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Effect of thermal injury on the kinin system in rabbit hind limb lymph

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Summary

1. The kinin-forming activity of hind limb lymph and of plasma has been examined in rabbits before and after thermal injury.

2. Neither plasma nor lymph contained much active kallikrein activity but the enzyme was evident in samples treated with glass or with acid.

3. There was little or no increase in the activity of enzyme activated by glass after thermal injury, but an increase in the activity of enzyme activated by acid regularly occurred.

4. There were two increases in the activity of enzyme activated by acid—one about 2 h and the other 4–6 h after thermal injury. They corresponded to increases in vascular permeability as indicated by increases in the concentration of lymph protein.

5. There was considerably more kininogen in the lymph and plasma than was used in the assays of kallikrein activity, showing that the increased kinin-forming activity in lymph was not the result of the passage of kininogen from the plasma.

6. The increase in activity in lymph was not usually accompanied by a similar increase in the plasma. However, an increase in the activity of enzyme activated by acid sometimes occurred in the plasma simply as a result of prolonged anaesthesia.

7. It is suggested that whereas the enzyme activated by glass is a measure of prekallikrein, the acid activatable enzyme appears as a result of the dissociation of a kallikrein-inhibitor complex. An increase in the concentration of this complex is therefore an indication of the preceding activation of kallikrein.

Introduction

Activation of the plasma kinin system leads only to a transient appearance of active enzymes and of pharmacologically active peptides. It is therefore difficult to obtain direct evidence of the participation of the kinin system in local tissue injury.

Several authors have found kinins in body fluids during injury but the presence of the kinins in a fluid which contains a high peptidase activity and in which added kinins disappear rapidly seems to have doubtful relevance.

Edery & Lewis (1963) examined the kinin-forming activity of the lymph draining dogs' hind limbs and found 3 to 9-fold increase after various forms of injury.

This increase was the result of several reactions going on in the incubation mixtures. Some of the difficulties were pointed out by Jacobsen & Waaler (1966) who reported that the increase of kinin forming potential in lymph after injury was due to an increase in kininogen concentration resulting from an increase in vascular permeability.

Kinin-forming enzyme is normally present in plasma and lymph in an inactive form which can be activated by several procedures such as contact with a foreign surface, dilution, or by treating plasma with acid or organic solvents.

In the experiments described here two methods of activation have been used to examine the kinin-forming system in lymph and plasma before and after thermal injury. First, samples of plasma or lymph were brought into contact with a glass surface which causes activation of a precursor of kallikrein to the active enzyme (Armstrong, Jepson, Keele & Stewart, 1957). Second, samples were activated by treatment with acid. The active kallikrein formed by either method was estimated by kinin formation. It cannot be assumed, however, that the active enzyme formed in each case is the same, since plasma appears to contain more than one kinin-forming enzyme (Frey, Kraut & Werle, 1950 ; Beraldo, 1950 ; Lewis, 1958 ; Armstrong & Mills, 1964 ; Vogt, 1966). The results of these experiments show that thermal injury causes a pronounced increase in the kinin-forming activity elicited by treatment with acid.

Methods

Collection of lymph and blood samples

New Zealand White (NZW) rabbits were anaesthetized with pentobarbitone sodium (40 mg/kg) or an ether-oxygen mixture. Cannulation of femoral lymph ducts and production of thermal injury were carried out as described by Lewis & Westcott (1968) for acute experiments and Lewis (1969) for chronic experiments.

Blood samples (2·0–2·5 ml) were taken from the ear artery and centrifuged for 10 min at 3,000 r.p.m. To prevent clotting, a few crystals of heparin were added to the collection tube. The lymph and plasma were kept at room temperature and used as soon as possible, within 1 hour.

Rat uterus

Wistar rats (150–200 g) were injected with 0·1% stilboestrol in ethyloleate (0·2 ml, i.p. and 0·2 ml s.c.). After 30–36 h the rats were killed and the uterus removed. One horn was suspended in a 5 ml organ bath in Munsick's solution at 28° C and aerated with oxygen. Samples were tested against bradykinin standard (10 ng/ml in 0·9% NaCl) and a new standard solution was made from a stronger solution every 2 hours. Threshold response was usually less than 0·5 ng bradykinin. To antagonize 5-hydroxytryptamine (5HT), 0·6 mg bromolysergic acid diethylamide (BOL) was added per litre.

Glass activation

Plasma or lymph (0·6 ml) was shaken by a motor at 37° C with 0·1 ml O-phenanthroline-HCl solution (1% in 0·9% NaCl) and 100 mg ballotini (0·5 mm diameter) for 5 min (after which time kinin formation proceeded more slowly). Samples were assayed immediately on the rat uterus.

Acid activation

Method 1

Plasma or lymph (0·4 ml) was acidified with 0·33 N HCl to about pH 2·0 and with 0·2 N HCl/KCl adjusted to exactly pH 2·0. After incubation for 30 min at 37° C the samples were neutralized with 0·33 N NaOH and after addition of 0·1 ml O-phenanthroline (1% solution) the pH was adjusted with phosphate buffer to 6·4. Samples were incubated for a further 20 min at 37° C (after which time kinin formation proceeded more slowly), and tested immediately on the rat uterus.

Method 2

Lymph (0·5 ml) was collected directly into 0·1 ml N HCl standing in ice and left for 15 min at room temperature. Plasma (0·5 ml) was added to 0·1 ml N HCl and kept for 15 min at room temperature.

The samples were then neutralized with N NaOH and after addition of 1 mg kininogen and 40 mg O-phenanthroline the volume was adjusted to 2 ml with 0·2 M phosphate buffer, pH 7·4. This was incubated for 30 min at 37° C (after which time kinin formation proceeded more slowly) and boiled for 10 minutes. Samples were then stored at −10° C overnight and assayed on the rat uterus the following day.

Protein

The method of Lowry, Roseborough, Farr & Randall (1951) was used for protein estimations.

Kininogen preparation

Kininogen was prepared after the method of Brocklehurst & Mawer (1966).

Kininogen estimation

In order to ensure that there was sufficient kininogen present in both plasma and lymph to act as substrate for the activated kallikrein, several estimations were carried out as follows. The sample of plasma or lymph was incubated together with either pancreatic kallikrein (Bayer) or trypsin in the presence of the kininase inhibitor, O-phenanthroline. The kinins so formed were assayed on the isolated rat uterus. As the lymph and plasma were not denatured for the assay of kininogen as described by Diniz, Carvalho, Ryan, & Rocha e Silva (1961), the values of kininogen in our investigation might be apparently low due to interference by kallikrein inhibitor. However, in all these experiments there was always considerably more than sufficient kininogen present during the estimation of glass and acid kallikrein.

Substances used

Heparin (180 units/mg) (Pulayin) Evans Medical Ltd., bradykinin triacetate, Sigma ; hog pancreatic kallikrein, Farbenfarbriken Bayer A.G. ; O-phenanthroline-HCl, Koch Light Lab. Ltd. ; stilboestrol dipropionate (10 mg/ml) Burroughs Wellcome Co. (diluted 1:10 in ethyl oleate) ; BOL 148 Sandoz Ltd.

Results

Levels of activity in normal plasma and lymph

The terms glass kallikrein and acid kallikrein will be used here to describe the activities induced by glass or acid activation without implication as to whether or not they are the same enzyme.

The mean values and ranges of the acid kallikrein, estimated by two methods, and glass kallikrein activities in normal plasma and lymph are given in Table 1. Two features are apparent from these results. First, in the experiments in which both glass and acid (Method 1) kallikrein were estimated in plasma and lymph, the activity produced by activation with acid was greater than that produced by glass. Second, the activity levels of both the acid kallikrein and the glass kallikrein were higher in plasma than those in lymph.

The levels of acid kallikrein in plasma were more variable and it might well be that these alterations were at least partially the result of the anaesthetic since as shown in the following experiments anaesthesia itself sometimes affected the plasma or acid kallikrein.

Pentobarbitone anaesthesia

Intravenous injection of pentobarbitone sodium (40 mg/kg) caused in the plasma of two out of four rabbits an increase in the activity of acid kallikrein estimated by Method 1. In these two experiments the levels increased within 2 h to 15 and 20 times the levels found in the plasma before injection of the anaesthetic. In the other two experiments the activity of plasma acid kallikrein did not change significantly. There was not a corresponding change in the activity of lymph acid kallikrein which remained unchanged in all four experiments. In none of these experiments was there a change in the activity of glass kallikrein in the plasma or lymph.

Ether anaesthesia

As with pentobarbitone, anaesthesia with ether did not result in the alteration of the activity of glass kallikrein, but affected the activity of acid kallikrein of plasma in a variable way. The effect of ether was studied in eight experiments. The preanaesthetic level of acid kallikrein activity increased 4, 7·5 and 9·5 times within 2 h in three experiments, and decreased by 3 and 4 times in two experiments. There was no change in three experiments. In four of the experiments in which lymph was collected from one hind limb, no significant change occurred in the activity of acid or glass kallikrein of the lymph even though in one of these the plasma acid kallikrein rose from 25 to 240 ng/ml.

TABLE 1. *Bradykinin equivalent (ng/ml) formed in normal plasma and lymph after acid and glass activation*

	Plasma			Lymph		
	Acid		Glass	Acid		Glass
	Method 1	Method 2		Method 1	Method 2	
No. of expts.	20	8	20	18	8	18
Mean	66	22	27	7·6	8·5	3·9
±S.E.	8	5	4	2	2·6	0·8
Range	10–140	3–52	4–58	1–25	2–25	1–10

Effect of thermal injury

Acute experiments using pentobarbitone anaesthesia

Here, only acid kallikrein was measured. The technique of activation and assay used was Method 2 in which samples of lymph were collected directly into acid every 10–30 min depending upon the flow rate. In all eight experiments there was an increase in the activity of acid kallikrein in the lymph after the hind limb had been scalded at 80° C for 15–20 seconds. Maximum levels attained were 23, 7, 13, 22, 3, 23, 16 and 24 times the resting level estimated before injury. There were 1·5, 3·5, 2, 2, 2, 1, 3, and 4-fold increases in the corresponding plasmas. The increase in activity in the lymph occurred in two peaks—the first within 2 h and the second 4–6 h after the burn. The values at each peak are given in Table 2, and Fig. 1 illustrates that the two peaks were quite distinct. Figure 1 also shows that the changes in the lymph did not simply reflect changes occurring in the plasma since the plasma level remained below 6 ng/ml whereas the activity in the lymph reached 26 and 33 ng/ml during the peaks of increase.

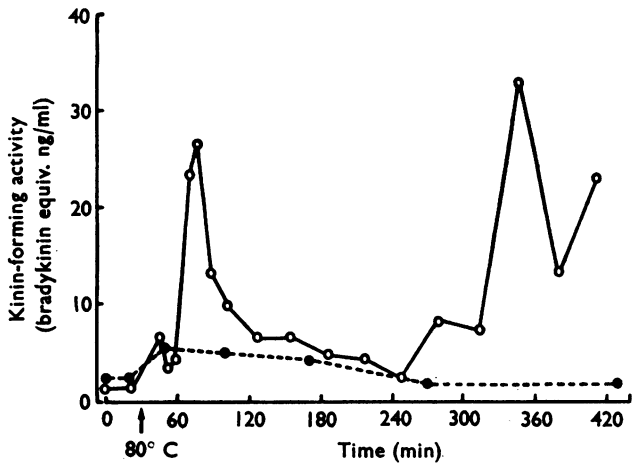

FIG. 1. Rabbit, male, 4·1 kg, anaesthetized with pentobarbitone sodium. Acid kallikrein activity, expressed as ng/ml bradykinin formed after incubation for 30 min, was estimated by Method 2, in lymph collected from the right hind limb (○——○) and in plasma collected from the carotid artery (●– – –●). At the arrow the right hind limb was scalded with water at 80° C for 15–20 seconds.

TABLE 2. *Bradykinin equivalent (ng/ml) formed by acid kallikrein in lymph and plasma before and after the hind limb had been burned at 80° C for 15–20 s under pentobarbitone anaesthesia*

		Lymph after burn			Plasma after burn	
Expt.	Control	0–2 h	4–6 h	Control	0–2 h	4–6 h
1	14	100	315	52	78	62
2	6	9	40	25	82	10
3	5	65	37	23	50	58
4	1·5	26	33	3	5	2
5	7	14	15	7	14	7
6	3	14	70	21	24	40
7	9	90	140	39	110	116
8	25	590	540	40	150	50

Acute experiments using ether anaesthesia

Here the rabbits were maintained under ether anaesthesia throughout the whole experiment and the hind limb from which the lymph was collected was burned at 60° C for 1 minute. The results are summarized in Table 3. In the three experiments in which lymph was collected, the level of activity of acid kallikrein estimated by Method 1 was raised; in two of these experiments the second peak of activity was larger than the first. The acid kallikrein in plasma also increased in most experiments but only in one was there evidence of a second peak of activity

TABLE 3. *Bradykinin equivalent (ng/ml) formed by acid kallikrein and glass kallikrein in lymph and plasma before and after the hind limb had been burned at 60° C for 1 min under ether anaesthesia throughout the experiment*

		Lymph after burn			Plasma after burn	
Acid kallikrein	Control	0–2 h	4–6 h	Control	0–2 h	4–6 h
1	3	15	25	22	32	11
2	2	45	—	10	68	—
3	4	15	73	60	110	110
4	—	—	—	140	110	40
5	—	—	—	29	210	20
6	—	—	—	31	40	13
Glass kallikrein						
7	2	2	5	17	14	11
8	3	2	2	10	20	5
9	—	—	—	48	30	47

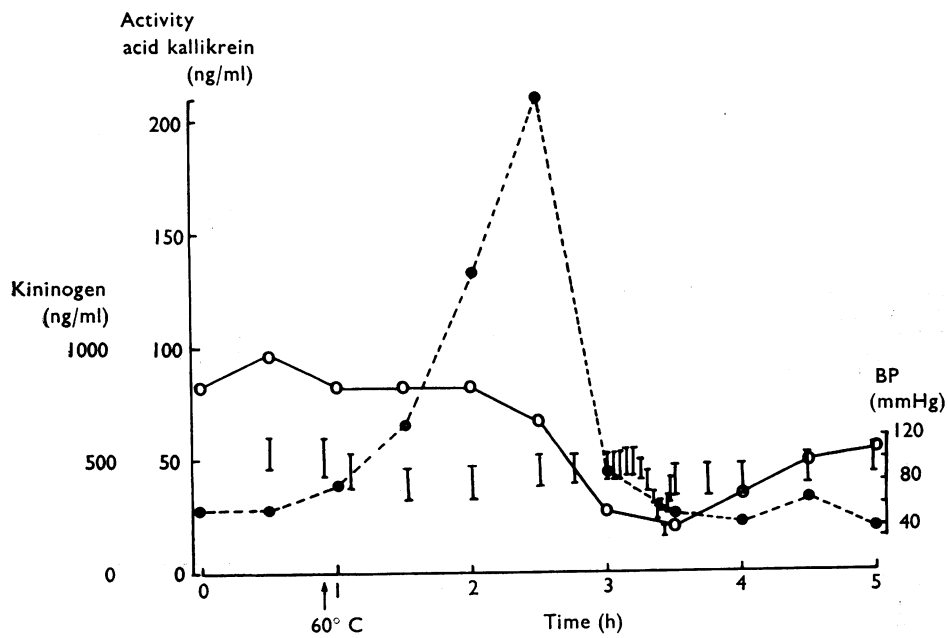

FIG. 2. Rabbit, male, 3·5 kg, anaesthetized with an ether/oxygen mixture. At the arrow the right hind limb was immersed in water at 60° C. A cannula was inserted in the carotid artery via which blood samples were taken and arterial blood pressure recorded continuously. The content of plasma kininogen (○——○) and acid kallikrein (●- - -●) (Method 1), both expressed as ng/ml bradykinin formed during incubation, were estimated as described in **Methods.**

4–6 h after injury. There was no consistent change in the activity of glass kallikrein either in plasma or lymph.

In one experiment illustrated in Fig. 2 the activity of kininogen in plasma was measured as well as that of acid kallikrein in plasma. The latter rose to the high level of 210 ng/ml at about 1·5 h after the hind limb was burned. As this acid kallikrein returned sharply to the normal value there was a sudden decrease in the concentration of plasma kininogen, accompanied by a vascular collapse in which the blood pressure fell to 30 mmHg (1 mmHg≡1·333 mbar) and the pulse pressure to one-third of normal.

Several attempts were made to repeat this experiment under prolonged ether anaesthesia when anoxia was deliberately induced, but the vascular collapse together with the decreased kininogen levels could not be repeated.

Chronic experiments using ether anaesthesia

In these experiments one hind limb was burned at 60° C for 1 min during a short period of ether anaesthesia of less than 10 min 24–48 h after a cannula had been implanted in the main femoral lymph duct. The results in Table 4 show that there was a marked increase in the activity of acid kallikrein (Method 1) in the lymph 4–6 h after injury; however, only in one of four experiments was there an increase during the first 2 hours. The absence of this first peak of activity might well be the result of using only a short period of anaesthesia. There was an increase in the activity of acid kallikrein in plasma in three experiments but only in one of them did the activity rise to that in the lymph. The glass kallikrein activity of the lymph increased in two out of three experiments but unlike the acid kallikrein, did not increase further at 4–6 h after injury.

Figure 3 shows the result of an experiment in which lymph was collected for 4 days and in which there was an early peak of activity as well as a later one. Although there was an increase in the level of acid kallikrein in the plasma as well as in the lymph, they did not coincide since the level in the lymph increased 2 h earlier than that in plasma.

The increase in activity of acid kallikrein is not associated with an increased lymph flow since as seen in Fig. 3 the flow increases gradually after the limb is injured and the increase is maintained during the next 24–48 hours. The increased

TABLE 4. *Bradykinin equivalent (ng/ml) formed by acid kallikrein and glass kallikrein in lymph and plasma before and after the hind limb had been burned at 60° C for 1 min under ether anaesthesia in chronic experiments*

Expt.	Lymph after burn				Plasma after burn			
Acid kallikrein	Control	0–2 h	4–6 h	24 h	Control	0–2 h	4–6 h	24 h
1	2	3	38	7	5	—	10	8
2	9	—	67	—	49	2	17	—
3	6	10	315	200	42	38	170	200
4	2	3	22	5	15	62	20	6
5	3	90	210	45	75	115	365	45
Glass kallikrein								
1	2	16	11	9	3	—	9	9
2	3	—	4	—	3	3	4	—
3	2	—	10	40	30	—	—	24
4	10	10	4	2	22	11	17	6
5	5	27	20	5	23	35	47	27

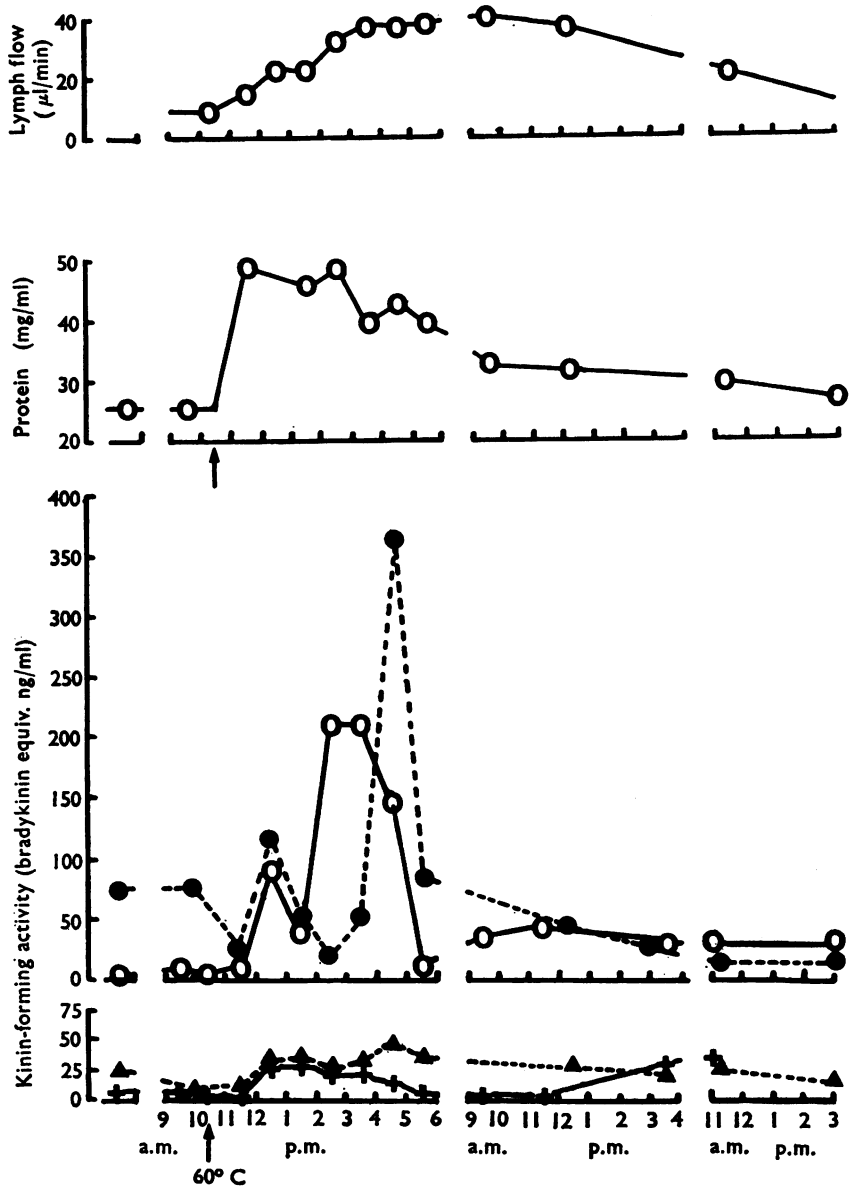

FIG. 3. Rabbit, male, 3·7 kg. Upper record: lymph flow in μl/min. Second record: protein concentration in lymph in mg/ml. Third record: activity of acid kallikrein (assayed by Method 1) in ng/ml bradykinin formed on incubation of lymph (○——○) and plasma (●- - -●). Lower record: activity of glass kallikrein in ng/ml bradykinin formed on incubation of lymph (△——△) and plasma (▲- - -▲). On day 1 the main femoral lymphatic of the right hind limb was cannulated while the rabbit was anaesthetized with ether. The animal recovered in less than 2 hours. On day 2, at the arrow the right leg was immersed in water at 60–62° C for 1 min, while the animal was again anaesthetized with ether. After recovery on day 2, and subsequently on days 3 and 4 the rabbit was allowed to move freely in the laboratory.

concentration of lymph protein which occurs rapidly after injury probably indicates an increased vascular permeability. But it was not possible to see from this experiment whether there was a second increase in protein concentration corresponding to the second increase in activity of acid kallikrein because the lymph protein remained high for at least 7 h, which included the time of the second kallikrein peak. However, in several experiments in which the concentrations of lymph protein were studied over prolonged periods of time a second peak was

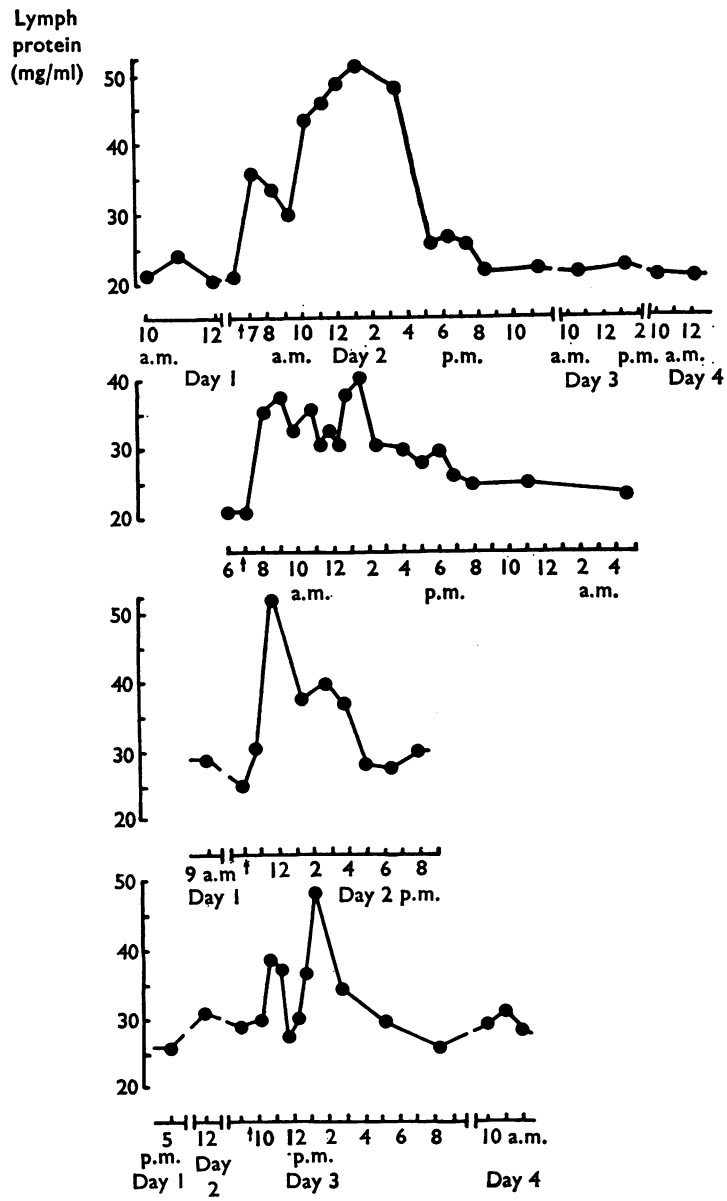

FIG. 4. Protein concentration in mg/ml of hind limb lymph collected from four rabbits. In each experiment the hind limb was immersed in water at 60–62° C for 1 min, at a time indicated by the arrow.

observed 4–6 h after the scald. The precise time and magnitude of the two protein peaks was variable like those of acid kallikrein activity. Figure 4 illustrates, in four experiments, the changes in the total concentration of protein in lymph after the limbs had been scalded at 60° C for 1 minute.

Discussion

These experiments show that after thermal injury there is a pronounced increase in the lymph of an inactive form of kallikrein which can be activated by acid treatment but not by contact with a glass surface. It is not known whether treatment with acid and contact with a foreign surface lead to activation of the same enzyme. However, it seems likely, as illustrated in Fig. 5, that in addition to the activation of Hageman factor shown by Eisen (1963) acidification leads to the dissociation of the complex formed by kallikrein and its inhibitor (Kraut, Frey & Werle, 1930), whilst a foreign surface acts only by activating Hageman factor which in turn activates the precursor of kallikrein (see Eisen & Vogt, 1970). The increase in the activity of kallikrein activated by acid found in the lymph after injury would therefore represent an increase in the kallikrein-inhibitor complex, resulting from an activation of kallikrein in the injured tissue. The formation of such a complex has been demonstrated recently by Trautschold, Fritz & Werle (1966) between a tissue kallikrein inhibitor, aprotinin, with kallikrein or trypsin. They found that the complex was formed at neutral pH but dissociated below pH4.

If this theory is correct any activation of prekallikrein would be expected to lead to an increase in the activity of acid kallikrein. But Eisen (1963) has shown that treatment of human plasma with glass ballotini does not increase its acid-activatable kinin forming activity. However, it is possible that the mechanism by which kallikrein is inactivated *in vivo*, particularly in the tissue space rather than plasma, might be different from that occurring in the test tube.

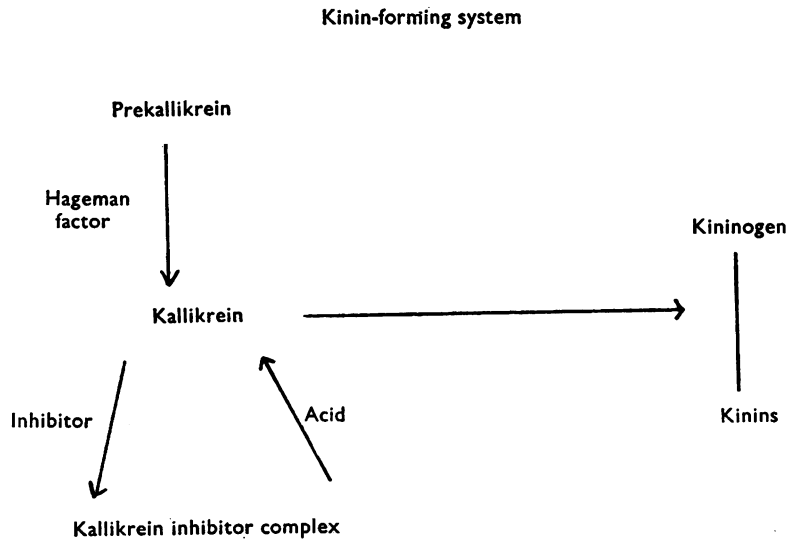

FIG. 5.

The importance of the kallikrein inhibitor in limiting the action the kinin-forming enzymes was clearly shown in the investigation by Landerman, Webster, Becker & Ratcliffe (1962) of a case of hereditary angioneurotic oedema. They showed that the disordered vascular permeability in the disease is an inherited hyperresponsiveness of the skin and of mucosal surfaces to a serum factor which was activated in an unknown way. They subsequently found that the patients were hyperresponsive to kallikrein because they were deficient in kallikrein inhibitor.

As the lymph in our experiments was collected after it has passed through the popliteal lymph node it is possible that the kallikrein inhibitor complex is formed in the node since Werle & Berek (1950) have shown that lymph nodes are particularly rich in kallikrein inhibitor.

It seems likely than an increase in vascular permeability, which occurs soon after injury, would lead to the passage of prekallikrein from the plasma into the interstitial space of the injured area. This precursor would be activated by contact with the damaged cells, or possibly by tissue activators which are released during injury (Lewis, 1959). The resulting kinin formation would be limited by inactivation of the kallikrein as a kallikrein inhibitor complex.

It appears that the change in the activity of acid kallikrein activity in the lymph is closely associated with a change in vascular permeability, although it is unknown whether the permeability change is the cause or the result of the change in the kinin system. Activation of the kinin system can occur in the circulating blood without a corresponding change in the hind limb lymph as shown in the experiments in which the activity of acid kallikrein in plasma increased as a result of anaesthesia. On the other hand the increase in concentration of lymph protein indicated that there were permeability changes which corresponded in time to the early and delayed changes in the activity of acid kallikrein in lymph.

There is no apparent explanation of why there should be a change in permeability and activity of acid kallikrein in the lymph 4–6 h after a thermal injury. However, Sevitt (1958) and Burke & Miles (1958) observed earlier that after the infliction of burns to guinea-pigs there were two phases of increased permeability—one developing almost immediately after the burn and the second after a delay of several hours.

The kininogen levels were measured in only a few experiments in our investigation. The object of these estimations was to ensure that during the assay of lymph kallikrein activity, more than sufficient kininogen was present without the addition of substrate prepared from other animals. There was no doubt that there was considerably more kininogen present than that utilized during the assay of kallikrein showing that the increase in the activity of acid kallikrein was not due to the passage of substrate into the lymph following an increase in vascular permeability, as suggested by Jacobsen & Waaler (1966). Further, in the experiments in which exogenous kininogen was added (see Table 2), the concentration of kinins formed was not higher than those in Tables 3 and 4 in which no additions of kininogen were made.

It seems unlikely that during local reactions at least, the circulating concentrations of kininogen would be appreciably altered during kinin formation. Only in one of our experiments (see Fig. 2) did it appear that the concentration of plasma

kininogen fell appreciably at a time when a high level of acid kallikrein suddenly decreased and when the animal suffered a vascular collapse. One possible interpretation of this finding is that the high concentration of kallikrein inhibitor complex, indicated by the high acid kallikrein activity, was suddenly dissociated perhaps by the rising concentration of ether in the blood stream. The liberated active kallikrein formed kinins thereby reducing the kininogen concentration and producing the vascular collapse. After dissociation of the kallikrein inhibitor complex there would have been an increase in the concentration of free inhibitor, which would be available once more to inactivate newly formed kallikrein.

The method used in this investigation for estimating kininogen does not exclude the interference of kallikrein inhibitor, and the values might well have been influenced by the presence of free inhibitor. In the method of Diniz & Carvalho (1963) the inhibitor should be destroyed by the acid denaturation, but the variable results obtained by different authors with the method suggest that the presence of the inhibitor might have influenced their results as well. For example, some workers have observed an apparent fall in kininogen during kinin formation in the circulating blood (Scharnagel, Greeff, Luhr & Strobach, 1965 ; Lecomte, 1961 ; others observed no change (Cirstea, Suhaciu & Butculescu, 1965 ; Back, Wilkens & Steger 1966) ; while others have found an increase in kininogen during haemorrhagic shock (Diniz & Carvalho, 1963).

REFERENCES

ARMSTRONG, D. A. J., JEPSON, J. B., KEELE, C. A. & STEWART, J. W. (1957). Pain-producing substance in human inflammatory exudates and plasma. *J. Physiol., Lond.*, **135**, 350–370.

ARMSTRONG, D. A. J. & MILLS, G. L. (1964). A highly purified kinin-forming enzyme from human plasma. *Biochem. Pharmac.*, **13**, 1393–1402.

BACK, N., WILKENS, H. & STEGER, R. (1966). Fibrinolysis and vasoactive peptides in anaphylaxis. *Hypotensive Peptides*, ed. Erdos, E. G., Back, N., Sicuteri, F. & Wilde, A. F., pp. 485–505. Berlin: Springer-Verlag.

BERALDO, W. T. (1950). Formation of bradykinin in anaphylactic and peptone shock. *Am. J. Physiol.*, **163**, 283–289.

BROCKLEHURST, W. E. & MAWER, G. E. (1966). The purification of a kininogen from human plasma. *Br. J. Pharmac. Chemother.*, **27**, 256–276.

BURKE, J. F. & MILES, A. A. (1958). The sequence of vascular events in early infective inflammation. *J. Path. Bact.*, **76**, 1–19.

CIRSTEA, M., SUHACIU, G. & BUTCULESCU, I. (1965). Bradykinin and anaphylactic shock in dogs, guinea-pigs and rabbits. *Archs int. Physiol.*, **73**, 231–40.

DINIZ, C. R. & CARVALHO, I. F. (1963). A micromethod for determination of bradykininogen under several conditions. *Ann. N.Y. Acad. Sci.*, **104**, 77–89.

DINIZ, C. R., CARVALHO, I. F., RYAN, J. & ROCHA E SILVA, M. (1961). A micromethod for the determination of bradykininogen in blood plasma. *Nature, Lond.*, **192**, 1194–1195.

EDERY, H. & LEWIS, G. P. (1963). Kinin-forming activity and histamine in lymph after tissue injury. *J. Physiol., Lond.*, **169**, 568–583.

EISEN, V. (1963). Observations on intrinsic kinin-forming factors in human plasma: The effect of acid, acetone, chloroform, heat and euglobulin separation on kinin formation. *J. Physiol., Lond.*, **166**, 496–513.

EISEN, V. & VOGT, W. (1970). Plasma kininogenases and their activators. *Handbook of Experimental Pharmacology. Bradykinin, Kallidin and Kallikrein*, ed. Erdos, E. G., Vol. **25**, pp. 82–130. Berlin: Springer Verlag.

FREY, E. K., KRAUT, H. & WERLE, E. (1950). *Kallikrein (Padutin)*. Stuttgart: Enke.

JACOBSEN, S. (1966). Substrates for plasma kinin forming enzymes in human dog and rabbit plasmas. *Br. J. Pharmac. Chemother.*, **26**, 403–411.

JACOBSEN, S. & WAALER, B. A. (1966). The effect of scalding on the content of kininogen and kininase in limb lymph. *Br. J. Pharmac. Chemother.*, **27**, 222–229.

KRAUT, H., FREY, E. K. & WERLE, E. (1930). Über die Inaktivierung des Kallikreins; uber dieses kreislaufhormon. *Hoppe-Seyler's Z. physiol. Chem.*, **192**, 1–2.

LANDERMAN, N. S., WEBSTER, M. E., BECKER, E. L. & RATCLIFFE, H. E. (1962). Hereditary angioneurotic edema. II. Deficiency of inhibitor for serum globulin permeability factor and/or plasma kallikrein. *J. Allergy*, **33**, 330–341.

LECOMTE, J. (1961). Consommation des Kininogenes plasmatique au cours du choc anaphylactique de lapin. *C.r. Soc. Biol.*, **155**, 1411–3.

LEWIS, G. P. (1958). Formation of plasma kinins by plasmin. *J. Physiol., Lond.*, **140**, 285–300.

LEWIS, G. P. (1959). Plasma-kinin-forming enzymes in body fluids and tissues. *J. Physiol., Lond.*, **147**, 458–468.

LEWIS, G. P. (1969). Changes in the composition of rabbit hind limb lymph after thermal injury. *J. Physiol., Lond.*, **205**, 619–634.

LEWIS, G. P. & WESTCOTT, B. (1968). Intracellular enzymes in rabbit hind-limb lymph after injury. *J. Physiol., Lond.*, **195**, 12–13*P*.

LOWRY, O. H., ROSEBOROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

SCHARNAGEL, K., GREEFF, K., LUHR, R. & STROBACH, H. (1965). Freisetzung von bradykinin beim toxischen, anaphylaktischen und anaphylaktoiden schock. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **250**, 176–177.

SEVITT, S. (1958). Early and delayed oedema and increase in capillary permeability after burns of the skin. *J. Path. Bact.*, **75**, 27–37.

TRAUTSCHOLD, I., FRITZ, H. & WERLE, E. (1966). Kininogenases, kininases and their inhibitors, *Hypotensive Peptides*, ed. Erdos, E. G., Back, N., Sicuteri, F. & Wilde, A. F., pp. 221–234. Berlin: Springer-Verlag.

VOGT, W. (1966). Demonstration of the presence of two separate kinin-forming systems in human and other plasma. *Hypotensive Peptides*, ed. Erdos, E. G., Back, N., Sicuteri, F. & Wilde, A. F., pp. 185–192. Berlin: Springer-Verlag.

WERLE, E. & BEREK, U. (1950). Über Kallidin *Birochem. Zeib.*, **320**, 136–145.

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