteria, from Witte's peptone, bactocasitone, casein, and from granulocytes and macrophages. They can be distinguished by their origin, mode of formation, and their chemical properties. The different cytotaxins may be of varying significance at different stages of a particular lesion and in different types of inflammatory reaction. This problem will now be discussed in more detail.

(a) *Arthus and other hypersensitivity reactions in relation to chemotaxis.* The mechanism of the Arthus reaction has been extensively studied and is relatively well understood. The early polymorph infiltration is a characteristic feature of the Arthus reaction and a prerequisite for its development[89–92]. In vitro studies by BOYDEN have shown that antigen-antibody complexes are chemotactic for polymorphs when incubated in fresh serum. This suggests that these complexes are responsible for the onset of the early polymorph infiltration. This assumption is supported by the finding that $7S\gamma_2$ antibodies of the guinea-pig together with the antigen induce chemotaxis in vitro and leucocyte accumulation in vivo, whereas $7S\gamma_1$ antibodies have no or little such effect in vitro or in vivo[46]. The mechanisms by which these antigen-antibody complexes act have been mentioned above. It has been shown that the influx of polymorphs will only cease when the immune complexes are removed by phagocytosis[93]. Phagocytosis can result in release of lysosomal products and even death of cells[94]. Since it has been shown that cells can release cytotaxigens from their lysosomes as well as cytotaxins it seems likely that these chemotactic factors are also of significance for the attraction of inflammatory cells.

At a later stage of the Arthus reaction, mononuclear cells appear. It has, however, been shown that the cytotaxins formed on interaction of the immune complexes with fresh serum are specific for polymorphs and can therefore not account for the accumulation of mononuclear cells[8]. This suggests that another still unknown cytotaxin(s) attracting mononuclear cells is also involved. In vivo studies have shown that the early influx of polymorphs and the infiltration with mononuclear cells are independent phenomena[90,91]. This indicates that at least under these particular conditions neutrophils are not a likely source of cytotaxins capable of attracting these mononuclear cells.

The participation of mononuclear cells seems to be of particular interest in delayed type hypersensitivity, homograft reactions, contact sensitivity and certain autoimmune diseases. It appears that chemotactic agents can also be involved in such reactions[95]. The identification of chemotactic mediators, particularly those attracting mononuclear cells which could possibly be involved in producing these reactions, has not been attempted, although this could be of great interest.

(b) *Bacterial infections and chemotaxis.* The various mechanisms which have been considered for induction of chemotaxis by antigen-antibody complexes may also apply for bacteria. In addition bacteria may themselves release cytotaxins which may also be of significance for the onset of the inflammatory reaction[35]. In vivo the following variations have been observed in the cellular composition of the inflammatory exudate, which can

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[95] H. RAMSEIER, Science *157*, 554 (1967).

best be explained by the complex host-parasite relationship. After injection of tubercle bacilli into the blood stream of animals, granulocytes collected where the bacilli lodged and this happened within 24 h. Afterwards these cells were replaced by monocytes. But again after a week or two the tubercle becomes necrotic and a second wave of granulocytes surrounded the bacteria[96,97]. One likely explanation for this seems that the host parasite interaction can determine which cytotaxins are released. It is conceivable that extracellular tubercle bacilli release cytotaxins similar to those found in their culture filtrates or induce formation of cytotaxins in serum in the manner described for tuberculoprotein. If the bacteria reside intracellularly such cytotaxins are probably not formed, but they could nevertheless induce release of macrophage cytotaxins from these cells. It is noteworthy that staphylococci which are mainly located extracellularly lead predominantly to the accumulation of granulocytes. It can be hoped that a further analysis of the mechanisms of the formation of the various cell specific cytotaxins will help to understand the varying histological pattern of inflammatory sites.

(c) *Mechanical and physical injury.* Besides chemical agents also other means such as mechanical or physical injury can lead to leucocyte accumulation in vivo[75,79]. In these cases exogenous cytotaxins play no part. It is likely that under these conditions cytotaxigens and/or cytotaxins are primarily released from damaged cells.

Chemotaxis and phagocytosis. Chemotaxis can direct phagocytes towards foreign matter such as bacteria, which will then be engulfed[6,7,11,63,98]. It is not known whether cytotaxins can also stimulate phagocytosis. Evaluation of this problem is complicated by the fact that phagocytic cells can themselves release chemotactic activity (unpublished results). For inhibition of phagocytosis, however, greater concentrations of chloroquine and of hydrocortisone are necessary than for inhibition of chemotaxis[57]. Following phagocytosis cells show also a decreased chemotactic response[22,54,99].

The significance of chemotaxis for histiolysis and repair. If we accept that chemotaxis plays a major role in the accumulation of leucocytes we have to consider that it can influence processes which occur at inflammatory sites as a consequence of their presence. LEBER[5] who first described chemotaxis of leucocytes has therefore suggested that it plays a role in histiolysis. Various workers have indeed shown that tissue damage in Arthus type reactions depends on the presence of polymorphs. Tissue damage is slight or absent if the influx of polymorphs is prevented by decreasing their number in the circulation[89,90]. Other data suggest that the histiolytic action of polymorphs is due to release of enzymes such as cathepsins[100].

Recent data show that cells as well as subcellular fractions induce chemotaxis by acting as cytotaxins or as cytotaxigens (BOREL, KELLER and SORKIN, to be published). This indicates that chemotaxis participates in the physiological removal of tissue cells and their breakdown products.

Also the possibility that chemotaxis can play a role in tissue repair should be considered. MEIER et al.[24,101] have provided some suggestive experiments on this point. They found that some lipopolysaccharides which have a pronounced stimulating effect on leucocyte emigration in vitro, stimulate granuloma formation in vivo, although they did not promote growth of fibroblasts in vitro. It was concluded from these experiments that the accumulation of leucocytes due to lipopolysaccharides is a cause for the increased granuloma formation. This seems to be a reasonable explanation as it has been shown that blood monocytes transform into fibroblasts[102,103].

Summary. It is the purpose of this review to provide a survey of some more recent work on chemotaxis of leucocytes. Attention was drawn to the existence of a variety of cytotaxins (chemotactic mediators) and in particular to their cell specificity. The significance of these different cytotaxins for the accumulation of leucocytes in inflammatory sites in vivo is discussed.

Zusammenfassung. Die Aktivität chemischer Substanzen, die Wanderungsrichtung von Zellen zu bestimmen, wird als Chemotaxis bezeichnet. Chemotaxis ist von erheblichem biologischem Interesse; sie dient der Reproduktion, der Ernährung, der zellulären Organisation oder der Vermeidung schädlicher Stoffe bei Pflanzen und Tieren.

Das Referat vermittelt einen kritischen Überblick über neuere Arbeiten über Chemotaxis von Leukozyten, und es wird die Bedeutung dieses Vorganges für die entzündliche Ansammlung von Leukozyten diskutiert.

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