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PMN-kinin and kinin metabolizing enzymes in normal and malignant leucocytes

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1. Studies have been carried out on the kinin-forming and kinin destroying activity of rabbit macrophages obtained from the lung before and after BCG injection and from the peritoneal cavity following mineral oil injection. A similar study was carried out with L-1210 leukaemic cells obtained from the peritoneal cavity of mice.

2. The macrophages and leukaemic cells contain enzymes that form kinins from purified kininogen substrates at acid pH. The kinin-forming activity is not limited to the lysosomal fraction of the cell since it is found in extra-lysosomal compartments. Delta-guanidovaleryl benzyl ester partially inhibits the kinin-forming activity. Trasylol does not inhibit the kinin-forming activity of these cells, but does inhibit the kininases of these cells. The lack of effectiveness of this agent as a general anti-inflammatory agent is thus explained.

3. The kininases of the normal and malignant cells are also inhibited by chloromethyl ketones such as tosyl-lysine chloromethyl ketone (TLCK) and tosyl-phenylalanine-chloromethyl ketone (TPCK) as well as by copper salts. Hydroxyquinoline has no inhibitory action on these cells, indicating that they differ from the plasma kininases.

4. Investigation of the kinins produced by enzymes in rabbit and human polymorphonuclear (PMN) cells has demonstrated the formation of a kinin that differs from bradykinin and other known mammalian kinins in its pharmacological properties, molecular weight, and amino-terminal end group. This peptide has been named PMN-kinin.

5. Overall, the investigation has demonstrated the importance of white cells in contributing to the formation and destruction of " extra-plasma " sources of kinins by enzymes which differ from plasma enzymes. Anti-inflammatory agents may have different actions on these cell enzymes from those on plasma enzymes.

It is now widely accepted that chemical mediators such as bradykinin, 5-hydroxytryptamine, histamine, slow reacting substance, basic proteins, etc., are the agents responsible for many of the signs and symptoms seen in inflammatory disorders, tissue injury, endotoxin shock, carcinoid syndrome, etc. The individual roles of

these substances, their time of release, the manner in which they are released, and the cells from which they are released remain, however, unclear in the overall picture of their combined action. To complicate matters further, each individual mediator may have several different pathways of formation and destruction—that is to say, different enzymes from different sources are involved in their metabolism. In kinin metabolism, for example, most investigators presume that kinin formation during injury occurs by activation of plasma kallikrein which in turn releases bradykinin or related analogues from a plasma kininogen. Bradykinin levels are regulated by plasma kininases (Erdös, Sloane & Wohler, 1964 ; Yang & Erdös, 1967) and by inactivation in the lung circulation (Ferreira & Vane, 1967). While the location of plasma kallikrein and plasma kininase in close proximity to the plasma kininogen makes this theory reasonable, the situation is in reality more complex because injury and inflammation bring " extra-plasma " components containing various kinin-forming and kininase enzymes to the site of inflammatory response, for example, white cells and enzymes from broken tissue cells (Greenbaum, Yamafuji & Kim, 1966). Enzymes from these cells may be quite different from the plasma enzymes. Indeed, the role of plasma kallikrein in kinin formation in various phases of inflammation may be questioned by the fact that plasma kallikrein inhibitors such as Trasylol do not have proven effectiveness in the treatment of inflammatory disorders. In addition, the claim (Cline & Melmon, 1966) that steroids exert their anti-inflammatory activity by preventing the activation of plasma kallikrein has been questioned (Eisen, Greenbaum & Lewis, 1968). On the other hand, it has been shown that tissues such as spleen and liver contain kinin-forming and kinin-destroying enzymes which differ from their plasma counterparts in terms of their properties (Greenbaum & Yamafuji, 1966). It was also demonstrated that cellular elements of inflammation such as the polymorphonuclear leucocytes (PMN) contain kinin-forming activity which is not blocked by Trasylol (Greenbaum & Kim, 1967), thus, adding to the suspicion that " extra-plasma " enzymes which are not sensitive to Trasylol may play an important role in forming kinins in inflammation and injury. Additional evidence for this reasoning comes from the findings that in artificially induced inflammatory reactions in rabbits, kinin-forming activity in the inflammatory exudate (removed up to 24 hr after the initiation of inflammation) may be found in the leucocytes present in the exudate (Greenbaum, Carrara & Freer, 1968).

In the current studies, we have extended our investigations of leucocytes to determine the presence or absence of kinin-forming and kinin-destroying enzymes in macrophages and in leukaemic cells. In addition, we are reporting in some detail on the properties of the vasoactive polypeptide, PMN-kinin, the formation of which is catalysed by enzymes from polymorphonuclear leucocytes and the properties of which differentiate it from other known mammalian kinins.

Methods

Rabbit polymorphonuclear leucocytes. These were obtained from exudates produced in the peritoneal cavity of rabbits by glycogen administration (Cohn & Hirsch, 1960). The method of collection and lysis was carried out as previously described (Greenbaum & Kim, 1967).

Human PMN cells. Polymorphonuclear cells were obtained from the synovial joint of a patient with synovitis produced by a gonnococcal infection. The cells were lysed and, in general, treated as the rabbit PMN cells.

Mouse leukaemic cells. Cells were obtained from mice carrying the L-1210 strain (Hirschberg, Brindle & Semente, 1964). To collect the cells, ascitic fluid from the peritoneal cavity of ten animals was pooled and centrifuged at 500 *g*. Each animal gave approximately 10^8 cells/ml. of ascitic fluid. The cells in the pellet were washed twice with saline and twice with cold water. The cells were then suspended in 10 ml. of H_2O and subjected six times to freezing and thawing. The final suspension was centrifuged at 20,000 *g* and the supernatant fluid used as the source of enzymes.

Macrophages. These were obtained from rabbits as peritoneal, normal-alveolar or BCG-induced alveolar macrophages. Peritoneal cells were obtained after injection of mineral oil, normal-alveolar by washout of normal lung tissue and BCG-induced alveolar macrophages after intravenous injection of BCG-protein. The procedures used for collection and disruption of the cells were essentially those of Cohn & Wiener (1963) and Myrvik, Leake & Oshima (1961, 1962). $0{\cdot}8–2{\cdot}0 \times 10^8$ cells of the peritoneal or normal alveolar macrophages were obtained from each rabbit. Four rabbits were used in most experiments. "BCG-induced" macrophages in amounts of 2×10^9 were obtained from the rabbits. All cells were initially collected by centrifugation of the exudates at about 500 *g*. After the supernatant had been removed by aspiration, the cells were washed twice with saline with intermittent centrifugation. The saline-washed pellet was then suspended in cold 0·25 M sucrose, centrifuged and then suspended in isotonic sucrose and homogenized with a glass homogenizer by hand. The homogenate was centrifuged at 250 *g* to sediment nuclei and cell debris. The 250 *g* supernatant fluid was centrifuged at 15,000 *g* for 15 min at 4° C. The supernatant was called the "extra-lysosomal" fraction. The pellet is the "lysosomal fraction". The pellet was suspended in 5 ml. of isotonic sucrose and subjected to six serial freezing and thawing procedures. The final suspension was centrifuged at 15,000 *g* to remove the debris.

Kinin-forming activity. Kinin-forming activity was determined by incubating various cell fractions with human or rabbit kininogen purified from plasmas as previously described (Greenbaum & Hosoda, 1963). The kinin formed was assayed on the guinea-pig ileum or oestrous rat uterus as previously described (Greenbaum & Kim, 1967). The isolated tissues were usually pretreated with atropine and diphenhydramine. Carboxypeptidase-B or chymotrypsin inactivation of the activity was taken as the final indication that a peptide had been formed.

Preparation of PMN-kinin. It was of interest to investigate the type of kinin produced by the enzymes from white cells. Because of the difficulty in some of the technical aspects of this investigation, the initial study was undertaken with only one type of cell, the polymorphonuclear leucocyte, which had been shown previously (Greenbaum *et al.*, 1968) to form a kinin which differed from bradykinin. The polymorphonuclear leucocytes were obtained from the peritoneal cavity of rabbits pretreated with glycogen. The kinin-forming activity was obtained by lysing the washed cells in 0·005 N HCl and centrifuging at 15,000 *g*. The supernatant fluid was used as the source of enzyme. Such acidic treatment conveniently destroys the kininase activity (as measured with bradykinin). Human kininogen (500 mg) was incubated with 10 ml. of a 15,000 *g* supernatant fluid of a acid-lysed PMN cell preparation. The volume of the reaction mixture was 100 ml. and the incubation was carried out at pH 4·0 (acetate buffer 0·2 M) for 16 hr at 37° C. Boiling ethanol (400 ml.) was added to the reacting solution and the solution kept in a boiling water bath for 20 min. The cooled suspension was centrifuged and the supernatant now

containing *p*-toluene-sulphonic acid (*p*-TSA) (see later) was brought to dryness by rotary exaporation. The concentrated material was taken up in 20 ml. of water. An amount of *p*-toluene-sulphonic acid had been added to the alcohol extract before flash evaporation so that the concentrated solution contained 10^{-3}M. The *p*-TSA was found to be absolutely necessary to prevent loss of activity, presumably by adsorption of kinins to glass, during the rotary evaporation (Greenbaum, Yamafuji & Hosoda, 1965).

It should be noted that only the extract of the complete reaction mixture had activity as assayed on the rat uterus and the guinea-pig ileum. Control alcohol extracts of the kininogen incubated without enzyme or of the enzyme incubated without kininogen showed no activity.

The concentrated extract was then subjected to column chromatography on carboxy-methyl-cellulose (CMC) (Fig. 1). The activity was first eluted from a 2·5 × 3·0 cm column by stepwise changes in concentrations of ammonium acetate. Activity was followed by assay on both the guinea-pig ileum and the rat uterus. It will be noted that two peaks of activity are obtained by such procedures. The fractions from the largest peak (tubes 32-36) were pooled, diluted until the conductivity was 5 mmho and rechromatographed on a 0·9 × 30 cm column of CMC and subjected to gradient elution with ammonium acetate. After elution of a small

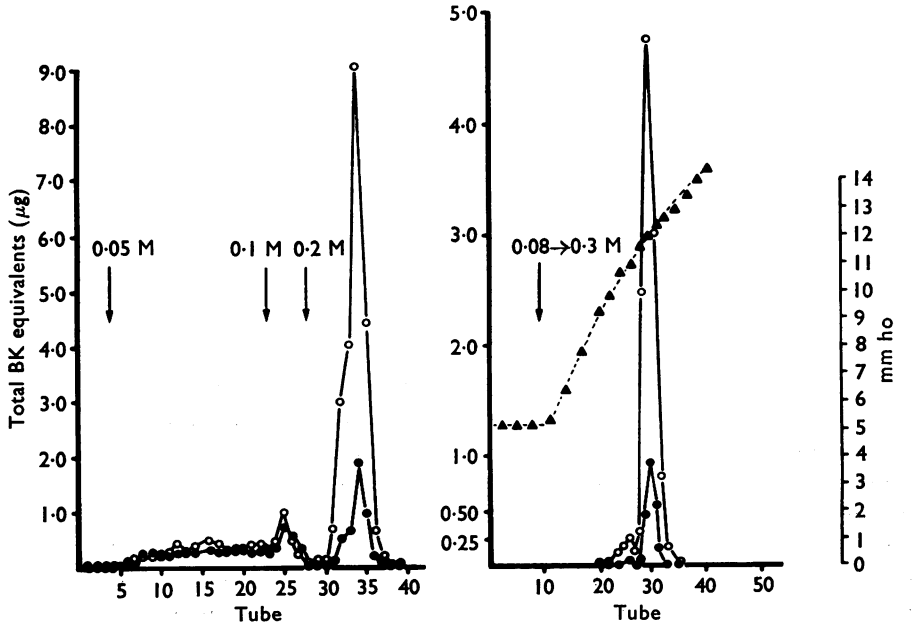

FIG. 1. Purification of PMN-kinin on carboxymethylcellulose columns. Left: stepwise elution from CMC. 300 ml. of water reconstituted ethanol extract (containing 35·2 μg bradykinin equivalents—rat uterus assay) was applied to CMC previously equilibrated with 0·05 M NH_4 acetate. Elution was by stepwise addition of increasing concentrations of NH_4 acetate at the indicated tubes. Flow rate was 40 ml./hr and fraction size was 4·0 ml. Column size, 2·5 × 3·0 cm. Right: re-chromatography on CMC by gradient elution. Tubes 31-36 from the stepwise eluted chromatogram (containing 22·2 μg bradykinin equivalents—rat uterus) were combined and diluted with distilled water to obtain a conductivity of 5·0 mmho. This was applied to CMC and eluted by a gradient from 0·08 M to 0·3 M NH_4 acetate. Flow rate was 25 ml./hr and 2·5 ml. fractions were collected. Column size, 0·9 × 30 cm. All fractions were assayed for activity on the isolated rat uterus (○——○) and guinea-pig ileum (●——●). Conductivity is represented by (▲——▲).

peak, a large symmetrical peak of activity appeared. The material from the peak was pooled and described here as PMN-kinin.

Kininase assays. Kininase was assayed by incubating bradykinin with various cell fractions at pH 8·0. At various times (30–60 min) a sample was added to the isolated ileum to determine the kinin remaining.

Chemical agents. Hydroxyquinoline was used as the HCl salt. Phenylalanine-arginine was a product of the Mann Corp. Tosyl-leucine-chloromethyl ketone (T-leu-CK), tosyl-phenylalanine-chloromethyl ketone (TPCK) and tosyl-lysine-chloromethyl ketone (TLCK) were products of the Cyclo Corporation, California. Solutions of these compounds were made daily and not stored. Trasylol was a product of the Bayer Corporation. Trypsin soybean inhibitor was a product of the Mann Corporation. Bradykinin-tri-acetate was a product of the Cyclo Corporation. Kallidin was a gift from the Sandoz Corporation. Methionyl-lysine-bradykinin was a gift of Dr. R. B. Merrifield, Rockefeller University. Delta-guanidovaleryl benzyl ester carbonate was a gift from Dr. Setsuro Fujii, Tokushima University, Japan.

Results

Macrophages are cellular elements which emigrate to the site of inflammation 3 or 4 hr or more after initiation of the injury. In some cases, as in the lungs, a homogenous group of macrophages is always present. Irritation to the lungs by substances such as BCG-protein produce a greatly enhanced population of " stimulated " macrophages. Since it is not clear if all macrophages are the same, three populations of rabbit macrophages were tested for their kinin-forming and kininase activities: peritoneal macrophages (produced by mineral oil injection), alveolar macrophages and BCG-stimulated alveolar macrophages. In all cases, the cells were lysed and the contents subjected to differential centrifugation to produce a lysosomal pellet and extra-lysosomal-supernatant fluid. The nuclear fraction (which also consists of cell debris) was also assayed. The results are seen in Table 1. It may be seen that all three types of macrophages have kinin-forming activity contained in both lysosomal and extra-lysosomal fractions. The activity is the same order or greater as that found in polymorphonuclear leucocytes (Greenbaum & Kim, 1967). The alveolar macrophages are particularly rich in kinin-forming activity as compared with the macrophages from other sources.

The alveolar kinin-forming activity in the lysosomal and extra-lysosomal fraction

TABLE 1. *Kinin-forming activity of subcellular fractions of macrophages*

Source of macrophages	Extra-lysosomal supernatant	Lysosomal pellet	Nuclei
Peritoneal	273±3	80±10	110±3
Alveolar	405±120	195±24	315±162
Alveolar* (BCG-induced)	220	150	0
Polymorphonuclear* leucocytes	140	47	ND

Values represent ng bradykinin/hr per 10^7 cells.
* Represents one preparation. Other values were obtained as the means of four separate preparations. ND, Not determined.
Kinin-forming activity was determined by incubating 0·2 ml. of each subcellular fraction (see **Methods**) with 5·0 mg of human kininogen in 0·05 M acetate buffer at pH 4·0 at 37° C for 3 hr. The incubation solution was then heated for 5 min at 100° C, neutralized, and a sample added to the guinea-pig ileum or rat uterus.

was subjected to the action of Trasylol and other agents to determine whether the kinin-forming activity was similar to plasma kallikrein. The results are seen in Table 2. Trasylol has no inhibitory activity on the kinin-forming activity of the macrophage cells, demonstrating their similarity to PMN cell kinin-forming enzymes

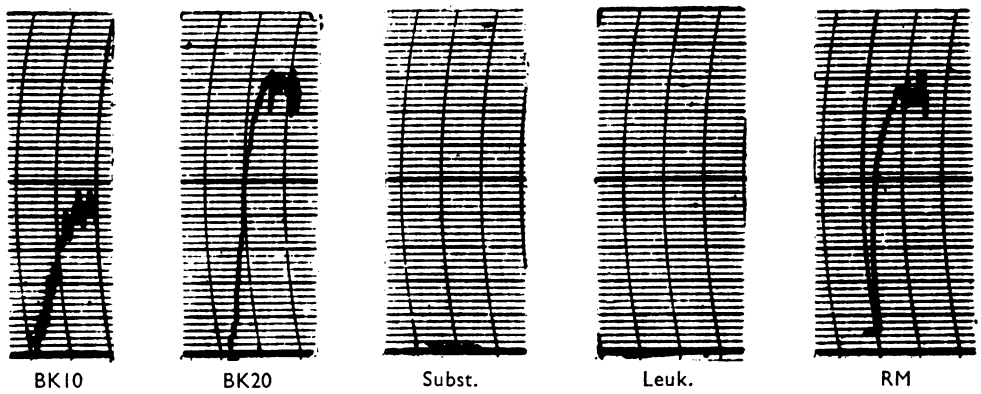

FIG. 2. Formation of kinin by leukaemic cell enzymes. Isolated guinea-pig ileum (35° C). BK, Bradykinin, 10 and 20 ng. Subst, Substrate alone—5 mg human bradykininogen in 1·0 ml. of 0·2 M acetate buffer pH 4·0 at 37° C for 3 hr ; 0·1 ml. of the boiled neutralized sample was assayed. Leuk, Leukaemic enzymes alone—0·1 ml. supernatant fluid from freeze-thawed leukaemic cells in acetate buffer at 37° C for 3 hr ; 0·1 ml. of boiled neutralized sample was assayed. RM, Complete reaction mixture—5 mg human bradykininogen and 0·1 ml. supernatant of leukaemic cells in acetate buffer pH 4·0 at 37° C for 3 hr, 0·05 ml. of boiled neutralized sample. In order to avoid destruction of kinin by kininases active at neutral pH, the incubating solutions were brought to 100° C for 10 min before neutralizing. Only neutral samples were added to the muscle bath to avoid untoward effects of acidity on the assay.

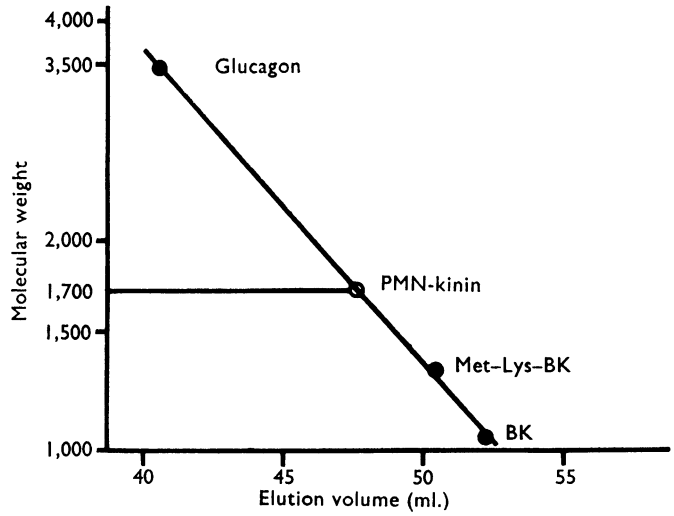

FIG. 3. Estimation of the molecular weight of PMN-kinin on Sephadex G-25. 1·0 ml. samples containing PMN-kinin (8 μg bradykinin equivalents—rat uterus), bradykinin (25 μg), Met-Lys-bradykinin (100 μg) and glucagon (1·5 mg) were applied to a 0·9 × 107 cm column of Sephadex G-25 previously equilibrated with 0·05 M NH_4 acetate, pH 3·0. Elution was carried out with 0·05 M NH_4 acetate pH 3·0 at a rate of 13–15 ml./hr. 1·5 ml fractions were collected. Elution volume was calculated as the volume to the peak tube. Kinins were determined by assay on the isolated rat uterus and glucagon was measured spectrophotometrically by its absorption at 280 mμ.

(Greenbaum & Kim, 1967) rather than to plasma kallikreins. Indole and soybean-trypsin-inhibitor have differential action on the lysosomal and extra-lysomal fraction of alveolar macrophages indicating that the kinin-forming activity in these cell fractions are actually different and not simply due to disruption of lysosomes during the preparation of the fractions. The finding that delta-guanidovaleryl ester is an inhibitor of the kinin-forming activity in both fractions is in keeping with the broad inhibiting activity of this compound whch has been recently shown to inhibit plasmin, trypsin and plasma kallikrein (Muramatu & Fujii, 1968).

Kinin-forming activity of leukaemic cells

It was of interest to compare the kinin-forming activity of malignant white cells with that of normal white cells. Mouse L-1210 cells provided a convenient source of such material. The cells were collected, washed and lysed as described in **Methods** and the supernatant fluid incubated with human kininogen. A sample of the incubation mixture as well as of control samples of kininogen alone and lysate alone were assayed on the guinea-pig ileum for kinin. As will be seen in Fig. 2, only the complete reaction mixture produced a contraction of the ileum. The activity was destroyed by brief incubation with carboxypeptidase-B (not shown) demonstrating the peptide nature of the material.

Trasylol, as in the case of macrophage and PMN cell kinin-forming enzymes, failed to inhibit the kinin-forming activity of the leukaemic cells.

Incubations were carried out at acid pH to prevent kininase activity.

Pharmacological and chemical properties of PMN-kinin

As can be seen from Table 3, purified PMN-kinin, prepared as described under

TABLE 2. *Inhibition of kinin-forming activity in alveolar macrophages*

Agent	% inhibition	
	Extra-lysosomal	Lysosomal
Trasylol (500 u./ml.)	0	0
Soybean trypsin inhibitor (1 mg/ml.)	3	37
Indole (0·001 M)	27	5
Delta-guanidovaleryl benzyl ester		
(0·002 M)	35	35
(0·01 M)	60	60

Incubations were carried out as in Table 1 but in the presence of the agents listed above.

TABLE 3. *Relative potency of mammalian kinins and PMN-kinin*

	BK	Lys-BK	Met-Lys-BK	PMN-kinin
Rat uterus/GPI	1·0	2·2	3·4	5·8
Rabbit B.P./GPI	1·0	6·0*	20–30*	28·5
Rat duodenum/GPI	1·0	1·7*	0·8–2·5*	2·5

* Calculated from data given by Erdös (1966).
GPI, Activity on the guinea-pig ileum. BK, Bradykinin; Lys-BK ,kallidin; Met-Lys-BK, methionyl-lysyl-bradykinin.
Figures represent the ratio of activities of the two assays listed to the left. All ratios of bradykinin activities were set at 1·0 for each set of assays.

Methods, differs from bradykinin in that it is some six times more active on the rat uterus than on the guinea-pig ileum when indices of discrimination are calculated with the bradykinin index set at 1. It may also be seen that in comparison with bradykinin and kallidin it has a greater index in causing the relaxation of the rat duodenum and in its hypotensive activity in the rabbit. Its relative potencies in the latter two bioassays resembles methionyl-lysyl bradykinin. Unlike methionyl-lysyl bradykinin, however, PMN-kinin is not affected by the action of trypsin. When methionyl-lysyl-bradykinin is incubated with trypsin the index of discrimination is lowered because bradykinin is released from the peptide (Table 4). In the case of PMN-kinin, however, bradykinin-like material is not released by trypsin from the peptide as seen by the lack of change in the index of discrimination. PMN-kinin does not inhibit trypsin in assays of the protease on synthetic substrates such as benzoyl-L-arginine ethyl ester indicating that the lack of activity by trypsin on the peptide was not due to inhibiting properties of the peptide but due to the structure of PMN-kinin which must differ from methionyl-lysyl-bradykinin.

In addition to contracting the rat uterus and guinea-pig ileum, and relaxing the rat duodenum, PMN-kinin, like other known mammalian kinins, was found to increase capillary permeability (blueing), was hypotensive, and caused joint pain in the dog. It is destroyed by chymotrypsin, carboxypeptidase-B and plasma and PMN kininases. The molecular weight is in the range of 1700 as determined on Sephadex G-25 (Fig. 3). Analysis of the amino-acid at the amino-terminal position of the most

TABLE 4. *Effect of trypsin on PMN-kinin and Met-Lys-BK*

	PMN-kinin	Met-Lys-BK
RU/GPI before trypsin	5·8	3·4
RU/GPI after trypsin	5·8	1·3

Reaction mixtures: PMN-kinin (assaying at 7·6 μg bradykinin equivalents/ml.–rat uterus) or Met-Lys-bradykinin (20 μg/ml.) were incubated with trypsin (20 μg/ml.) in 1·0 ml. 0·05 M Tris containing 0·05 M $CaCl_2$ at pH 7·5. Incubation was for 30 min at 38° C. Reaction was stopped by placing samples in boiling water bath for 10 min. RU/GPI represents ratio of activities on the rat uterus and guinea-pig ileum.

TABLE 5. *Action of various agents on leucocyte kininases*

Agent	PMN	Alveolar macrophages	L-1210
8-Hydroxyquinoline (10^{-4})	0	0	0
Phenylalanyl-arginine (10^{-3})	0	0	0
Soybean inhibitor	0	0	0
Trasylol (500–1,500 u./ml.)	+	++	+
TPCK (10^{-3})	++	++	+
TLCK (10^{-3})	++	++	+
T-Leu-CK (10^{-3})	++	++	+
$CuSO_4$ (10^{-4})	++	++	+
Pancreatic inhibitor (0·5 mg/ml.)	++	++	—

TPCK, Tosyl-L-phenylalanine chloromethyl ketone; TLCK, tosyl-L-lysine chloromethyl ketone; T-Leu-CK, tosyl-L-leucine chloromethyl ketone.
++ Represents 75–100% inhibition after 60 min; + represents 50% inhibition after 60 min; 0 represents no inhibition; — indicates not tested. Numbers in parenthesis refer to final concentrations in molarity or units.
The assays were carried out by incubating 0·010 mg bradykinin in 0·05 M Tris buffer at pH 8·0 with 0·01 to 0·1 ml. of the "supernatant fractions" of each type of leucocyte in a final volume of 0·5 ml. At zero time and at 30 and 60 min, 0·1 ml. sample was diluted with *p*-toluensulphonic acid (see **Methods**) to 1·0 ml. and 0·01 to 0·05 ml. assayed on the guinea-pig ileum strip.

purified preparation by dinitrophenylation indicated only one end group—that of glutamic acid.

Figure 4 demonstrates that the relative amounts of PMN-kinin and bradykinin-like peptides (ileum to uterus ratio of 1·0) produced by kinin-forming enzymes of PMN cells depends on the species of substrate and species of PMN cell used. Human cell enzymes catalysed the formation of more bradykinin-like material than PMN-kinin, when human kininogen was the substrate. The rabbit enzymes acting on its own kininogen seemed to produce about equal quantities of the two peptides. Rabbit enzymes acting on human substrate always produce a larger concentration of the PMN-kinin.

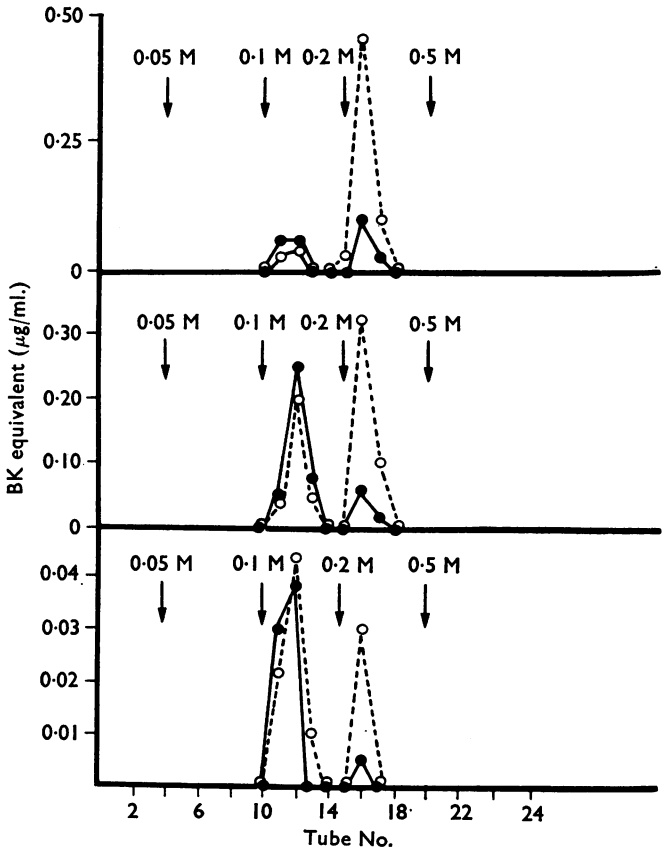

FIG. 4. Kinins produced by various enzyme-substrate combinations. Samples were applied to a 0·9 × 4·0 cm column of CMC and eluted by stepwise addition of NH_4 acetate as indicated. Each tube was assayed on the isolated rat uterus (○——○) and guinea-pig ileum (●——●). Peak eluting between 0·1 M and 0·2 M buffer, chromatographs and assays like the bradykinin standard. Peak eluting at 0·2 M buffer is PMN-kinin. Top: Rabbit PMN enzyme and human bradykininogen; 14 ml. of water reconstituted ethanol extract (containing 2·1 μg bradykinin equivalents—rat uterus) was applied and eluted at a rate of 13–18 ml./hr. Fraction size was 1·5 ml. Middle: Rabbit PMN enzyme and rabbit bradykininogen; 12.5 ml. water reconstituted ethanol extract (containing 1·4 bradykinin equivalents—rat uterus) was applied and eluted at rate of 15–17 ml./hr. Bottom: Human PMN enzyme and human bradykininogen; 5·0 ml. water reconstituted ethanol extract (containing 0·5 μg bradykinin equivalents—rat uterus) was applied and eluted at rate of 11–13 ml./hr.

Studies on leucocyte kininases

A study was carried out on the characteristics of the kininase enzymes in normal and malignant cells. It was of particular interest to determine whether inhibitors of plasma kininases also inhibited the white cell enzymes. Table 5 summarizes this study. Inhibitors of plasma kininases—for example, hydroxyquinoline and phenylalanine-arginine (Yang & Erdös, 1967)—had no effect on the white cell enzymes indicating that the plasma kininases and leucocyte kininases are different. The chloromethyl ketones and $CuSO_4$ are potent inhibitors of the leucocyte enzymes. Trasylol which is usually considered to be an agent which blocks kinin-formation was found to block the kininases of the leucocyte.

The relative activities of the kininases of the various leucocytes was studied and found to be in the same range.

Studies on the pH optimum of the various enzymes have indicated a broad range of activity from pH 6·0–10·0.

Discussion

The results of the present investigations showing that rabbit leucocytes of the monocytic series contain kinin-forming and kinin-destroying enzymes supplement previous findings that leucocytes of the granulocytic series contain such enzymes (Greenbaum & Kim, 1967). The finding that leukaemic cells of mice also contain kinin-forming and destroying enzymes lends additional support to the conclusion that leucocytes may play a role of importance as " extra-plasma " sources of kinin-metabolizing enzymes. One question which is difficult to answer is whether the properties of the kinin-forming and destroying enzymes of the white cells allow " net " kinin formation to take place *in vivo*. In the *in vitro* experiments carried out, there was " net " kinin formation because the reaction was allowed to proceed at acid pH at which kininase activity was known to be negligible. Since the substrates used were purified, an additional complication in extrapolating to the *in vivo* situation would be the influence of other blood components on the activity of the kinin-forming enzymes. Nevertheless, the presence at inflammatory sites of large numbers of macrophages and other leucocytes such as PMN cells containing kinin-forming enzymes of activators of kinin-forming enzymes (Cline & Melmon, 1966) would seem to indicate that under appropriate conditions of pH or in the presence of appropriate drugs which inhibit kininase activity, kinin formation and its resulting physiological effects would occur. In this regard it is of interest that Trasylol has been found in the present study to inhibit kininase activity of macrophages, PMN cells, and leukaemic cells. Trasylol has always been thought of as an agent which exerts anti-inflammatory activity by inhibiting plasma kallikrein and consequently kinin formation. Trasylol is not, however, a very effective anti-inflammatory agent. The reasons for this, as shown here, may be (*a*) Trasylol does not inhibit kinin-forming activity of the leucocytes of the granulocytic and monocytic series, and (*b*) it inhibits leucocyte kininase activity, allowing accumulation of kinins. Thus its use in chronic inflammatory conditions might even exacerbate the condition rather than alleviate it.

The finding of kinin-forming enzymes in leukaemic cells is of interest from several points of view. First, because kinin-formation from this large source of enzymes in leukaemic patients may play a role in some of the localized circulatory changes as

well as pain, etc., of the diseases. It is of interest that in Hodgkin's disease, plasma kininogen concentrations appear to be reduced (Eilam, Johnson, Johnson & Greger, 1968). The current findings that leukaemic cells contain kinin forming activity would provide a logical explanation for such a reduction.

The inability of agents which are known to inhibit plasma kininases such as hydroxyquinoline, argininyl-phenylalanine, etc., to inhibit the kininases of the types of leucocytes tested indicates that these kininases differ from the plasma kininases. It should be pointed out that in many laboratories hydroxyquinoline and other chelating agents are used to inhibit kininase activity when studying kinin formation. The present findings indicate that these inhibitors would not be effective if leucocyte kininases are present. Copper sulphate was found to be a most consistent leucocyte kininase inhibitor along with the chloromethyl ketones TPCK and TLCK. The latter are a group of agents which are known to act on histidine residues in proteins (Schoellmann & Shaw, 1963 ; Shaw, Mares-Guia & Cohen, 1965) and to inhibit trypsin and chymotrypsin. The results obtained would seem to indicate that histidine residues may have an important function as part of the kininase active site although the crude nature of the enzymes prevent any definitive statement in this regard.

While bradykinin and its analogues kallidin and methionyl-lysyl-bradykinin have been thought of as the kinins prevailing in inflammatory reactions, the present investigation clearly indicates that a polypeptide differing from bradykinin or its analogues may be formed by enzymes of polymorphonuclear leucocytes acting on a plasma kininogen of man or rabbit. This kinin, which we have termed PMN-kinin, has been differentiated from bradykinin and related analogues on the basis of column chromatography, indices of discrimination (on isolated tissues, blood pressure, relaxation of rat duodenum), molecular weight and end-group analysis. The pharmacology of this kinin will be reported in detail in a future publication but it can be stated that it has many of the properties of bradykinin including the production of joint pain and increasing capillary permeability. It differs from leucotaxine (Menkin, 1936) in that it is not destroyed by trypsin although it will be of interest to determine if PMN-kinin-like bradykinin and leucotaxine causes the emigration of leucocytes. Molecular weight studies indicate it is in the order of twenty amino-acids. The discovery that PMN cell enzymes catalyse the formation of a kinin differing from all known mammalian kinins indicates that it will be reasonable to assume that additional kinins differing from bradykinin and its immediate analogues in structure will eventually be found. It is possible that they may represent large peptides containing bradykinin as in the case of wasp-kinin (Pisano, 1968) or peptides differing in amino-acid sequences. Studies are proceeding with PMN-kinin to elucidate these points. Overall, it should be noted that drugs acting on " extra-plasma " enzymes may have as much or more influence on the inflammatory response as those acting on plasma enzymes.

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L. M. G. is a Career Scientist, Health Research Council, City of New York. Studies on various aspects of this investigation have been submitted to the Graduate Faculties of Columbia University as part of the Dissertation of R. F. in partial fulfilment for the Ph.D. degree.

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