THE GENERATION OF KININS IN THE BLOOD OF DOGS DURING HYPOTENSION DUE TO HAEMORRHAGE

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SUMMARY

1. Circulating kinins were detected and continuously assayed during hypotension due to haemorrhage in dogs, using the blood-bathed organ technique and isolated strips of cat jejunum as the assay tissue.

2. In arterial blood kinin concentrations of 1–5 ng/ml were attained after a hypotension of 35–65 mmHg had been maintained for 10–190 min. When portal venous blood was simultaneously assayed kinins appeared earlier and in concentrations 1–2 ng/ml higher than in arterial blood. No differences in time course of kinin generation or in concentration were found when mixed venous blood and arterial blood were compared. In those instances in which the blood pressure was restored to normal by returning the shed blood, kinin formation stopped.

3. Kinin generation was due to the presence in the circulation of a kinin-forming enzyme, such as kallikrein. When kallikrein was infused into the portal vein, it was partially inactivated by the liver.

4. Prolonged intravenous infusions of kallikrein (20–60 μg kg⁻¹ min⁻¹) generated kinins in the circulation in concentrations (1–5 ng/ml) which were well maintained throughout the infusion, demonstrating that kinin generation is not limited by depletion of the precursor kininogen; nevertheless, the effects of kallikrein infusions on the blood pressure and central venous pressure waned.

5. It is concluded that in hypotension due to haemorrhage, an active kallikrein appears in the portal circulation. Delay in the appearance of kallikrein in the systemic circulation may be due to the kallikrein inactivating mechanism of the liver. This inactivating mechanism may fail during shock. Kinins are generated in amounts sufficient to have a substantial effect on the circulation and an influence on the course of events in shock.

Kinins have been implicated in the pathogenesis of experimental shock but methods for their estimation have mainly been indirect, through measurement of kininogen concentrations in

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plasma (Diniz, Carvalho, Ryan & Rocha e Silva, 1961). Decrease in concentration of circulating kininogen has been shown in endotoxin shock (Webster & Clark, 1959; Diniz, Carvalho, Reis & Corrado, 1966) and anaphylactic shock (Diniz & Carvallo, 1963). Webster & Clark (1959) measured the plasma kininogen of dogs killed by irreversible haemorrhagic shock and suggested that small amounts of kinins may have been released, and Diniz & Carvalho (1963) found that bradykininogen fell to 60% of the resting levels during haemorrhage in dogs.

Direct estimation of kinins in the circulation (for example, Brocklehurst & Zeitlin, 1967) has depended on lengthy and difficult extraction procedures to minimize destruction or activation of kinins in the sample. With either the direct or indirect methods of estimating kinins, the intermittent blood sampling procedure makes it difficult to relate any release of kinins to the time course of the haemorrhage. The picture is further complicated by the fact that kinins are inactivated not only in blood but also in the pulmonary circulation and peripheral vascular beds (Ferreira & Vane, 1967a) so that venous blood samples do not necessarily reflect the kinin concentrations in arterial blood.

The blood-bathed organ technique (Vane, 1964) is a direct and continuous assay technique which avoids many of these disadvantages. It was used to assay the concentration of circulating kinins in different parts of the circulation during hypotension due to blood loss.

**METHODS AND MATERIALS**

When superfused with blood, strips of cat jejunum are selectively sensitive to kinins and do not respond to other substances, such as angiotensin and vasopressin, known to be released in shock. The enzyme kallikrein has no direct effect on cat jejunal strips (Ferreira & Vane, 1967b). The jejenum was removed from the animal, opened longitudinally and washed in Krebs' solution (NaCl 118 mM; KCl 4.7 mM; CaCl₂ 2.5 mM; KH₂PO₄ 1.2 mM; MgSO₄ 1.17 mM; glucose 5.6 mM; NaHCO₃ 25.0 mM; gassed with 95% O₂; 5% CO₂) in which it was stored at 4°C. Longitudinal strips (2-4 mm wide and 6-8 cm long) were cut and used as needed during the next 2-7 days. As previously described (Ferreira & Vane, 1967b), the strips become more sensitive to bradykinin with storage. They also lose their spontaneous activity, making it easier to detect small concentrations of bradykinin. Strips were suspended in polypropylene chambers and superfused (Gaddum, 1953) with Krebs' solution, gassed with 95% oxygen and 5% CO₂ and maintained at 37°C. Contractions of the assay tissues were detected by auxotonic levers (Paton, 1957) attached to Harvard transducers and recorded on an 8-channel Beckman Offner dynograph. The initial load on the strips of jejunum was 1-2 g. In many experiments a rat stomach strip (Vane, 1957) and a chick rectum (Mann & West, 1950) were included in the assay system to detect catecholamines (Armitage & Vane, 1964). The assay tissues were arranged in two separate banks with three tissues in series in each bank.

Dogs of either sex weighing 12-30 kg were anaesthetized with halothane delivered from a Goldman vaporizer. A femoral vein was cannulated and chloralose (100 mg/kg) was given intravenously. The anaesthetic was supplemented with pentobarbitone sodium (5-10 mg/kg intramuscularly, subcutaneously or intravenously) as required. In three experiments, pentobarbitone sodium (30 mg/kg intravenously) was used as the only anaesthetic. The trachea was cannulated and the lungs ventilated mechanically throughout the experiment. Polyethylene cannulae were inserted into the femoral or carotid artery for removal of blood. To sample mixed venous blood, a catheter was inserted into the right jugular vein until its tip lay in the
right ventricle as judged by the pulse wave recorded through it; the catheter was then withdrawn until it lay in the right atrium. In those experiments in which portal venous blood was superfused over the tissues the abdomen was opened by a midline incision. A fine catheter was then inserted into the portal vein via the splenic vein, which was ligated. The splenic arteries were also ligated or splenectomy performed.

Fig. 1. Diagram of the blood-bathed organ technique. Blood is continuously withdrawn from the dog by a roller pump and kept at 37°C by water jackets; it then superfuses the assay tissues, arranged in two banks. Movements of the tissues are detected by transducers and recorded on a dynograph. In some experiments blood was diverted through an incubating circuit (as shown) before being assayed by the isolated tissues.

To sample blood draining from the small intestine, a catheter was passed via a small tributary in the ileal mesentery into the superior mesenteric vein. To sample venous blood from a hind leg, a catheter was passed up a saphenous vein until the tip lay in the femoral vein.
Mean arterial blood pressure was recorded from an arterial cannula connected to a pressure transducer. Central venous pressure was recorded from a catheter inserted into the vena cava. The bladder was continuously drained by a urethral catheter.

Dogs were given heparin (1000 i.u./kg) intravenously every 4 h. This completed the preparation of the dog and the assay tissues were then superfused with blood at 37° instead of Krebs’ solution. Each bank of assay tissues was superfused at a rate of 10 ml/min, kept constant and delivered by a roller pump or Harvard peristaltic pump. The blood was then collected in a reservoir from whence it drained by gravity into the femoral vein (Fig. 1). When superfusion was started, dextran solution (30 ml) was infused intravenously to replace the blood contained in the extracorporeal circuit. Additional amounts (2–5 ml/kg) were given when required to compensate for blood lost during surgery. In some experiments each of two banks of assay tissues were superfused with a different stream of blood chosen from portal venous, mixed venous or arterial catheters. An arrangement of cross connections allowed the source of blood bathing each bank of assay tissues to be interchanged.

The transit time of the blood from the dog to the first of the assay tissues was 10–20 s; this could be increased by up to 2 min by the inclusion of an ‘incubating circuit’ (Fig. 1) which consisted of a coil of silicone tubing kept at 37° by a heated water bath (Ferreira & Vane, 1967b).

The assay tissues were calibrated by infusions of bradykinin into the blood as it left the dog (about 15 s before reaching the assay tissues). The infusions were made at this point to compensate for any change in concentration due to destruction of kinins between the cannula and the assay tissues. Infusions of catecholamines, antagonists and other substances, were made at the same site. Infusions of kallikrein were made through catheters into either the portal vein or the inferior vena cava.

The dogs were bled from a femoral or carotid arterial cannula attached by silicone tubing to a polyethylene reservoir. The blood loss was continuously measured by recording the weight of the reservoir with a strain gauge. At the beginning of the haemorrhage, the reservoir was suspended approximately 150 cm above the operating table. It was then intermittently lowered during the early stages of the haemorrhage until it reached a level of 55–90 cm, which provided a stable blood pressure of 35–60 mmHg. The shed blood could return to the dog by autotransfusion or by elevation of the reservoir. The rate of return was sometimes increased by using a 50 ml plastic syringe as a pump.

The following drugs were used: adrenaline bitartrate (British Drug Houses), synthetic bradykinin (Parke-Davis), bradykinin potentiating factor (BPF) prepared as described by Ferreira (1965), a pentapeptide PCA-Lys-Trp-Ala-Pro (SQ 20, 475) isolated from BPF (Greene, Stewart & Ferreira, 1970) kindly supplied and synthesized by Dr M. Ondetti and J. Pluscec of the Squibb Institute for Medical Research, 2-3 dimercapto-l-propanol (BAL, British Drug Houses), heparin (Pularin, Evans), kallikrein (pig pancreatic, Glumorin, Bayer AG), mepyramine maleate (May & Baker), methysergide bimaleate (Sandoz), propranolol hydrochloride (Inderal, ICI). Doses of salts are expressed as base.

RESULTS

Sensitivity of assay system

Strips of cat jejunum respond to kinins by contraction and by an increase in spontaneous activity. They were usually sensitive to bradykinin infusions which gave initial concentrations
of 1–2 ng/ml. Bradykinin has a half-life in dog’s blood of about 15 s (Ferreira & Vane, 1967b, c) which was equivalent to the delay before the infusions reached the assay tissues. Thus, the actual kinin concentrations at the jejunum were one half (0.5–1 ng/ml) of those at the infusion point. This difference is important when estimating the amounts of kinins generated by kallikrein infusions. Pojda & Vane (personal communication) have shown that during kallikrein infusions concentrations of kinins generated in dog’s blood are constant over a wide range (15–120 s) of incubation times.

![Diagram](image)

**Fig. 2.** 17-kg dog. The release of catecholamines during haemorrhage and their influence on the assay of bradykinin.

Isolated tissues superfused with arterial blood at 10 ml/min. Upper trace shows the relaxations of a rat stomach strip, calibrated by infusions of adrenaline made into the bathing blood as it left the dog (IBB) to give concentrations of 10, 5 and 20 ng/ml (inset). Lower traces show contractions of cat jejunum calibrated by IBB infusions of bradykinin to give concentrations of 1, 2 and 3 ng/ml. An intravenous (IV) infusion of adrenaline (10 µg/min) gave a concentration in the circulating blood of approximately 20 ng/ml. In the presence of circulating adrenaline in this concentration the cat jejunum strip failed to respond to an infusion of bradykinin (2 ng/ml IBB). When circulating in a concentration of 5 to 10 ng/ml (5 µg/min IV) adrenaline did not depress the tissue response to bradykinin. Sudden haemorrhage of the dog produced a release of catecholamines at a rate of between 5 µg/min and 10 µg/min (equivalent to a blood concentration of 10 ng/ml). The contraction of the cat jejunum to bradykinin (2 ng/ml) was depressed. This inhibitory effect of catecholamines was blocked by propranolol (100 ng/ml IBB), as, to some extent, was the relaxation of the rat stomach strip. Time, 10 min; vertical scale, 5 cm.

The contractions of the cat jejunum induced by bradykinin were depressed by catecholamines when present in concentrations of more than 10 ng/ml. This is illustrated in Fig. 2 which shows a duplicate assay of bradykinin on two strips of cat jejunum. The presence of circulating catecholamines (equivalent to >10 ng adrenaline/ml) following a rapid haemorrhage is shown by relaxation of the rat stomach strip. This concentration of adrenaline inhibited the response of the cat jejunum strip to bradykinin (2 ng/ml). Propranolol (100 ng/ml) was then infused into the assay system. This partially reduced the relaxation of the rat stomach strip induced by
the circulating catecholamines and prevented their inhibitory effects on the response of the cat jejunum to bradykinin. Propranolol itself did not contract or relax the cat jejunum. Intermittent infusions of propranolol (100 ng/ml for 5 min) into the blood superfusing the assay tissues were therefore used to test whether catecholamines were interfering with the registration of circulating kinins, and also as a means of blocking this effect. When such an

![Graph](image)

**Fig. 3.** 16-kg dog. The effect of an intravenous (IV) infusion of kallikrein (0.5 units/min) for 60 min.

Tracings from top to bottom of a rat stomach strip and two strips of cat jejunum (all superfused with arterial blood at 10 ml/min), mean arterial blood pressure (mean BP) and central venous pressure (CVP). The sensitivity of the assay tissues to adrenaline (ADR 5 ng/ml) and bradykinin (BK 1 ng/ml and 2 ng/ml) is shown on the vertical scales on the right. A short release of catecholamines at the onset of the kallikrein infusion, to give blood concentrations in excess of 5 ng/ml is shown by the relaxation of the rat stomach strip. Note that on each strip of jejunum, the kinin release assayed at about 1.5 ng bradykinin/ml. The constant generation of kinins (1–2 ng/ml) throughout the kallikrein infusion is shown by the contractions of the strips of cat jejunum. In contrast, the precipitous fall in arterial pressure and rise in venous pressure is followed by partial recovery during the infusion. Time, 10 min; vertical scales; ng/ml adrenaline; ng/ml bradykinin, mmHg, and cm H2O.

infusion was made, the total amount of propranolol reaching the dog (4–5 µg) was far less than that necessary (10 µg/kg) to have an effect on the circulation or on the cardiovascular effects of catecholamines (Black, Duncan & Shanks, 1965).

**Kallikrein infusions**

The effects of prolonged intravenous infusions of kallikrein (20–60 µu kg⁻¹ min⁻¹)
Kinins in haemorrhage

were studied in three experiments. These infusions generated concentrations of kinin in the circulating blood equivalent to those produced by bradykinin infusions of 1–5 ng/ml. Fig. 3 shows an intravenous infusion of kallikrein (31 μg kg⁻¹ min⁻¹) lasting for 60 min. There was an immediate fall in blood pressure and rise in central venous pressure; these were associated with a generation of kinins in the circulation as shown by contractions of the strips of cat jejunum. There was also a short burst of catecholamine secretion at the start of the infusion, as shown by relaxation of the rat stomach strip. The concentration of circulating kinins (1–2 ng/ml) remained constant throughout the infusion, whereas the arterial and venous pressures both tended to return towards the previous level. This experiment also illustrates the ability of the cat jejunum to maintain its response during a prolonged exposure to kinins.

The influence of the liver on kallikrein infusions was studied in four experiments. The kinin concentration in arterial blood during intravenous or intraportal infusions of kallikrein (5–28 μg kg⁻¹ min⁻¹) were compared. The ability to detect small differences in kinin concentration depended on the sensitivity of the assay tissues and the steepness of their dose response curves. Changes in concentration of less than 10% would not have been discernible. In two experiments, higher concentrations of kinins were detected during intravenous infusions than during intraportal infusions, but in the other two no difference was detected. Fig. 4 is from the experiment in which the greatest decrease in kinin concentration (approximately 50%) was observed when the kallikrein was infused into the portal vein rather than into the inferior vena cava.

Haemorrhage

In all experiments (thirty) in which arterial blood was assayed, kinins were demonstrated in the circulation during hypotension due to haemorrhage. The concentration usually attained was 1–5 ng/ml. Kinins were only detected in the systemic circulation when a blood pressure of 35–60 mmHg was maintained for a period which varied from 10 to 190 min.
That the generation of kinins is a reversible process was demonstrated by those experiments (eight trials in six dogs) in which the blood pressure was restored by transfusion of the shed blood after a relatively short period of hypotension. Fig. 5 illustrates such an experiment in which kinin generation fell to undetectable levels after transfusion.

Three dogs were anaesthetized with pentobarbitone instead of chloralose. When these animals were bled kinins appeared in the arterial blood 29, 36 and 78 min later. These were all within the range found with chloralose anaesthesia, as were the maximum concentrations of kinins achieved.

![Graph](image)

**FIG. 5.** Fall in circulating kinin after transfusion of shed blood following a short period of hypotension, in a 15-kg dog.

From top to bottom, are tracings of a cat jejunum preparation, bathed in venous blood, mean arterial blood pressure and the weight of the shed blood. The cat jejunum gave graded contractions to direct infusions of bradykinin (1–4 ng/ml). The reservoir was then opened to start the haemorrhage. Within 25 min, at a time when the reservoir was still filling, kinins appeared in the blood. The kinin concentration reached an equivalent of bradykinin of just under 4 ng/ml; the reservoir was then raised and the blood returned to the dog. Kinin generation decreased. Time 20 min; vertical scales 5 cm, mmHg and g.

When hypotension was prolonged and the blood pressure was not restored by returning the shed blood, maximum blood loss occurred in 70 min (range 50–90 min). In that time 3.3% (range 1.7 to 3.9%) of the body weight was shed. The volume of blood in the reservoir remained constant for 60–120 min and the mean arterial pressure steady between 35 to 60 mmHg. Spontaneous return of blood then occurred at a rate which varied from dog to dog. In one in which the blood loss was small, all the shed blood returned. In the remainder (eight dogs) an average of 22% of the shed blood was taken up within a period of 60 min. Furthermore, the
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kinin generation continued despite the complete return of the blood, either spontaneously or by elevation of the reservoir. In eight experiments the increased kinin concentration was observed to continue for up to 1 h after complete return of the blood, at which time the animal was killed.

When comparison was made between kinin concentrations in arterial and mixed venous blood (four trials in three dogs), kinins appeared during haemorrhage simultaneously in both streams (three trials in two dogs). In the third dog, unequal sensitivity of the assay tissues precluded comparison of the appearance time. In all these experiments the kinins in both arterial and mixed venous blood reached similar concentrations. In three dogs, kinin concentrations during haemorrhage were compared in femoral arterial and femoral venous blood. The peak concentrations of kinins obtained were similar in each stream, but in two dogs the kinins appeared in the arterial blood a few minutes before they appeared in the venous blood from the leg. In the other, the appearance was simultaneous.

In fifteen experiments, portal venous blood and arterial blood were simultaneously assayed for kinins. In twelve of these the sensitivities of the assay tissues allowed direct comparison of the time at which kinins appeared in each stream. Kinins appeared in portal blood before arterial blood by up to 160 min. Furthermore, the concentration of kinins in portal blood was greater than in arterial blood, usually by 1 to 2 ng/ml. Fig. 6 shows an experiment in which kinins were detected in the portal blood approximately 30 min after the start of the haemorrhage and 110 min before they appeared in arterial blood. The kinin concentration in portal blood gradually increased, but there was no sudden change concomitant with the appearance of kinins in the arterial circulation. This figure also illustrates one of the nine experiments in which spontaneous return of blood occurred. When two banks of assay tissues were bathed simultaneously with blood from different sources, interchange at intervals during the course of a prolonged hypotensive haemorrhage confirmed the different concentrations of kinins in the different streams.

In one experiment blood was withdrawn from the superior mesenteric vein. The tip of the catheter was positioned in the vein at a point proximal to its termination, to ensure that blood draining from the pancreas was not sampled. Kinins were detected in this blood draining the small intestine in concentrations similar to, and possibly higher than in the portal venous blood with which it was compared.

The possibility that the kinins detected in the dog’s circulation were generated in the reservoir was excluded. In one experiment superfusion of the assay tissues with blood withdrawn directly from the dog was alternated with blood taken from the reservoir, at a time when kinins were circulating in high concentration. Substitution of reservoir blood resulted in an immediate and complete relaxation of the cat jejunum strips, showing no kinins to be present in the shed blood.

The generation of kinins was also not due to blood from the reservoir returning to the circulation. This is shown clearly in Fig. 5 in which kinin formation starts whilst the animal is still bleeding. Furthermore, in three experiments, blood was removed from the dogs and not allowed to return. Kinin generation in these experiments was similar to that found where autotransfusion was possible.

The inclusion of an incubation circuit which delayed the transit of blood from the dog to the assay tissues by 2 min, had no effect on the concentration of kinins in the blood bathing the tissues (five experiments).
Fig. 6. 17-kg dog. The simultaneous assay of kinins in arterial blood and portal venous blood during hypotension due to haemorrhage.

Upper tracings are of strips of cat jejunum (CJ) superfused with arterial and portal venous blood, calibrated by infusions of bradykinin (1, 2 and 5 ng/ml) into the blood bathing the tissues (IBB). The lower tracings record the weight of the blood lost and mean arterial pressure. The generation of kinins in portal blood began about 30 min after the start of the rapid haemorrhage as shown by the contraction and increase in spontaneous activity of the cat jejunum strip. Kinins were not detected in arterial blood until 110 min later. The unchanged sensitivity of the strip bathed in arterial blood was confirmed by infusions of bradykinin (2 ng/ml IBB). In this experiment spontaneous return of blood to the dog commenced 110 min after the start of the haemorrhage and all the blood returned during the following 70 min. Time 10 min; vertical scales 5 cm; 500 g and mmHg.
Kinins in haemorrhage

Substances which are known to influence the kallikrein-kinin system were infused into the blood bathing the assay tissues. Fig. 7 illustrates one such experiment in which the kininase inhibitor SQ 20, 475 was infused. This is a synthetic pentapeptide with the same structure (Greene et al., 1970) as one of the active components of 'bradykinin potentiating factor' (Ferreira, 1965).

Before blood loss from the dog infusions of this pentapeptide (100 ng/ml) into the blood superfusing the assay tissues had no effect on the strips of cat jejunum. However, the contractions of the jejunum induced by bradykinin infusions were potentiated, as were the contractions of the jejunum which appeared after blood loss.

Similar results were obtained in three other experiments in which the natural BPF extract was used. Potentiations of the contractions of the cat jejunum were also obtained in three experiments with dimercaptopropanol (50 µg/ml) another kininase inhibitor (Ferreira & Rocha e Silva, 1962).

Fig. 7. 15 kg dog. The generation of kinins in arterial blood during hypotension due to blood loss and the potentiation of the contractions of the assay tissues by a bradykinin potentiating factor (SQ 20, 475).

The tracings show the movements of a cat jejunum superfused with arterial blood and the mean arterial blood pressure. The first section shows contractions of the cat jejunum induced by bradykinin (2 and 4 ng/ml). SQ 20, 475 (100 ng/ml) by itself had no action on the cat jejunum but when given at the same time as bradykinin (2 µg/ml) it increased the contraction of the cat jejunum. A haemorrhage was then started and kinins gradually appeared in the arterial blood. Infusions of SQ 20, 475 (100 ng/ml) increased the contractions of the cat jejunum. Time 20 min; vertical scales 5 cm and mmHg.

When kinins were present in the circulation, trasylol (20–200 µl/ml) was incubated with the bathing blood for 1 min before assay, but this did not reduce the contractions of the cat jejunum. Soya bean trypsin inhibitor (250 µg/ml) was also without effect. Methysergide (200 ng/ml) and mepyramine (100 ng/ml) did not antagonize the responses of the assay tissues during haemorrhage, confirming that neither 5-hydroxytryptamine nor histamine, both of which in high concentrations contract cat jejunum (Ferreira & Vane, 1967b) could have been present in the blood.
Fig. 8. Correlation between kinin output and spontaneous return of shed blood. In nine dogs the blood loss into the reservoir was allowed to progress past the time when the shed blood started to return passively to the dog. The points on the graph show the time to the first spontaneous return of blood plotted against the time when kinins first appeared in arterial blood. The correlation coefficient was 0.808 and the slope of the calculated regression line was 0.42, crossing the y axis at 49 min. The correlation was highly significant ($P < 0.01$).

In the nine experiments in which spontaneous return of blood from the reservoir to the dogs occurred, a significant correlation ($P < 0.01$) existed between the time when kinins were first detected in arterial blood and the time when spontaneous return commenced (Fig. 8). The correlation coefficient was 0.808 and the slope of the calculated regression line was 0.42, crossing the $y$ axis at 49 min.

Two further dogs were bled into the reservoir set at a height to give a mean arterial pressure of 100–110 mmHg. In one of these dogs the blood loss stopped after 33 min, when 210 ml of blood had been shed. Ten minutes later, an infusion of kallikrein (0.5 u/min intravenously for 25 min) was given; this led to a generation of kinins in the arterial blood equivalent to bradykinin (5 ng/ml). At the same time, the blood started to return to the dog from the reservoir and by the end of the infusion, 130 ml blood had returned to the dog. After the infusion was stopped, haemorrhage into the reservoir occurred again. A second trial gave similar results, as did a third trial (in the second dog) except that in these all the blood returned to the dog during the kallikrein infusions.

Changes in the intestine

The animals’ intestine was inspected at the conclusion of the experiments. The macroscopic appearance was normal when hypotension had been maintained for less than 90 min. With more prolonged hypotension, however, there was congestion of the mucosa which sometimes had progressed to necrosis and haemorrhage.
DISCUSSION

This study has demonstrated that during hypotension due to haemorrhage, kinins appear in the blood in concentrations sufficient to lower a normal blood pressure. The estimation of kinins during haemorrhage has previously been attempted by the measurement of their precursor, plasma kininogen. However, changes of kininogen concentration of less than 3% cannot be reliably estimated and such small changes are probably all that are required for the detection of kinins by the blood-bathed organ technique. In addition, the observation of Urbanitz & Habermann (1970) that the fall in kininogen levels in shock is accompanied by a fall in other plasma proteins, makes the ‘kininogen’ method for kinin estimation difficult to interpret.

Our evidence that kinins were generated is direct; the method depends upon the specificity and sensitivity of the cat jejunum preparation to kinins. Ferreira & Vane (1967b) showed that the cat jejunum is relatively insensitive to other hormones known to circulate during haemorrhage although the effects of bradykinin are masked by catecholamines. This interference with the assay of kinins can be effectively blocked by exposing the assay tissue to the β-receptor blocking drug, propranolol.

In many of our experiments, two or three strips of cat jejunum were used for assay of kinins in the circulation. This provided simultaneous duplicate or triplicate results on tissues with differing sensitivities to bradykinin. Thus, the specificity of the assay was increased, making it more likely that the substance released was bradykinin-like. Additional evidence for the presence of a bradykinin-like substance during haemorrhage was obtained by the use of kininase inhibitors. Both dimercaptopropanol (Ferreira & Rocha e Silva, 1962) and bradykinin-potentiating-factor (Ferreira, 1965; Ferreira & Rocha e Silva, 1965), augmented the tissue contractions not only to infused bradykinin but also during haemorrhage. This potentiation by BAL and BPF was just as effective in the later as in the early stages of kinin output during a prolonged hypotension. This observation does not support the suggestion that in shock the production of kinins may be due to depletion of circulating kininase (Thal & Sardesai, 1965).

The use of an incubating circuit allowed us to distinguish between release of a kinin alone into the blood and release of a kinin-forming enzyme. Bradykinin has a mean half-life in dog’s blood of 17 s (McCarthy, Potter & Nicolaides, 1965; Ferreira & Vane, 1967a); the inclusion of a 2-min incubation circuit would, therefore, have reduced the concentration of a circulating bradykinin-like substance to undetectable levels. In fact, inclusion of the incubating circuit did not alter the concentration of kinin reaching the assay tissue. This result strongly suggests that a kinin forming enzyme was present, and that equilibrium between kinin formation and kinin destruction had already been reached in the blood superfusing the assay tissues, whether it had been outside the dog for 15 or 120 s. Pig pancreatic kallikrein is relatively stable in the circulation of dogs (half-life 2-4 min, Ferreira & Vane, 1967c) and when incubated with blood before assay for times varying from 15 s to 3 min it produces the same kinin concentration (Pojda & Vane, personal communication).

It can be concluded therefore that kinins are generated in the circulation by an active kallikrein and that the generation continues in the extracorporeal circuit between the dog and the assay tissue. The levels of kinin assayed reflect the concentration of circulating kallikrein. This interpretation is supported by the fact that there was a similar concentration of kinins in mixed venous blood and arterial blood. If kinin alone were present in the blood more than
90% would have disappeared in the transit through the pulmonary circulation (Ferreira & Vane, 1967a). Kallikrein, however, is not inactivated by the lungs.

Trasylol is known to be ineffective against pancreatic and urinary kallikrein of dogs (Trautschold, Werle & Zickgraf-Rüdel, 1967). In our experiments, it was also ineffective as an antagonist of the kallikrein-like enzyme released by haemorrhage.

When pig pancreatic kallikrein is infused intravenously there is an initial fall in mean blood pressure, followed by partial recovery while the infusion is still in progress (Webster & Clark, 1959). To explain this phenomenon it has been suggested that the levels of kinin precursor (kininogen) in the blood decreases progressively during the infusion, and that the recovery is due to waning levels of kininogen (Werle, 1964). Our experiments show that the concentration of circulating kinins is maintained for the duration of the kallikrein infusion. Thus, waning of the cardiovascular effect of kallikrein cannot be explained on the basis of depleted levels of kinin precursors.

The finding that kinins were detected earlier and in higher concentrations in portal blood than in systemic blood suggests that the site of activation or release of kallikrein is the gastrointestinal tract. Rothe & Selkurt (1961) demonstrated a vasodilating substance in portal venous blood during haemorrhagic shock but were unable to identify it. Polypeptide-like substances (Kobold & Thal, 1963) and adrenaline-inhibiting substances (Baez, Hershey & Rovenstine, 1961) have also been demonstrated in portal blood after release of a temporary occlusion of the superior mesenteric artery. However, more precise information as to the exact nature of the vasodilating substances has been lacking. The present experiments suggest that these agents are probably kinins, generated by kallikrein which first appears in the portal circulation.

The finding of kinins were detected earlier and in higher concentrations in portal blood than in systemic blood suggests that the site of activation or release of kallikrein is the gastrointestinal tract. Rothe & Selkurt (1961) demonstrated a vasodilating substance in portal venous blood during haemorrhagic shock but were unable to identify it. Polypeptide-like substances (Kobold & Thal, 1963) and adrenaline-inhibiting substances (Baez, Hershey & Rovenstine, 1961) have also been demonstrated in portal blood after release of a temporary occlusion of the superior mesenteric artery. However, more precise information as to the exact nature of the vasodilating substances has been lacking. The present experiments suggest that these agents are probably kinins, generated by kallikrein which first appears in the portal circulation.

The mechanism by which kallikrein is activated or released remains obscure. Several factors may be involved. The reduced tissue perfusion in shock results in cellular anoxia. Catheptic enzymes released from cytoplasmic lysosomes (de Duve, 1959) under such conditions have been shown to generate kinins in plasma (Greenbaum & Yamafuji, 1965). In turn, the acidosis which occurs as a result of disturbed cellular metabolism may also contribute, for acidification of plasma will also release kinins (Werle, Kehle & Koebke, 1950). The fact that acidosis is most marked in the splanchnic circulation may account for the early detection of kinins in portal blood.

The formation of plasmin from plasminogen also occurs during severe haemorrhage (Back, 1966). Plasmin, like kallikrein, can generate kinins from their inactive precursors in blood (Lewis, 1958). This mechanism, therefore, may also contribute to the kinins detected after haemorrhage. Finally, kallikrein present in argentaffin cells in the mucosa of the small intestine may be released into the blood. In many of our experiments secretion of adrenaline was observed in the early stages of hypotension (see Fig. 2) and this always preceded kinin generation. Adrenaline has been shown to release kallikrein from carcinoid tumours, which arise from argentaffin cells (Oates, Melmon, Sjoerdsma, Gillespie & Mason, 1964); it is possible, therefore, that kinins originate from a similar mechanism after haemorrhage. The detection of higher concentrations of kinins in the portal blood than in the arterial blood certainly points to intestine or pancreas being a major source of the circulating kallikrein.

In those experiments in which hypotension was prolonged, haemorrhagic necroses of the intestinal mucosa were observed, typical of 'irreversible shock' in dogs (Lillehei, Longerbeam, Bloch & Manax, 1964). However, kinins were often detected in the circulation early in the hypotensive period, at a time before any macroscopical evidence of ischaemic damage to the intestine was seen. Furthermore, the generation of kinins was discontinued in those experi-
ments in which the blood pressure of the dog was restored by retransfusion of the shed blood. This reversible output of kinins suggests that physiological stimuli rather than cell death are the most likely cause of kallikrein release.

The time lag between the detection of kinins in portal venous blood and their appearance in the systemic circulation can in part be accounted for by inactivation of kallikrein in the liver. There is a disruption of normal architecture of the liver leading to various cellular changes in haemorrhagic shock (Shoemaker & Fitch, 1962). A gradual deterioration after haemorrhage of the kallikrein inactivating mechanism in the liver could explain the delayed appearance of kinins in the arterial blood. In addition, dilution by systemic venous blood of the kallikrein which survives passage through the liver would result in delay in the detection of kinins, until the concentration rose to within the range of detection by the assay tissues.

It is probable that the spontaneous uptake of blood which occurs when dogs are subjected to a prolonged hypotension due to haemorrhage is associated with the appearance of kinins in the circulation. This was shown in two ways. First, by the striking correlation between the time for spontaneous return of blood to start and the time for kinins to appear in the arterial blood (Fig. 8). The fact that the regression line cuts the y axis at 49 min is presumably a reflection of the time it takes for maximum blood loss to occur. Secondly, infusions of pig pancreatic kallikrein which gave similar concentrations of kinins in the circulation to those found after haemorrhage caused a return of blood to the dog. Thus, in these experiments, and perhaps in those of others, the spontaneous return of blood to the dog was most likely due to the appearance of kinins in the circulation.

The exact role of kinins in the course of events observed in shock remains difficult to interpret, partly because the effects of prolonged infusions of pig pancreatic kallikrein on arterial and venous pressure tended to wane. However, this does not necessarily imply that all the cardiovascular effects of kinins decrease during a prolonged exposure. Circulating kallikrein leads to a dilatation of capillaries and the smaller arterioles (Keatinge, 1966), especially in the mesenteric circulation of the dog. This action, which would have the effect of improving the inadequate perfusion of the peripheral vascular bed, may in part be nullified by the ability of kinins to stimulate the release of catecholamines from the suprarenal medulla (Feldberg & Lewis, 1964; Staszewska-Barczak & Vane, 1967). By further increasing the levels of circulating adrenaline arteriolar constriction is increased and tissue perfusion reduced. The main influence of kinins in shock may lie in their ability to increase vascular permeability (Lewis, 1962) and by this action they would contribute to the fluid shifts which occur. Whatever the eventual contribution of kinins is to the aetiology of haemorrhagic hypotension, the fact that they are amongst the most potent vaso-active substances known suggests that they play a major role.

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