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THE EFFECT OF SCALDING ON THE CONTENT OF KININOGEN AND KININASE IN LIMB LYMPH

BY

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Edery & Lewis (1962, 1963) reported that kinin-forming activity was present in lymph collected from hind limbs of dogs, and that this activity increased after various types of injury, especially scalding. Rocha e Silva & Rosenthal (1961) found bradykinin, together with histamine and other pharmacologically active substances in the wash fluid from air pockets in burned skin of the rat. Jacobsen & Waaler (1965) were unable, however, to demonstrate kinin-forming activity in the limb lymph of anaesthetized rabbits and dogs before as well as after scalding. In freshly collected lymph samples from non-injured hind limbs of dogs and rabbits Jacobsen (1966b) similarly found no kinin-forming activity and also no kinin activity. In the lymph samples from dogs he could demonstrate, however, the presence of an inactive kinin-forming enzyme. The two different substrates for kinin-forming enzymes which are present in plasma (Jacobsen, 1966a) could also be detected in limb lymph samples from both animals. The concentration of the substrates was lower in the lymph than in the plasma.

In the present work we have examined the effect of scalding upon the concentration in limb lymph of these various factors related to plasma kinin formation. Dogs and rabbits have again been used as the experimental animals.

METHODS

Animals and anaesthetics. Albino rabbits of both sexes weighing from 2.5 to 3.5 kg, and male mongrel dogs weighing from 15–23 kg were used. Rabbits were anaesthetized with pentobarbitone (Nembutal Natrium V.M., Abbott Laboratories Ltd., London) and chloralose, and dogs with morphine and pentobarbitone as has been described before (Jacobsen, 1966b).

Collection of lymph through one cannulated femoral lymphatic vessel was carried out as has been described in a previous paper (Jacobsen, 1966b).

Scalding of the hind limb was carried out by covering the limb with cotton wool and pouring water of 85° C over it for 15 sec, as described by Edery and Lewis (1963).

Plasma kinin-activity, using the rat uterus preparation, *substrates for kinin-forming enzymes* and *kininase activity* in lymph and plasma samples were estimated as described by Jacobsen (1966b).

Plasma samples were obtained from citrated blood (Jacobsen, 1966b).

Pseudoglobulin from human plasma was prepared as described by Jacobsen (1966a).

Stable plasma substrate preparation was prepared as described by Amundsen, Nustad & Waaler (1963).

Protein content in plasma and lymph samples were estimated using the Biuret method (Gornall, Bardawill & David (1949)).

Bradykinin. Synthetic bradykinin (BRS 640), (Sandoz, Basel, Switzerland) was used.

Glandular kallikrein. Human saliva without kininase-activity (Amundsen & Nustad, 1964) was used as the source of glandular kallikrein.

Histamine inhibition. Mepyramine maleate (Anthisan, May & Baker, Ltd., Dagenham, England) was used as histamine antagonist.

Kininase inhibition. Disodium edetate dihydrate was used *in vitro* as described by Jacobsen (1966b). Calcium disodium edetate (Fluka A.G., Buchs, Switzerland) in a solution of *p*H 7.35 was used for intravenous injection. Phenanthroline (Sigma Chemical Company, St. Louis, Missouri) was used in a stock solution of 4×10^{-3} M at *p*H 7.35 in 0.1 M Tris-HCl buffer.

Haemoglobin estimation, paper electrophoresis, p*H measurements* and *counting of erythrocytes* were carried out as has been described previously (Jacobsen, 1966b).

RESULTS

In five experiments the flow from the cannulated main inguinal lymph vessel in a dog increased from about 1–2 ml./hr to between 6 and 12 ml./hr upon scalding the limb. All other visible inguinal lymph vessels had been tied off, and the limb was exposed to gentle passive movements all the time. In ten similar experiments on rabbits the lymph flow through the main inguinal vessel increased from 0.7–1.1 ml./hr to between 4 and

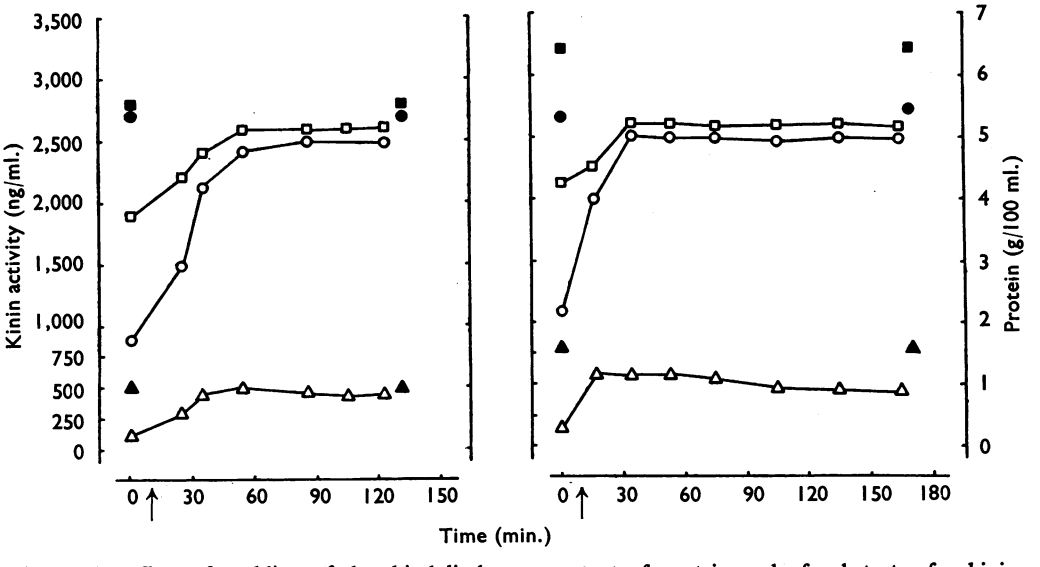

Fig. 1. The effect of scalding of dog hind limbs on content of protein and of substrates for kinin-forming enzymes in the limb lymph. Observations from two separate dog experiments are shown. Scalding carried out at arrows, as described in Methods. Substrate contents are expressed in terms of kinin that could be developed/ml. lymph or plasma (see Methods), synthetic bradykinin used as reference. Triangles give amount of substrate for plasma kallikrein in plasma (▲) and in lymph (△). Squares give amount of substrate for glandular kallikrein (obtained by subtracting the amount formed with plasma kallikrein from total amount formed with saliva) in plasma (■) and in lymph (□). Circles give amount of total protein in plasma (●) and in lymph (○).

6 ml./hr upon scalding. Only a moderate increase in lymph flow was seen in the rabbits as well as in the dogs during the first 15–20 min after scalding. Maximal flow was reached after about 1 hr.

The *p*H of freshly collected limb lymph did not change after scalding. The protein content of the collected lymph increased markedly, however, reaching is highest values between 30 and 60 min after the injury. The maximal level of lymph protein approached the protein level found in plasma at the same time (Fig. 1). Clot formation was never observed in rabbit lymph collected after scalding, whereas clots were sometimes formed in dog lymph samples if no anticoagulant were added. Dog lymph also had a tendency to clot in the collection cannula.

Lymph samples from scalded limbs showed plasma-like distribution of the different proteins on paper electrophoresis. The albumin-globulin ratio decreased after scalding and became the same as in plasma.

Some haemolysis was seen and an increased number of erythrocytes observed in lymph samples from scalded limbs of both dogs and rabbits. The number of erythrocytes usually increased from 3,000–5,000 to between 8,000–12,000/mm^3 lymph. The haemoglobin content of lymph from scalded limbs could amount to 0.1 g/100 ml. and the highest values were reached about 45–90 min after the injury.

Free plasma kinins. Plasma kinin activity could not be detected in freshly collected lymph samples from the scalded rabbit or dog hind limbs, not even when the samples were collected into a disodium edetate solution or directly into boiling ethyl alcohol (Hamberg & Rocha e Silva, 1957).

In one experiment 8 mg calcium disodium edetate/ml. calculated plasma volume were injected intravenously into a rabbit after scalding its hind limb. No plasma kinin activity could be detected in subsequently collected lymph samples.

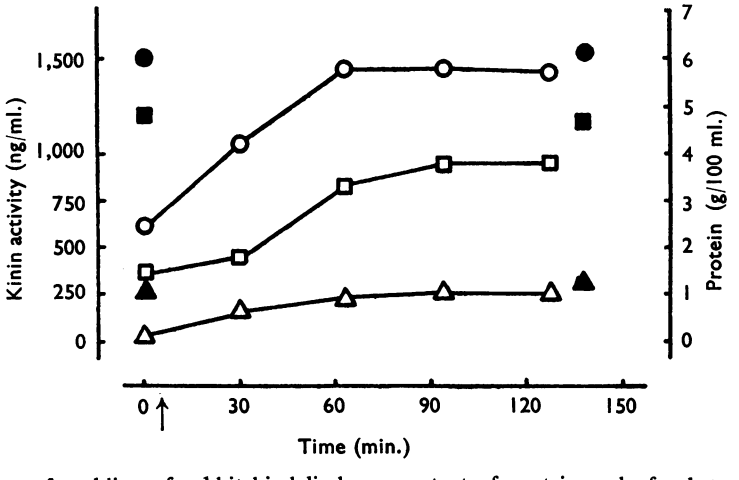

Fig. 2. The effect of scalding of rabbit hind limb on content of protein and of substrates for kinin-forming enzymes in the limb lymph. Explanation and signs as in experiments of Fig. 1.

Plasma kinin-forming activity in lymph. Lymph samples were incubated in siliconized glass tubes with various portions of fresh human or dog plasmas, with human plasma that had been heated to 56° C for 1 hr or with the stable plasma substrate preparation of Amundsen, Nustad and Waaler (1963) (Jacobsen, 1966b). Plasma kinin forming activity was never detected with lymph samples from scalded hind limbs of dogs or rabbits by any of the described incubation procedures, not even when disodium edetate was present as a kininase inhibitor.

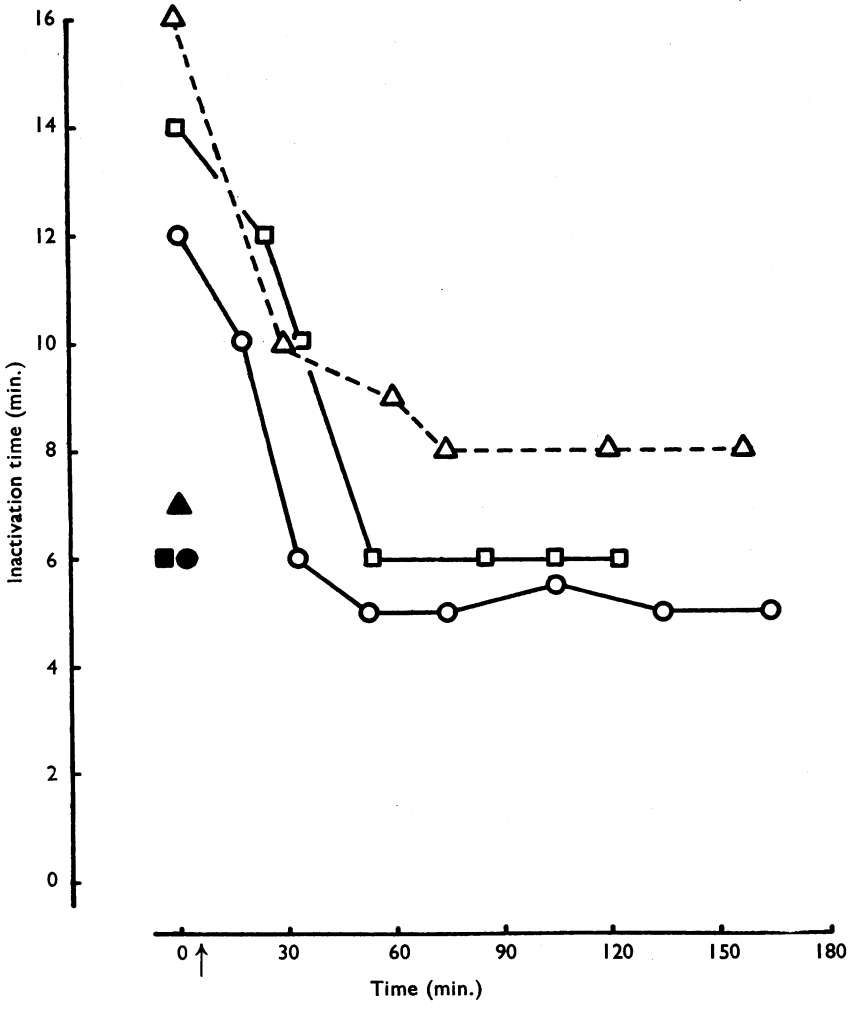

Fig. 3.—The effect of scalding of hind limbs of dogs on kininase activity in limb lymph from the animals. Results from three separate dog experiments are shown. Kininase activity was evaluated as the time needed for inactivation of 250 ng synthetic bradykinin in 0.35 ml. 0.1 M Tris-HCl buffer at *p*H 7.35 and at 37° C when incubated with 0.1 ml. lymph or plasma. Along the ordinate is given inactivation time (min) whereas the abscissa is a time axis. Scalding carried out at arrow. Open triangles, squares and circles represent inactivation times for lymph samples from three different experiments. Closed marks give inactivation times in the corresponding plasmas.

Substrates for plasma kinin forming enzymes. The plasma concentrations in dogs and rabbits of the two types of substrates for kinin forming enzymes did not fall in the 2–3 hr period subsequent to the scalding of their hind limbs. The amounts of substrates in dog hind limb lymph increased markedly after scalding and reached maximal values about 30 to 60 min after the injury. The concentrations in lymph of both types of substrates increased towards, but never exceeded, the plasma concentrations. The values obtained from two experiments with dogs are shown in Fig. 1. Similar results were obtained in another three dog experiments.

In the rabbit similar changes were seen upon scalding the limb. It was difficult to evaluate and to follow the amount of substrate for plasma kallikrein, however, because of a relatively high kininase activity and a marked kallikrein inhibition by the lymph (Jacobsen, 1966b). Values from a scalding experiment in a rabbit are shown in Fig. 2. Similar results were obtained in another two experiments with rabbits. In ten more experiments, where the level of substrate for plasma kallikrein was not followed, the level of substrate for glandular kallikrein and the protein content of the lymph were found to change as shown in Fig. 2.

Kininase activity. Kininase activity of hind limb lymph from dogs as well as rabbits increased upon scalding the limb (Figs. 3 and 4). In plasma and in lymph samples collected before scalding, the kininase activity was well inhibited by disodium edetate in a final concentration of 0.68×10^{-2}M. In lymph samples collected after scalding, kininase activity was less well inhibited by disodium edetate. Addition of phenanthroline to a concentration of 3×10^{-4}M caused a more marked, although not complete inhibition.

Effect of antihistamine. Edery & Lewis (1963) observed that injection of the antihistaminic drug mepyramine maleate prevented the increase in kinin-forming activity,

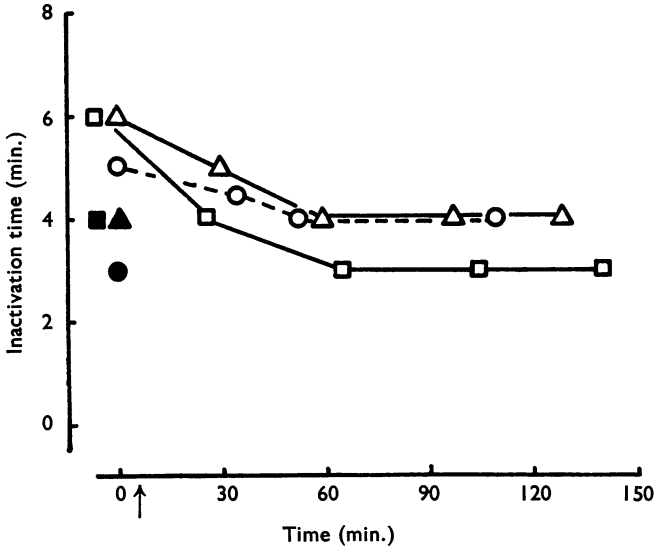

Fig. 4. The effect of scalding of hind limbs of rabbits on kininase activity in limb lymph from the animals. Results from three different rabbit experiments are shown. Explanation of signs as in Fig. 3.

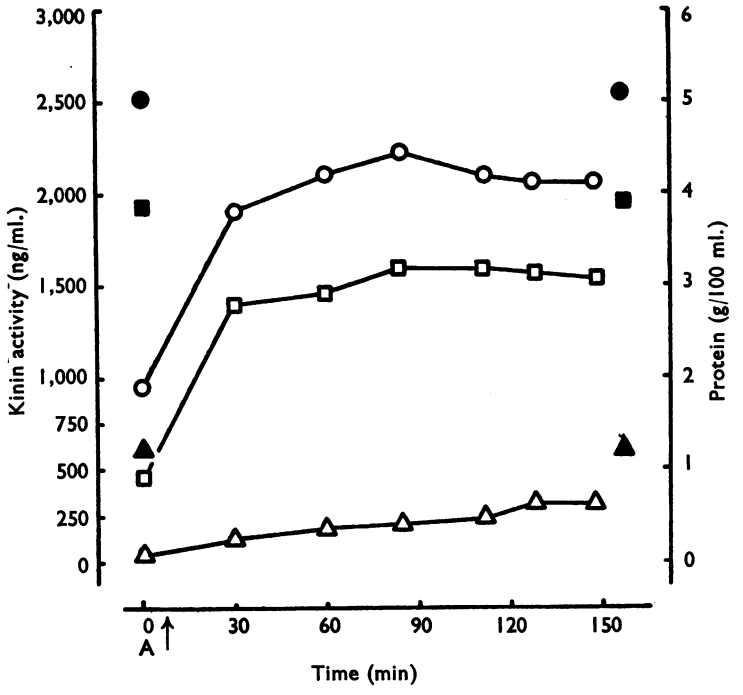

Fig.5. The effect of scalding a dog hind limb after intra-arterial administration of antihistamine on content of protein and of substrates for kinin-forming enzymes in the limb lymph. After collection of some lymph from the non-injured limb mepyramine maleate, 1 mg/kg was injected into the femoral artery (at A), and 5 min later the limb was scalded (at arrow). For explanation and signs see Fig. 1.

but not the increase in flow and in protein content of the lymph after scalding. In two experiments on dogs we injected 1 mg mepyramine maleate/kg body weight into the femoral artery prior to scalding the limb. The increase in lymph flow occurred as in the other experiments, but again no plasma kinin activity or kinin-forming activity could be detected in the lymph samples. The increase in content of the substrate for plasma kallikrein seemed to develop more slowly and somewhat less markedly, however, than in experiments where no antihistaminic drug had been given (Fig. 5).

DISCUSSION

Scalding of hind limbs of dogs and rabbits leads to the well-known increase in lymph flow (Starling, 1894 ; Field, Drinker & White, 1932 ; Edery & Lewis, 1963) and in lymph content of total protein, with a decrease in the lymph albumin-globulin ratio (Perlmann, Glenn & Kaufman, 1943). The concentration of protein in lymph approaches that in plasma.

The content of the two substrates for plasma kinin-forming enzymes also increases towards the corresponding levels in the animal's plasma. The substrate for plasma kallikrein, which has the larger molecular weight (Jacobsen, 1966a), and which is more poorly represented in lymph from a non-injured limb, thereby shows the more marked

increase after scalding. These observations seem simply to reflect the increased escape of plasma protein from blood vessels into interstitial fluid and lymph after tissue injury. The relatively great restriction of the capillary wall to the passage of the larger substrate molecule is markedly reduced after the injury.

No plasma kinin activity could be detected by us in lymph collected from scalded limbs. Even if kinins are formed in the tissue after scalding, their detection in lymph may be difficult to achieve because of the kininase activity of this fluid, which is also increasing after the injury. Any kinins formed may well be destroyed during the relatively slow collection.

Edery & Lewis (1963) observed that kinin formation takes place on incubation of lymph with a pseudoglobulin preparation, and that this formation is markedly increased when lymph from a scalded limb is used. We have confirmed this finding, but we interpret the increased formation after scalding as reflecting the increasing content of substrate in the lymph for the plasma kallikrein which is present in the pseudoglobulin (Jacobsen, 1966b).

Rocha e Silva & Rosenthal (1961) found that a kinin-like substance is formed in interstitial issue during thermal injury of the skin in rats. The plasma kinin formation in plasma from rats seems to differ from that seen in plasma of dogs and rabbits (Fasciolo & Halvorsen, 1964). In rats the kinin-forming enzyme in plasma seems to be more easily activated, and its substrate is apparently more unstable than that from the plasma of several other animals (unpublished results).

Edery & Lewis (1963) were able to prevent the increased kinin formation on incubation of pseudoglobulin with lymph from a scalded limb by intra-arterial injection of an antihistaminic drug before scalding. In similar experiments we observed that the amounts in lymph of substrates for kinin-forming enzymes increase after scalding. For the substrate with the larger molecular weight, however, this increase is apparently slower and less marked than in other experiments. Our observations here do not permit any definite conclusion. It appears as if the antihistamine may retard and diminish the escape of large plasma proteins into the interstitial space after scalding. This may indicate that histamine liberation plays a role in the change in blood vessel permeability upon this type of injury.

The increase in the number of red cells and in the content of haemoglobin in lymph on scalding, described by Glenn, Peterson & Drinker (1942) and De Witt & Stahl (1964), demonstrates increased escape of cells from blood vessels and increased destruction of red blood cells. The kininase activity of lymph also increases after scalding. Plasma kininase does probably enter the interstitial space together with other plasma components. The kininase acivity was, however, sometimes higher in lymph collected after scalding than in plasma taken at the same time. Plasma kininase, but not erythrocyte kininase, is inhibited by disodium edetate, whereas phenanthroline inhibits both types of kininase (Erdøs, Renfrew, Sloane & Wohler, 1963). The findings that kininase activity is less well inhibited by disodium edetate in lymph collected after than in lymph collected before scalding, and that the kininase activity in the former is well inhibited by phenanthroline, indicate that part of the kininase activity in lymph from a scalded limb may originate from destroyed cells such as, for example, haemolysed erythrocytes.

SUMMARY

1. Lymph was collected from hind limbs of dogs and rabbits before and after scalding the limbs. The scalding effected an increase in lymph flow and in lymph content of total protein and of the two substrates for kinin-forming enzymes. This increase in substrate probably reflects the increased escape of plasma proteins from blood vessels into tissue spaces.

2. Kininase activity in lymph also increased after scalding. The kininase activity seemed to originate partly from plasma and partly from destroyed cells.

3. Free plasma kinins and kinin-forming activity could not be detected in the lymph, before or after scalding.

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