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The release and fate of vaso-active hormones in the circulation

J. R. VANE

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England

> "The Pharmacologist has been a 'jack of all trades,' borrowing from physiology, biochemistry, pathology, microbiology and statistics—but he has developed one technique of his own and that is the technique of bioassay."
>
> Gaddum, 1964

Some of Gaddum's first publications were on the development of specific and sensitive methods for biological assay, and he maintained a deep interest in this subject for the rest of his life (see Feldberg, 1967). One of the methods which he developed was that of superfusion, first described by Finkleman (1930). In this technique the bathing fluid runs over the external surface of the tissue, and Gaddum (1953) called it "superfusion" by analogy with "perfusion," in which the fluid runs through the tissue.

The blood-bathed organ technique

The principle of superfusion is used in the blood-bathed organ technique (Vane, 1958, 1964). This is a dynamic bioassay in which isolated assay organs are superfused with a stream of blood rather than with an artificial salt solution. Heparinized blood is removed continuously from an anaesthetized animal, assayed for its hormone content by superfusion over a series of isolated smooth muscle preparations and then returned intravenously (Fig. 1). The choice of isolated organs depends on the hormones to be assayed and also on the circumstances of the assay. Since there are no steps of purification or extraction the assay must gain specificity in some other way. It is usually possible to find a piece of smooth muscle which is particularly sensitive to the hormone under investigation and relatively insensitive to other substances. For instance, the rat colon (Regoli & Vane, 1964) is very sensitive to the contractor action of angiotensin but is relatively insensitive to other substances likely to be found in the circulation, such as 5-hydroxytryptamine and bradykinin.

The cat jejunum (Ferreira & Vane, 1967a) is specifically sensitive to bradykinin and the chick rectum (Mann & West, 1950) to the relaxing effects of adrenaline. The simultaneous use of the rat stomach strip (Vane, 1957) and the chick rectum allows estimation of the proportion of adrenaline and noradrenaline in a mixture of these drugs (Armitage & Vane, 1964). The rat stomach strip relaxes to both catecholamines whereas the chick rectum relaxes to adrenaline but is almost insensitive to noradrenaline. This increase in specificity takes advantage of the principle of parallel pharmacological assay. Gaddum often used the differing sensitivities of different pieces of smooth muscle to the same substance to help identify that substance (Chang & Gaddum, 1933 ; Feldberg & Gaddum, 1934 ; Gaddum & Kwiatkowski, 1939) and he regarded parallel pharmacological assay as strong evidence for the identity of a compound (Gaddum, 1959).

The specificity of the bioassay can be still further increased by the use of antagonists. Circulating 5-hydroxytryptamine can be distinguished from other substances which contract the rat stomach strip, such as prostaglandins, by the use of a specific antagonist for 5-hydroxytryptamine, such as methysergide. This will abolish the contraction induced by 5-hydroxytryptamine but not that due to prostaglandins. Antagonists can also be used to abolish unwanted responses. For instance, the rat colon is relaxed by catecholamines but is contracted by angiotensin ; if both are

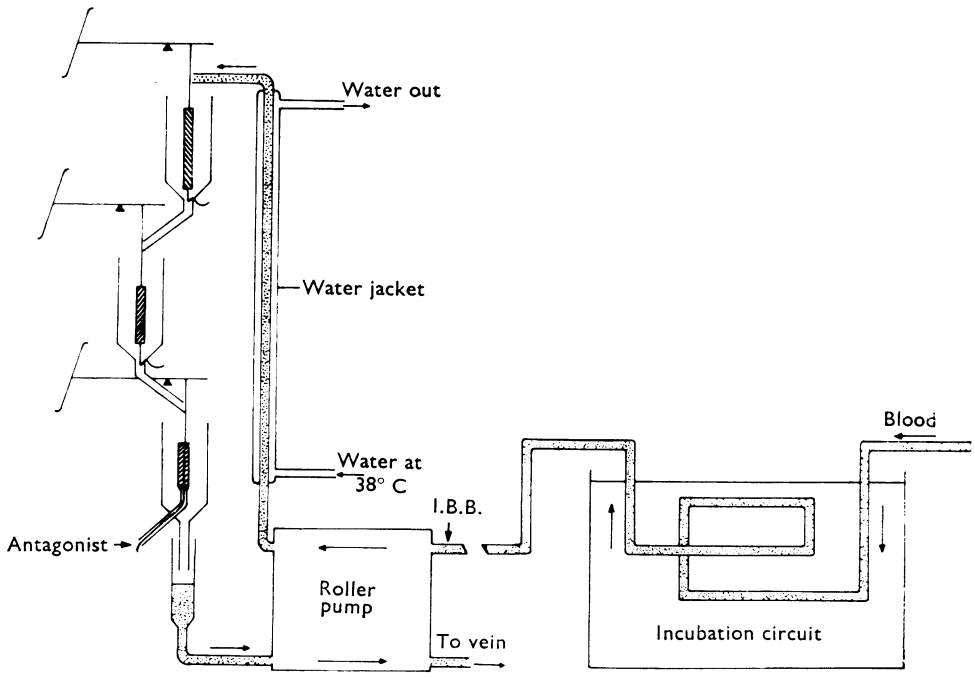

FIG. 1. Diagram of the blood-bathed organ technique. Blood is continuously withdrawn from a convenient vessel by a roller pump, kept at 37° C by a water jacket and then allowed to superfuse a series of isolated organs, the movements of which are recorded by levers on a kymograph (as shown) or by transducers and a dynograph. The blood is then collected in a reservoir and returned to the animal. When necessary, one or more of the isolated organs can be treated by intraluminal infusions of antagonists. In some experiments (see later) the blood flows through a length of silicone tubing in a water bath (incubating circuit) before superfusing the isolated tissues. Drugs can be applied directly to the isolated tissues by infusions or injections into the bathing blood (IBB).

present in the superfusing blood the catecholamines reduce the contraction produced by angiotensin. This unwanted interference can be prevented by antagonizing the actions of catecholamines with a β-receptor antagonist perfused through the closed lumen of the rat colon (Hodge, Lowe & Vane, 1966a); in this way the blocking agent is localized to the assay organ and the whole animal is not affected. For tissues with a high spontaneous activity, like the rat colon and rabbit rectum, pronethalol is better than propranolol, for it also damps down the spontaneous movement (Regoli & Vane, 1964).

Up to three tissues can be conveniently superfused in series when using a double kymograph. With an 8 channel polygraph two banks of assay organs can be used, with three tissues in each bank; hormone concentrations in the venous and arterial blood of the same animal can then be compared. As the blood cascades down from one tissue to the next there is a temperature drop of approximately 1° C. This fall in temperature does not interfere with the assay as long as it is kept constant by maintaining a constant rate of blood flow. The jackets which surround the isolated assay organs are made from polypropylene centrifuge tubes and protect the tissues from changes in temperature due to draughts.

The reactions of some blood-bathed organs to various endogenous substances are shown in Fig. 2. Combinations of two or more of these assay tissues allow the quantitative determination in the circulation of adrenaline, noradrenaline, angiotensin, bradykinin, prostaglandins, 5-hydroxytryptamine and antidiuretic hormone. So far we have been unable to find a specific assay organ for histamine. Although in some early experiments (Vane, 1964) the guinea-pig ileum superfused with blood showed a good sensitivity to histamine, in more recent ones the guinea-pig ileum does not react well to histamine when superfused with blood: no explanation for this has been found.

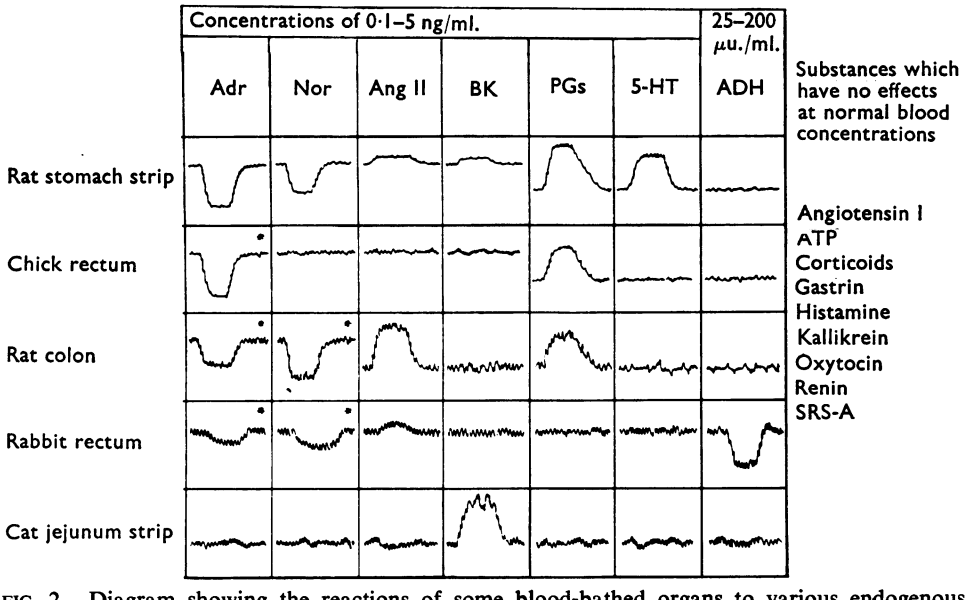

FIG. 2. Diagram showing the reactions of some blood-bathed organs to various endogenous substances in concentrations likely to be found in circulating blood. Adr, Adrenaline; Nor, noradrenaline; Ang II, angiotensin II; BK, bradykinin; PGs, prostaglandins; 5-HT, 5-hydroxytryptamine; ADH, vasopressin. * Response may be abolished with propranolol or pronethalol.

Since the assay tissues are superfused in series with the same stream of blood, one tissue might liberate a substance which affects the next assay organ in line. We have tried to detect such effects in several ways. Changing the order of the assay organs in the cascade does not affect their reactions to infusions of different substances. Similarly, making an infusion of a hormone directly to the last assay tissue in the series, rather than to all three, does not change the effect produced on that organ. Thus the first two do not add or substract sufficient substances to and from the blood to affect the reactions of the third.

Calibration and uses

(a) Fate of exogenous substances

The method can be used to study the fate of a substance infused into the circulation and as long as the substance infused does not release endogenous hormones which affect the blood-bathed organs, there is no problem of specificity. Thus the rat stomach strip can be used to assay changes in blood concentrations following injections or infusions of acetylcholine, 5-hydroxytryptamine or prostaglandins (which contract it) or the catecholamines (which relax it). For studying the fate of substances in the circulation, we have preferred to use infusions rather than injections. The infusions are made for as long as is necessary (usually 3–6 min) to set up steady-state conditions, as shown by the maintained contraction or relaxation of the assay organs. Infusions into the bathing blood (IBB) after it has left the animal calibrate effects in terms of concentration (15 ng/min infused into a flow of 15 ml./min gives a concentration change of 1 ng/ml.). Such infusions can also be used to measure the rate of inactivation of a substance in the circulating blood outside the body. To increase the contact time with the drug infused the blood is passed through a length of silicone tubing kept at 37° C (Fig. 1). Infusions can be made into this additional incubating circuit so that the drug is in contact with the circulating blood for any desired period of time (up to 4 min) before being assayed by the tissues. If contact with blood reduces the activity of a substance, the contact time required to reduce activity by 50% can be measured ; this will be referred to as the half-life.

Infusions of hormones can also be made into different parts of the circulation of the animal. By comparing the responses of the assay tissues after an intravenous infusion with those after an infusion into the arterial supply of a particular vascular bed the disappearance of the hormone in one circulation through that vascular bed can be determined. For example, infusion catheters can be placed in the right atrium and in the ascending aorta: when the assay tissues are bathed in arterial blood, matching their reactions to infusions into the right atrium and into the aorta will determine the rates of infusions on either side of the pulmonary circulation required to give the same arterial blood concentration. In this way, the amounts of a drug which disappears in a single passage through the pulmonary circulation can be calculated. Thus, when an infusion into the aorta of 1 μg/min gives the same response as an infusion into the right atrium of 10 μg/min, 90% of the drug must have been removed in the 3–6 sec (Spector, 1956) that it takes for the blood to pass through the pulmonary circulation of the cat or the dog.

Infusions can also be made into a peripheral vascular bed, such as the kidney or the hindquarters and the amount of the drug which passes through can be

measured by comparing the reactions of the blood-bathed organs with those produced by intravenous infusions.

The fate of a substance in one circulation through the total peripheral vascular beds can also be traced by the use of a coaxial polyethylene catheter (Ferreira & Vane, 1967b). This is introduced retrogradely through the carotid artery, so that the tip of the inner catheter lies in the ascending aorta just above the aortic valves. Blood for supplying the arterial assay circuit is withdrawn through this catheter; the drug under study is infused into the outer catheter which is about 2 cm shorter and therefore opens downstream with respect to the inner one. In this way the infusion mixes with the blood in the ascending aorta just *after* it has been sampled, so that the drug passes once round the body before being assayed by the blood-bathed organs.

(b) Release of endogenous substances

The release of endogenous substances can also be detected, but the specificity of the combination of the blood-bathed organ has to be carefully delineated. When the release is short it can be calibrated by giving an intravenous injection of the same substance to the animal. When the release is prolonged, calibrating infusions can be made. Sometimes, as with bradykinin, the blood concentration of the hormone may be a resultant of both generation and destruction within the circulation. Matching the responses of the assay organs to the endogenous hormone with their reactions to intravenous infusions still gives a measure of the generation rate if the endogenous and exogenous hormones are inactivated at similar rates.

Limitations of the method

(a) In measuring fate of substances

The assay technique measures changes in concentration of a substance in a continuous sample of the circulating blood, taken at 10–15 ml./min. This represents a proportion of the cardiac output and indeed, when a stable substance is infused such as adrenaline or angiotensin II, the sensitivity of the assay organs to intravenous infusions as compared with infusions directly into the bathing blood will indicate what proportion of the cardiac output is being sampled. From this, an estimate of the cardiac output can be made. However, when the technique is used to measure the fate of substances in the circulation, errors will be introduced if the cardiac output changes, for this will change the dilution of the infused substance. Errors due to variations in flow in a particular vascular bed can be eliminated by sampling, not the venous blood from that bed, but the mixed central venous or arterial blood.

(b) In measuring release of substances

The assay tissues are usually superfused with Krebs solution while the animal is being prepared. When they are first superfused with blood there is often a change in resting tone. For instance, in cat blood, the rat stomach strip contracts and maintains a much higher resting tone; in dog blood the contraction is not so great. This contraction, which is much stronger in the rat stomach strip than in other assay tissues, may be a reaction to an unknown substance in the blood or to a change in oxygen tension, or to a change in ionic composition. After starting superfusion

of the assay organs with blood, they usually take 30–60 min to reach a stable base line. Because of the disturbances in tone of the assay tissues when the superfusion fluid is changed from Krebs solution to blood, the actual concentration of a substance in the blood cannot be determined. Thus calibration of the blood-bathed organs must be in terms of *changes* in blood concentration.

If more than one substance is liberated into the circulating blood at the same time the reactions of the assay organs become more difficult to interpret. For instance, although noradrenaline and adrenaline can be assayed in the presence of each other, or simultaneously with angiotensin II, it is much more difficult to detect angiotensin II in the presence of prostaglandins (see Fig. 2). As will be seen later, the use of both venous and arterial blood partially overcomes this problem, for prostaglandins disappear in the pulmonary circulation whereas angiotensin II does not.

The dynamic nature of the blood-bathed organ technique makes it well suited to study rapid or transitory changes in hormone concentrations in the circulation, but slow changes, taking longer than 20–40 min to develop, cannot easily be distinguished from drifts in the resting tones of the assay tissues unless the changes are experimentally reversible.

Measurements of release and fate of vaso-active hormones

The release into the circulation, distribution and fate of several vaso-active hormones will now be considered. No attempt will be made to review comprehensively the physiological and pathological stimuli for the release of each substance. However, recent work on control mechanisms will be quoted, especially when the hormone is likely to escape into the circulation. The concepts of " tissue " hormones (Feldberg & Schilf, 1930) or " local " hormones (Gaddum, 1936, 1950) as distinct from " circulating " hormones will be developed.

Amines

(a) Acetylcholine

Acetylcholine is a chemical transmitter released by several types of nerve endings. Its action is usually terminated by cholinesterase situated close to the site of release. The blood also contains abundant cholinesterase both on the surface of the red cells and in the plasma. Thus if acetylcholine enters the circulation it is very rapidly destroyed. The rapidity of the destruction can be demonstrated by assaying the acetylcholine on a rat stomach strip bathed in carotid arterial blood from a dog. When acetylcholine was in contact with blood for 16 sec before being assayed, rather than being assayed immediately, up to 80 times the infusion rate had to be used. The half-life of acetylcholine in blood was therefore less than 2 sec. In addition to the extremely rapid breakdown of acetylcholine in blood, Eiseman, Bryant & Waltuch (1964) have shown that dog isolated lungs perfused with a dextran-saline solution rapidly inactivated large amounts of acetylcholine. Thus, if acetylcholine leaks into the venous circulation, it is very quickly destroyed and is unlikely to reach the arterial side of the circulation in effective amounts (Fig. 3). It can be concluded that although acetylcholine is highly important as a local hormone, it is unlikely to have a significant role as a circulating one. Indeed, its rapid metabolism, both in the blood and in the pulmonary circulation, suggests that the body has developed protective mechanisms to prevent such a rôle.

(b) 5-Hydroxytryptamine

Release. 5-Hydroxytryptamine (5-HT) is released into the circulation from carcinoid tumours, although Oates, Melmon, Sjoerdsma, Gillespie & Mason (1964) and Zeitlin & Smith (1966) have suggested that it is likely to be the simultaneous generation of bradykinin and not the release of 5-HT which causes the flush. Zeitlin & Smith (1966) could not detect an increase in 5-HT in peripheral venous blood during the vasomotor symptoms of the dumping syndrome, but there was an increase in a bradykinin-like peptide and a fall in plasma kininogen. In dogs increased 5-HT concentrations in venous blood draining the intestine have been found during dumping (Drapanas, McDonald & Stewart, 1962; Peskin & Miller, 1962; Sloop, Johnson & Jesseph, 1962; Walker, Turner & Hardy, 1962), and Burks & Long (1966a, b, 1967a, b) have shown that 5-HT is released from the dog isolated perfused intestine by drugs and by muscular contraction. 5-Hydroxytryptamine released from platelets has also been implicated in the airways constriction that develops in dogs after either pulmonary emboli (Thomas, Stein, Tanabe, Rege & Wessler, 1964) or administration of bacterial endotoxin (Stein & Thomas, 1967).

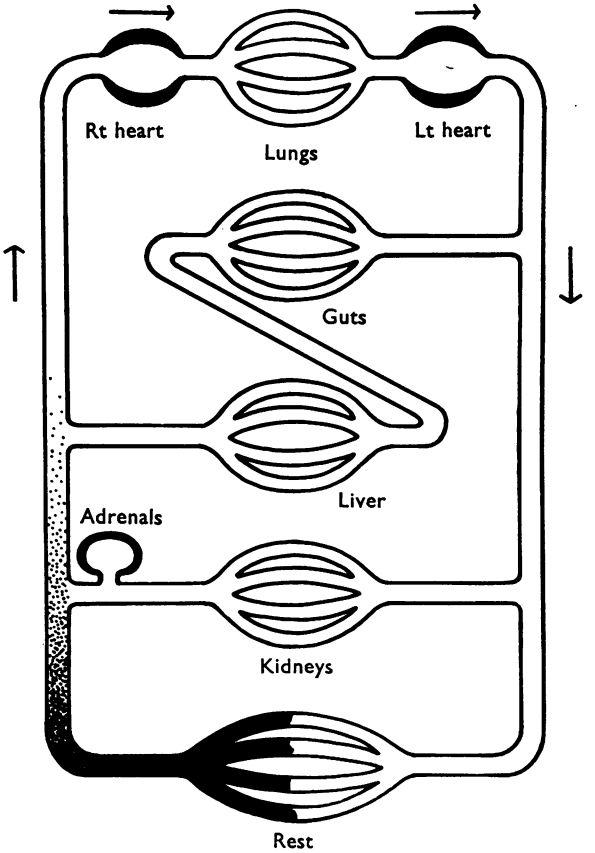

FIG. 3. Fate of acetylcholine in the circulation. In this and subsequent diagrams the removal of drug is shown by a lessening of the shading. "Guts" refers to that part of the viscera drained by the portal vein. With a half-life in blood of less than 2 sec, acetylcholine, if released into the venous circulation from a vascular bed, is unlikely to reach the arterial side of the circulation.

The respiratory symptoms of pulmonary embolism in man have also been ascribed to the release of 5-HT from platelets in the embolus (Comroe, Van Lingen, Stroud & Roncoroni, 1953).

Fate. What happens when 5-HT is released, say, into the portal circulation ? First it comes into contact with blood, where it has a half-life of 1–2 min (Thomas & Vane, 1967). Thus, uptake of 5-HT into platelets (Stacey, 1961 ; Born, 1963) does not bring about rapid inactivation in the blood stream. The amine then reaches the liver ; Drapanas & McDonald (1963) and Thomas & Vane (1967) have found that 20–80% of the 5-HT which enters the portal circulation disappears there. The 20–80% of the blood 5-HT which emerges in the hepatic vein next reaches the lungs, where it encounters a tremendously active removal process. The first indication of this came from Starling & Verney (1925), who found it impossible to maintain adequate circulation through an isolated perfused kidney with a simple perfusion circuit. As soon as the defibrinated blood reached the kidneys, the vessels closed down. However, they could overcome this effect by perfusing the kidneys from a heart-lung preparation ; they concluded that the blood was " detoxicated " in the lungs. The identification of the serum vasoconstrictor substance as serotonin or 5-HT came in 1948 (Rapport, Greene & Page, 1948a, b). Gaddum, Hebb, Silver & Swan (1953) then showed that 5-HT was inactivated by cats' isolated lungs perfused with blood. Isolated perfused lungs of the dog also metabolize 5-HT (Eiseman, Bryant & Waltuch, 1964). Davis & Wang (1965) measured the removal of injected 5-HT by dogs' lungs *in vivo* and found 30–40% was removed in one circulation. Thomas & Vane (1967) used infusions of 5-HT rather than injections in order to study the steady-state effects. They found that up to 98% of an intravenous infusion of 5-HT into the anaesthetized dog disappeared in a single passage through the pulmonary circulation.

These results indicate that the major site for inactivation of 5-HT in the body is the lungs and not the liver. Predominantly right-sided heart lesions associated with carcinoid tumour may be linked with pulmonary removal of 5-HT (Goble, Hay & Sandler, 1955) and there is evidence that the removal mechanism in man may sometimes fail (Davis, 1968). On what does the dramatic removal of 5-HT in the lungs depend ? It does not seem to be amine oxidase, for inhibition of the enzyme does not decrease the disappearance of 5-HT in the dog lung (Davis & Wang, 1965 ; Thomas & Vane, 1967). Axelrod & Inscoe (1963) found that after intravenous injections of labelled 5-HT in the mouse and rat most radioactivity was in the spleen, lungs and heart. Moreover, unchanged 5-HT was retained in the lungs and the spleen for up to a week. Drugs such as imipramine, which prevented uptake of catecholamines, also decreased storage of 5-HT by the lungs. Potentiation of the cardiovascular actions of 5-HT by substances like methylphenidate (Rutledge, Barrett & Plummer, 1959) may, therefore, be through inhibition of this removal process in the lungs, allowing more 5-HT to reach the arterial side of the circulation.

Thus it seems that 5-HT is primarily removed from the circulation not by enzymic inactivation but by an uptake and storage process. The storage may be associated with reticulo-endothelial cells, for Gershon & Ross (1966a, b) found that after infusion of labelled precursor, some 5-HT was localized in reticulo-endothelial cells in the spleen, liver and lungs ; these cells do not contain monoamine oxidase (Gershon, personal communication). Eiseman, Bryant & Waltuch (1964) in their experiments

on blood-perfused lungs found that added 5-HT disappeared from the re-circulating blood and there was a concomitant rise in 5-hydroxyindoleacetic acid. Thus there may be two different removal mechanisms for 5-HT in the lungs, one depending on storage in cells and the other depending on transfer of the 5-HT to other cells where it can be metabolized. It is possible, for instance, that the stored 5-HT is gradually transferred to platelets. The lung capillaries are a rich source of platelets, for 20–50% of the mature megakaryocyte population ultimately reaches the lungs and in man 7–17% of platelets are released from them in the pulmonary capillaries (Kaufman, Airo, Pollack & Crosby, 1965).

Whatever the intimate details of the mechanism, the body has two lines of defence which prevent 5-HT released in the gut from reaching the arterial circulation. The liver and lungs together can reduce 5-HT concentration in arterial blood to less than 1% of that in portal blood in one circulation (Fig. 4). Only in situations where this protective mechanism is overwhelmed or disturbed, as may happen in carcinoid, or by-passed, as may happen when 5-HT is released from platelets or from lungs during anaphylaxis (Waalkes, Weissbach, Bozicevich & Udenfriend, 1957) is 5-HT ever likely to be effective as a circulating hormone. It can be concluded that the

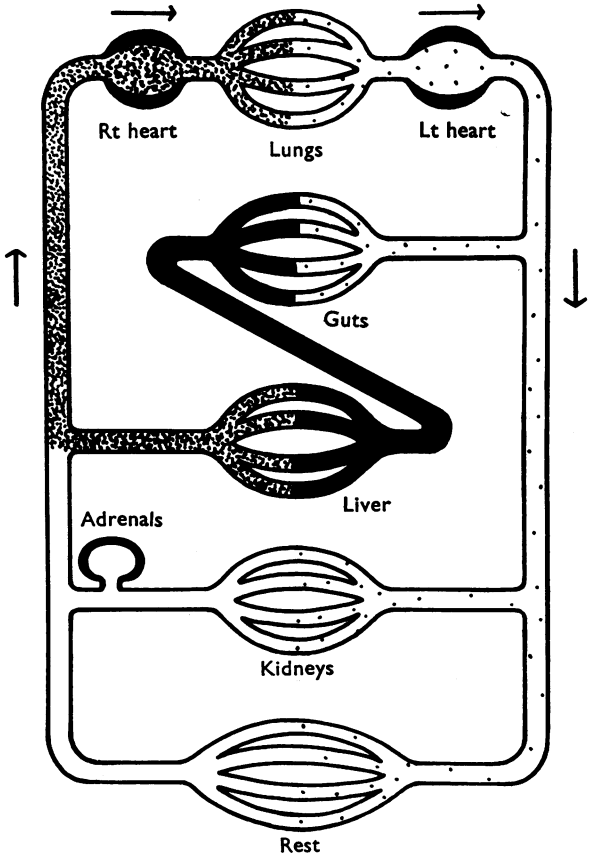

FIG. 4. Fate of 5-hydroxytryptamine in the circulation. If 5-hydroxytryptamine is released from the intestine, about 65% will be removed by the liver and more than 95% of that remaining will be removed by the lungs. Thus the amount reaching the arterial circulation will be reduced to less than 1% of that released.

role of 5-HT in the body must be as a local hormone and that the body has developed protective mechanisms to prevent 5-HT from reaching the arterial circulation.

The process in the lungs responsible for the uptake and storage of 5-HT seems to show some structural specificity. Although melatonin (N-acetyl-5-methoxytryptamine) is closely related in structure to 5-HT, injected melatonin was mostly found in liver, kidney and spleen rather than in the lungs (Kopin, Pare, Axelrod & Weissbach, 1961). The specificity of the uptake process for 5-HT also excludes some other amines. For instance, histamine is not inactivated in heart–lung (Steggeda, Essex & Mann, 1935) or perfused lung (Eiseman, Bryant & Waltuch, 1964) preparations from dogs. Heart–lung–liver preparations of cats rapidly eliminated histamine from the blood, but heart–lung preparations alone did not (Lilja & Lindell, 1961).

The lack of removal of histamine on passage through the pulmonary circulation *in vivo* would not be predicted from *in vitro* experiments. Histamine is inactivated by chopped or homogenized lung tissue from man (Lilja, Lindell & Saldeen, 1960), guinea-pig and rat (Bennett, 1965), and enzymes from cat lungs are as efficient as those from cat liver in N-methylating histamine *in vitro* (Brown, Tomchick & Axelrod, 1959). Lung extracts from most species contain diamineoxidase activity and some contain histamine N-methyltransferase activity (Buffoni, 1966). The contrast between the *in vitro* inactivation of histamine by lung tissue and the lack of inactivation on passage through the pulmonary circulation *in vivo* is a striking illustration of the danger of extrapolating results from studies with enzymes *in vitro* to the physiological situation.

Studies of distribution of a substance may also lead to erroneous predictions about its route of inactivation. In mice and rats 1 hr after injection of labelled histamine, the lungs contained approximately four times the radioactivity of the serum, but only one-third of this was in the form of histamine. Although the level in the lungs was higher than that in the brain, skin and skeletal muscle, there was 10 times more radioactivity in the kidney and two to three times more in the liver (Snyder, Axelrod & Bauer, 1964; Snyder & Axelrod, 1965). Similar high levels of histamine in kidney and liver of guinea pigs were found after injections; the lungs contained three to four times the amount of radioactivity in the plasma (Andrejewski & Cohen, 1966). How do these experiments fit with those quoted earlier, showing that little or no histamine was removed on passage through the pulmonary circulation? Clearly, tissue uptake of a drug or its metabolites does not necessarily reflect the ability of that tissue effectively to remove the drug in one circulation. Distribution of a drug is often measured 60 min (or 100 circulations) after injection. The distribution found at this time may be quite different from that which occurred during the first few circulations of the drug and may not in any way reflect the primary mechanism of inactivation. This conclusion may well be important in discussing the fate of the catecholamines (see later).

(c) The catecholamines

Release. Adrenaline and noradrenaline are released from the adrenal medulla and from sympathetic postganglionic nerve fibres, but the reflex control mechanisms, especially for the adrenal medulla, are still not fully understood. For instance, catecholamines released into the circulation from the medulla contribute little to the

rise in blood pressure during the strong reflex sympathetic stimulation induced by carotid occlusion (Driver & Vogt, 1950; Schaepdriver, 1959; Vane, 1964); even when the vagi are cut to prevent reaction of aortic baroreceptors the release of adrenaline induced by carotid occlusion is still comparatively small.

We have recently used the blood-bathed organ technique to assay arterial blood concentrations of catecholamines in dog and man during activation of arterial baroreceptor reflexes (Hodge, Lowe & Vane, unpublished). In the anaesthetized dog, carotid occlusion caused an average pressor response of 65 mm Hg and the change in secretion of catecholamines was equivalent to less than 2 μg adrenaline/min. When infused, this dose had a pressor effect of 2–7 mm Hg. In man, the experiments were carried out in volunteers and the arterial blood was run to waste after superfusing the assay organs. Each subject was stressed to the limit of tolerance by lower-body suction, which reduced mean arterial pressure sometimes to as low as 14 mm Hg. Changes in catecholamine secretion were detected in only two of the six subjects, amounting to 5 μg/min in each, when fainting was imminent.

Circulating catecholamines, therefore, appear to play little or no part in arterial baroreceptor reflexes. These results are difficult to reconcile with the concept that adrenal medullary secretion is reflexly induced by arterial baroreceptor stimulation, as suggested for the hypotension associated with haemorrhage (Glaviano, Bass & Nykiel, 1960) or with endotoxin shock (Spink, Reddin, Zak, Peterson, Starzecki & Seljeskog, 1966). The selective activation of sympathetic nerves by carotid occlusion is also difficult to reconcile with the concept of generalized sympatho-adrenal activation (Malmejac, 1964; Irving, 1968).

Under what conditions is there strong stimulation of adrenal medullary secretion? Catecholamines are released into the circulation during anaphylaxis, but it is not known whether this is through nervous or humoral stimulation of the adrenal medulla or both (Piper, Collier & Vane, 1967). Of the substances thought to be released by the anaphylactic action (Collier, 1968) histamine, bradykinin and SRS-A will all release adrenaline when injected intravenously, although there may be species differences in the mechanisms of action and in the sensitivity of the adrenal medulla (Staszewska-Barczak & Vane, 1965a, b, 1967).

Angiotensin also releases medullary adrenaline in the cat (Feldberg & Lewis, 1964, 1965) and the guinea-pig (Piper & Vane, 1967) but is not very potent in the dog (Staszewska-Barczak & Vane, 1967). It is interesting that the adrenal medulla has a different specificity for chemical stimulation from that of sympathetic nerve endings. Tyramine, which depends for its action on release of noradrenaline from the sympathetic nerve ending (Burn & Rand, 1958) causes little or no release of catecholamine into the blood stream (Vane, 1960). Other examples are given by Hagen (1959), who points out that anoxia (presumably for brief periods) causes release of catecholamines from the adrenal medulla (Büibring, Burn & De Elio, 1948) but not from sympathetic nerves (Konzett & Rothlin, 1952). Evidence is therefore accumulating that the adrenal medulla and other parts of the sympathetic nervous system can be selectively and individually activated, not only by central control (Folkow, Heymans & Neil, 1965) but also by drugs. In this context it is interesting that both bradykinin (Lang & Pearson, 1968) and angiotensin (Lowe & Scroop, 1969) can produce hypertension when injected into the blood supply to the brain.

Evidence that adrenal medullary secretion may be initiated by as yet undefined reflexes arises from the work of Staszewska-Barczak & Ceremuzynski (1968). They have shown in the dog that production of cardiac infarction by sudden coronary occlusion leads within a few minutes to a prolonged output of adrenaline into the circulation. This adrenaline comes from the adrenal medulla and the outpouring occurs without any change in mean arterial pressure ; presumably, therefore, there is no stimulation of arterial baroreceptors. The adrenaline secretion may be mediated by hormonal or nervous changes. Ceremuzynski, Staszewska-Barczak & Cedro (1969) have also shown a striking correlation between the secretion of medullary adrenaline and cardiac arrhythmias after infarction. In a few dogs, coronary occlusion did not induce a secretion of adrenaline and there were no cardiac arrhythmias. When adrenaline was infused intravenously in these dogs at rates equivalent to those secreted in the other experiments, cardiac arrhythmias were produced. The same rate of infusion of adrenaline before coronary occlusion did not produce arrhythmias.

In many experiments on catecholamine release into the circulation, adrenaline is found to be the only hormone released, whereas in others noradrenaline release is also detected. In endotoxin shock, for instance, Spink, Reddin, Zak, Peterson, Starzecki & Seljeskog (1966) found only adrenaline ; in haemorrhage, Glaviano, Bass & Nykiel (1960) occasionally found both catecholamines and Marley & Paton (1961) and Marley & Prout (1965) found mixtures after stimulation of the splanchnic nerve. A possible explanation of these differences arises from a study of the fate of the released catecholamine in the circulation.

Fate. In 1905 Elliott found that adrenaline did not disappear in the lungs but did so in other vascular beds. He concluded that " adrenalin disappears in tissues which it excites "; since then there have been many studies of distribution of catecholamines and their removal from the circulation. After injecting radioactive adrenaline or noradrenaline unchanged catecholamines were found mostly in the heart, spleen and glandular tissues, whereas skeletal muscle took up the least (Axelrod, Weil-Malherbe & Tomchick, 1959 ; Whitby, Axelrod & Weil-Malherbe, 1961). Two minutes after the intravenous injection of high doses of adrenaline or noradrenaline into cats the lungs contained less than the plasma level of labelled adrenaline but four times the plasma level of labelled noradrenaline, a concentration which equalled that of labelled noradrenaline in the adrenal glands (Axelrod, Whitby, Hertting & Kopin, 1961). There were considerable amounts of labelled metanephrine and normetanephrine in the lungs. The relatively high levels of noradrenaline and normetanephrine found in the lungs after 2 min had almost completely disappeared after 2 hr, even though at this time the heart, spleen and adrenal glands contained high concentrations of unchanged labelled noradrenaline (Whitby, Axelrod & Weil-Malherbe, 1961). These results suggest that either the uptake or storage process for noradrenaline in the lungs is different from those associated with nervous tissue in the heart and spleen and from those in the adrenal gland. It is conceivable that noradrenaline has some affinity for the uptake and storage processes for 5-HT in the lungs, discussed earlier.

Adrenaline and noradrenaline have a half-life in the circulation of less than 20 sec (Ferreira & Vane, 1967b), which is probably less than one circulation time (15–30 sec in the cat and dog ; Spector, 1956). The shortest time at which distribution of adrenaline and noradrenaline was measured by Axelrod and his colleagues

was 2 min. This represents several circulation times; to determine whether a single circulation through different vascular beds would remove catecholamines, experiments in cats and dogs were performed using the blood-bathed organ technique (Ginn & Vane, 1968). Intravenous infusions of adrenaline (0.5–5 μg/min) passed through the pulmonary circulation without loss; however, up to 30% of the infusions of noradrenaline disappeared in the lungs. The disappearance of noradrenaline in the pulmonary circulation has also been demonstrated in other ways. Dog isolated lungs removed noradrenaline from the perfusing blood (Eiseman, Bryant & Waltuch, 1964). Labelled noradrenaline infused into saline perfused rat lungs was concentrated by the lung tissue (Hughes & Gillis, 1968). The uptake was a saturable process and about 70% of the radioactivity held in the lung was noradrenaline.

These results allow two interesting conclusions. First, if a mixture of adrenaline and noradrenaline is released from the adrenal medulla, the lungs may preferentially remove some of the noradrenaline, thereby increasing the proportion of adrenaline in the mixture of catecholamines which reaches the arterial circulation. Measurements of catecholamine content in adrenal venous blood will therefore give different proportions of adrenaline and noradrenaline from measurements of content in arterial blood. Indeed, noradrenaline may have little significance as a circulating hormone; its release from the medulla may only be a means of replenishing stores in the lungs. Secondly, drugs which interfere with uptake or storage of noradrenaline may cause some of their potentiating effects on the cardiovascular system not only by preventing uptake in peripheral tissues but also by interfering with uptake in the lungs, thereby allowing more of the injected noradrenaline to reach the arterial circulation. The potentiation of the effects of noradrenaline by antihistaminic compounds, for instance (Johnson & Kahn, 1966), may be because the antihistamines enter the catecholamine storage particles in the lungs and elsewhere in the body. Way & Dailey (1950) have shown that tripelenamine, one of the antihistamines which potentiates catecholamines, is preferentially taken up in the lungs. Another antihistamine, diphenhydramine, is found in highest concentration in the lungs and spleen of rats and guinea-pigs (Glazko & Dill, 1949; Glazko, McGinty, Dill, Wilson & Ward, 1949).

If the mechanism of potentiation of catecholamine effects by these substances is through their ability to compete for the catecholamine stores, it would be interesting to investigate the actions of mepacrine, for this substance is bound to an extraordinary degree by tissues, such as lungs, spleen, adrenal glands and liver (Dearborn, Kelsey, Oldham & Geiling, 1943; Shannon, Earle, Brodie, Taggert & Berliner, 1944; Brodie, 1964). When mepacrine is added to the blood in heart-lung preparations of rabbits, 77% is found in the lungs after 2 min and 93% after 2 hr (Chen & Geiling, 1944).

Fate in peripheral vascular beds. It both cats and dogs 70–95% of an intra-arterial infusion of adrenaline or noradrenaline disappears in one passage through the hindquarters (Vane, 1966; Ginn & Vane, 1968). Part of an experiment on a dog is shown in Fig. 5. The relaxation of the rat stomach strip during intra-arterial infusion of adrenaline (25 μg/min) showed that less than 5 μg/min were passing through the hindquarters (>80% removal). Steady-state conditions were reached within a few minutes and even though the infusion was maintained for 20 min the hindquarters continued to abstract adrenaline, removing a total of about 400 μg

Similarly, from an intra-arterial infusion of adrenaline at 50 μg/min given for 10 min, about 35 μg/min or 350 μg disappeared (70% removal). These results confirm those of Celander & Mellander (1955), who studied the disappearance of adrenaline and noradrenaline in vascular beds of the cat. They found that up to 90% of an arterial infusion of catecholamine disappeared in the hindquarters; similar results were obtained in the spleen, kidneys and intestine. All these results contrast strikingly with the experiments on distribution of catecholamines (Axelrod, Weil-Malherbe & Tomchick, 1959; Whitby, Axelrod & Weil-Malherbe, 1961), in which uptake into skeletal muscle was small.

What process could be responsible for the immediate disappearance of catecholamines from the circulation? Disappearance of catecholamines into tissue was found by Elliott in 1905. In 1932, Burn suggested that this was an uptake process and since then many investigations have suggested that uptake is important in the disposition of circulating catecholamines (see Iversen, 1967). The demonstrations that tissue uptake of noradrenaline can be correlated with the richness of adrenergic innervation (Whitby *et al.*, 1961) and that sympathetic denervation by surgical (Hertting, Axelrod, Kopin & Whitby, 1961; Stromblad & Nickerson, 1961) or immunological (Zaimis, Berk & Callingham, 1965; Iversen, Glowinski & Axelrod, 1966) means reduces the uptake of noradrenaline have led to the conclusion that tissue uptake is mostly into sympathetic nerve fibres (see Iversen, 1967). It is in turn assumed that this uptake into nerve fibres accounts for the rapid removal of catecholamine from circulating blood. This assumption is probably unwarranted for several reasons. First, in experiments *in vivo,* uptake into nerve fibres has only been demonstrated minutes or hours after injection of catecholamines. These are long time intervals when compared with the rapid removal during a single passage through the hindquarters, and substantial redistribution may have taken place. Secondly, the relative importance of tissue uptake as measured by Axelrod, Weil-Malherbe & Tomchick (1959) and Whitby, Axelrod & Weil-Malherbe (1961) in the

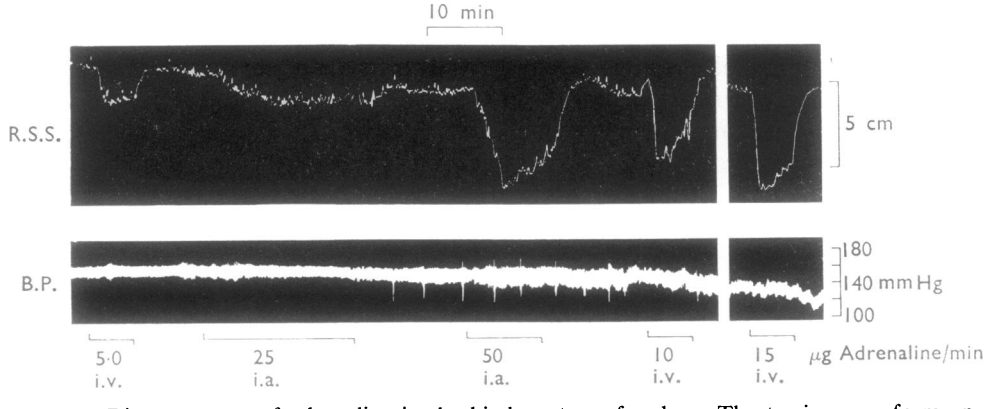

FIG. 5. Disappearance of adrenaline in the hindquarters of a dog. The tracings are from an experiment in a 24 kg dog. The lower record is of blood pressure (BP) and the upper one of the relaxation of a rat stomach strip (RSS) bathed in arterial blood. Infusions of adrenaline were made either into the lower aorta (intra-arterial) to reach the hindquarters or intravenously. From an intra-arterial infusion of adrenaline (25 μg/min for 20 min) about 5 μg/min traversed the hindquarters. Similarly, from an infusion of 50 μg/min for 10 min, only 15 μg/min came through the hindquarters. Time, 10 min: vertical scales 5 cm and mm Hg. (From Ginn & Vane, 1968.)

mouse was considerably greater for noradrenaline than for adrenaline, but both catecholamines are almost completely removed in a single circulation through the hindquarters. Thirdly, Gillespie & Hamilton (1966, 1967) and Avakian & Gillespie (1967, 1968) have shown that noradrenaline is taken up *in vitro* not only into adrenergic nerve fibres but also into smooth muscle, collagen and elastic tissue. The noradrenaline taken into smooth muscle resists washing out, as does that in nerve fibres, whereas that taken up by collagen and elastic tissue is easily washed out. Concentrations of phenoxybenzamine which prevent uptake into nerve terminals and smooth muscle have no effect on uptake into collagen and elastic tissue. Avakian & Gillespie (1968) suggested that smooth muscle cells have a transport mechanism for the intracellular uptake of noradrenaline. This uptake may be the same as that designated as Uptake$_2$ by Iversen (see Iversen, 1967). The almost complete removal from the blood stream of large quantities of adrenaline, noradrenaline or isoprenaline in a single passage through a peripheral vascular bed might be due to a similar process, but it seems unlikely that uptake into sympathetic nerves contributes greatly to the immediate physiological disposition of circulating catecholamines.

The fact that many organic bases are concentrated in adrenal glands, lungs, heart and spleen (see Vane, 1968b) raises the possibility that they are taken up and stored by the same processes which concentrate noradrenaline. This possibility has been discussed (Vane, 1962 ; Iversen, 1967) for those amines which have been shown to inhibit uptake of noradrenaline into tissues, but it may be a much more general phenomenon, the degree of uptake depending on the degree of structural resemblance

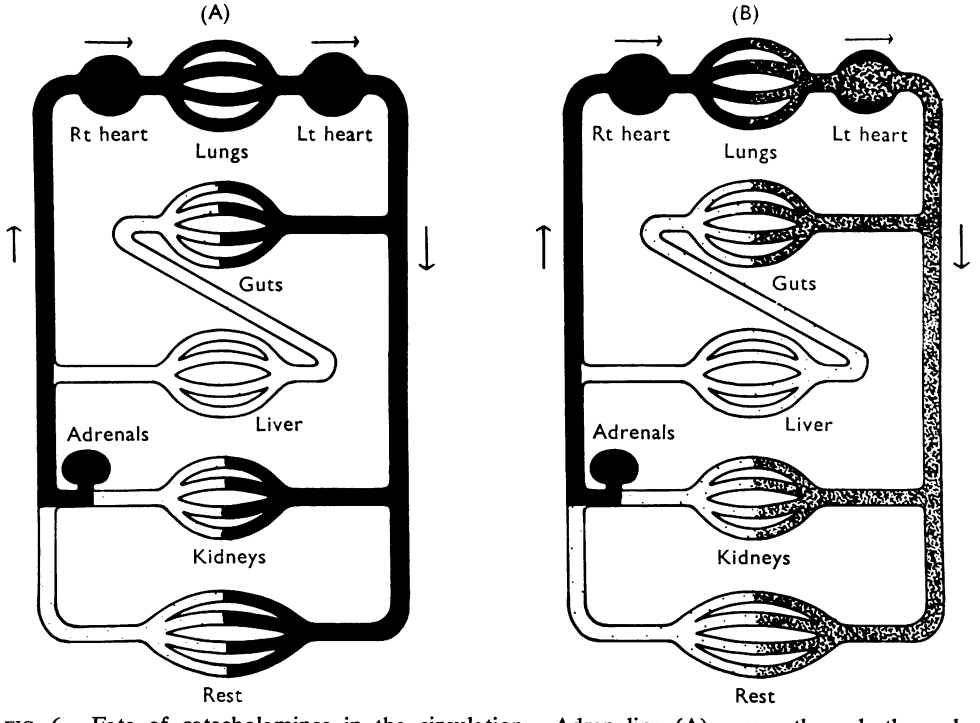

FIG. 6. Fate of catecholamines in the circulation. Adrenaline (A) passes through the pulmonary circulation unchanged, but up to a third of the noradrenaline (B) is removed. Both catecholamines disappear in peripheral vascular beds.

to noradrenaline. This could apply not only to the " specific " storage sites but also to " non-specific " storage sites (Vane, 1962) such as reticulo-endothelial cells or negative charges on proteins. The fates of adrenaline and noradrenaline in the circulation are shown in Fig. 6.

Peptides

(a) Bradykinin

Release. The discovery of bradykinin (Rocha e Silva, Beraldo & Rosenfeld, 1949) provided a new candidate for the rôle of endogenous vasodilating agent that histamine had held for so long. Subsequently, local release of bradykinin or kallidin has been implicated in the atropine-resistant vasodilatation associated with activity of the salivary (Hilton & Lewis, 1956) and other glands (Folkow, Heymans & Neil, 1965 ; Hilton, 1966), but this hypothesis is not yet fully accepted (Webster, 1966 ; Schachter, 1966 ; Skinner & Webster, 1968). Local formation of bradykinin has also been postulated in many types of inflammation (see Spector & Willoughby, 1968).

Activation of plasma kininogenase (kallikrein) leading to the formation of kinins in blood has been studied in several pathological conditions. Landerman, Webster, Becker & Ratcliffe (1962) showed that hereditary angioneuroticoedema is associated with a deficiency in inhibitor of plasma kininogenase. Activation of plasma kininogenase may also occur in asthma, pancreatitis, pulmonary oedema, anaphylaxis, reactions to blood transfusions, infection by pathogenic organisms, burns, endotoxin and haemorrhagic shock (see Erdös, 1966 ; Erdös, Back & Sicuteri, 1966). Release of kininogenase from metastases of carcinoid tumours has also been suggested to explain the associated flushes (Oates, Melmon, Sjoerdsma, Gillespie & Mason, 1964 ; Oates & Melmon, 1966). Bradykinin has also been implicated as a mediator of the circulatory changes in the foetus at time of birth (Melmon, Rudolph, Hughes, Nies & Cline, 1967). The pulmonary vascular bed of the foetal lamb is certainly highly

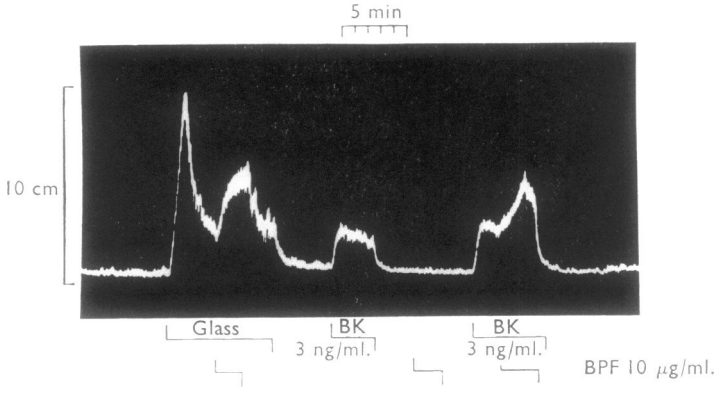

FIG. 7. Strip of cat jejunum superfused at 15 ml./min with arterial blood from a cat anaesthetized with chloralose. At the point marked, the blood was diverted through a tube containing 1 g of glass beads. There was an almost immediate generation of bradykinin which rapidly declined to a rate of 45 ng/min and a concentration of 3 ng/ml. Infusion of bradykinin potentiating factor (BPF) (10 μg/ml.) into the blood increased once more the contraction of the cat jejunum. The contraction caused by an infusion of bradykinin to give a concentration of 3 ng/ml. was similarly increased by BPF. Time in min: vertical scale 10 cm. (From Ferreira & Vane, 1967a.)

sensitive to bradykinin (Campbell, Dawes, Fisherman, Heyman & Perks, 1968). These authors also conclude that some vasodilator effects obtained in perfusion experiments using glass connections are due to glass-activated formation of a plasma kinin. Release of kinin by contact of blood with glass is shown in Fig. 7, in which arterial blood from a cat was diverted through a tube containing 1 g of glass beads. There was an almost immediate generation of kinin which rapidly declined to a rate of about 45 ng/min and a concentration of 3 ng/ml.

Fate. What happens if bradykinin escapes into the venous circulation? Bradykinin is fairly rapidly destroyed in blood and has a half-life in the blood stream of cat or dog of about 17 sec (McCarthy, Potter & Nicolaides, 1965; Ferreira & Vane, 1967c), making it likely that the released bradykinin will reach the liver and lungs. Ferreira & Vane (1967c) showed that whereas the liver inactivated about 50%, the lungs inactivated about 80% of a bradykinin infusion. The almost complete pulmonary removal of bradykinin has since been confirmed in rats and dogs (Biron, 1968; Stewart, 1968). Bradykinin is probably also inactivated in the lungs of man, for it was one-sixth as active as histamine when given intravenously but more potent when administered intra-arterially (Fox, Goldsmith, Kidd & Lewis, 1961). Lung inactivation now seems a more likely explanation of this effect than

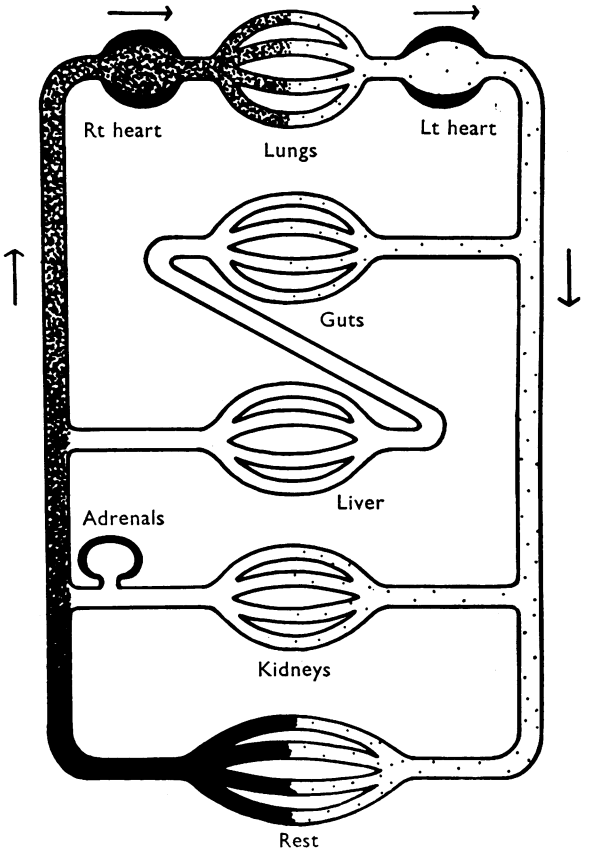

FIG. 8. Fate of bradykinin in the circulation. If bradykinin is released into the venous blood, some will be destroyed before reaching the lungs (half-life=17 sec). The lungs will remove 80% or more of the remaining bradykinin, allowing little to escape into the arterial circulation.

the authors' suggestion of rapid inactivation in blood. Intravenous bradykinin produced vasodilatation in the face and neck at dose levels which were too low to produce effects on forearm or hand blood flow (Fox *et al.*, 1961). The fact that bradykinin is removed in the lungs suggests the possibility that the vasodilatation of the face and neck were reflex effects arising from stimulation of sensory nerve endings in the pulmonary circulation. Such an action might also account for the flush associated with carcinoid syndrome.

That the ability of the lungs to remove bradykinin is a special one is shown by two types of results. First when bradykinin was infused in other sites, only about 30% disappeared in the hind legs, about 70% in the kidneys, 50% in the liver and 65% in the head (Ferreira & Vane, 1967c). Secondly, the activity of eledoisin (Ferreira & Vane, 1967c) and polisteskinin (Stewart, 1968) are unaffected by passage through the pulmonary circulation.

Ryan, Roblero & Stewart (1968) using radioactive bradykinin found that the radioactivity passed through the pulmonary circulation, whereas the bradykinin activity did not. The inactivation mechanism must therefore be enzymic. From a study of a series of bradykinin analogues, Ryan *et al.* (1968) concluded that the pulmonary enzyme had the properties neither of the carboxypeptidase-N which inactivates bradykinin in blood (Erdös, Renfrew, Sloane & Wohler, 1963) nor of the endopeptidase isolated from kidney (Erdös & Yang, 1967). The fate of bradykinin in the circulation is shown in Fig. 8. It can be concluded that the role of bradykinin in the body is likely to be one of a local hormone, for as with acetylcholine and 5-HT, there is an efficient mechanism for protecting the arterial circulation from bradykinin liberated into the venous circulation. This inactivation mechanism may be by-passed, however, when bradykinin is formed on the arterial side of the circulation by circulating kininogenase. The kininogenase kallikrein has a half-life in the cat's circulation of about 3 min (Ferreira & Vane, 1967b), so it probably recirculates several times. In this context it is of interest that the kallikrein inhibitor " trasylol " is found in particularly high concentrations in bovine lung (Trautschold, Werle & Zigrafrödel, 1967).

(b) The angiotensins

Release of renin. When renin is secreted into renal venous blood it acts upon angiotensinogen, a protein in the α-globulin fraction, to form the relatively inactive decapeptide angiotensin I. The octapeptide, angiotensin II, is made by the action of another enzyme (converting enzyme) on this decapeptide ; all the pharmacological activity of the renin-angiotensin system resides in the octapeptide angiotensin II. The control of the system, which is probably through changes in secretion of renin, has been studied for many years (see Peart, 1965 ; Brown, Davies, Lever & Robertson, 1966 ; Vander, 1967). For example, a fall of renal arterial pressure rapidly induces secretion of renin (Skinner, McCubbin & Page, 1963 ; Regoli & Vane, 1966). Thus any condition accompanied by a fall in blood pressure, such as severe haemorrhage, will lead to increased secretion of renin followed by increased generation of angiotensin (Scornik & Paladini, 1964 ; Regoli & Vane, 1966). The kidney can also secrete renin in response to conditions in which mean arterial blood pressure does not change. Thus, non-hypotensive reductions of blood volume led to increased secretion of renin and generation of angiotensin in the circulation through a reflex action involving the renal sympathetic nerves (Hodge, Lowe & Vane,

1966a, b ; Bunag, Page & McCubbin, 1966 ; Vander, 1967). The afferent limb is probably mediated by stretch receptors in the low pressure side of the circulation.

It has also been suggested that changes in tubular sodium concentration can effect renin release, but direct evidence for this is lacking (see Vander, 1967). In dogs, there is an increased rate of generation of angiotensin in the plasma within a minute or two of the intravenous injection of the diuretic frusemide (Hodge, Ng & Vane, unpublished) and this occurs without any fall in arterial blood pressure.

Release of angiotensin I and II. Renin has a half-life in the circulation of the dog of more than 15 min (Hodge, Lowe & Vane, 1966a). It will therefore recirculate many times, the gradual decrease in concentration probably being a result of inactivation in the liver (Heacox, Harvey & Vander, 1967). By its action on angiotensinogen in the blood, renin generates angiotensin I throughout the circulation. This decapeptide is then converted to angiotensin II by removal of the C-terminal dipeptide histidyl-leucine (Skeggs, Kahn & Shumway, 1956a, b). The conversion of angiotensin I to II *in vivo* is very rapid and it has long been assumed that this conversion is by an enzyme in blood. However, Ng & Vane (1967, 1968a, b) have shown in dogs that converting enzyme activity in blood is too slow to account for *in vivo* conversion of angiotensin I to angiotensin II: the rapid conversion *in vivo* takes place in the pulmonary circulation. Biron & Huggins (1968) have confirmed this conclusion by experiments in rats, cats, dogs and rabbits.

Conversion of angiotensin I to II in the lungs could be brought about by an enzyme fixed in the pulmonary circulation, or by activation of the mobile blood enzyme as it goes through the pulmonary circulation. Bakhle, Reynard & Vane

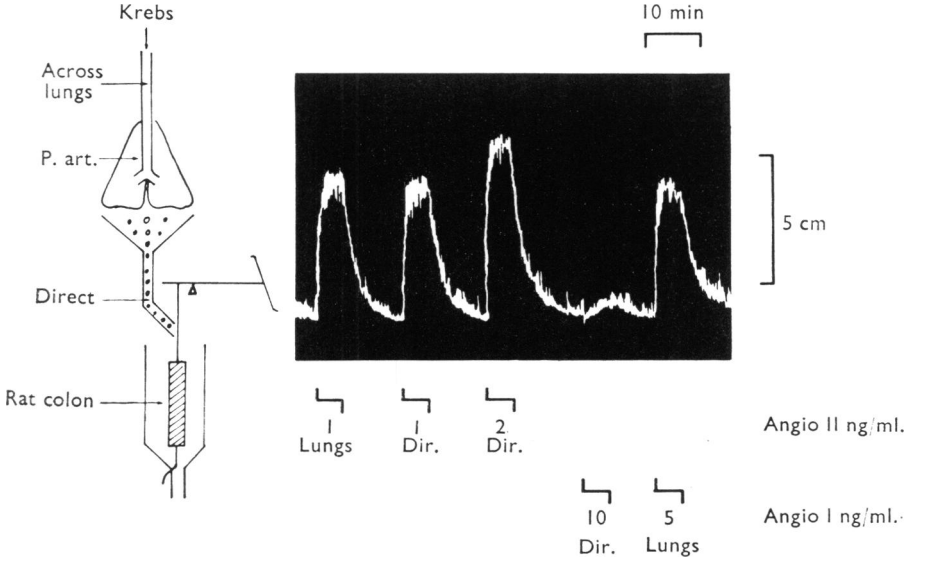

FIG. 9. Increased activity of angiotensin I when passed through guinea-pig isolated lungs. The diagram on the left shows the experimental procedure. In fusions of angiotensin II (Angio II) through the lungs or direct to the rat colon (Dir.) gave the same response of the rat colon, showing that there was no destruction in the lungs. An infusion of angiotensin I (Angio I) at 10 ng/ml. direct to the rat colon gave a minimal response, but when half this concentration (5 ng/ml.) was infused through the lungs there was a much greater contraction of the rat colon. Time, 10 min, vertical scale 5 cm. (From Bakhle, Reynard & Vane, unpublished.)

(unpublished) have distinguished between these possibilities by using isolated lungs perfused through the pulmonary artery with Krebs solution, the perfusate superfusing a rat colon. Angiotensin I or II was infused either directly into the Krebs solution superfusing the rat colon or so that they traversed the lungs before reaching the assay tissue. Fig. 9 shows that the activity of angiotensin II was unaffected by passage through lungs of a guinea-pig. However, when angiotensin I traversed the isolated lungs its activity was substantially increased, through conversion to angiotensin II. Similar results were obtained on isolated lungs or lobes of lungs from the rat, dog and cat. When angiotensin I was infused through isolated perfused kidneys from the same species, there was a net loss of activity. Thus the lungs contain converting enzyme, but none could be detected in the kidney using this perfusion technique.

Fate of angiotensin I. Since renin recirculates time and time again, some angiotensin I will be formed on the arterial side of the circulation ; most of this (50–70%) disappears in the peripheral vascular beds without conversion to angiotensin II (Ng & Vane, 1968b). Despite the fact that homogenates of many tissues exhibit converting enzyme activity (Page & Bumpus, 1961 ; Huggins & Thampi, 1968) we have been unable to demonstrate physiological conversion of angiotensin I to II in any of the peripheral vascular beds so far studied (Ng & Vane, 1968b).

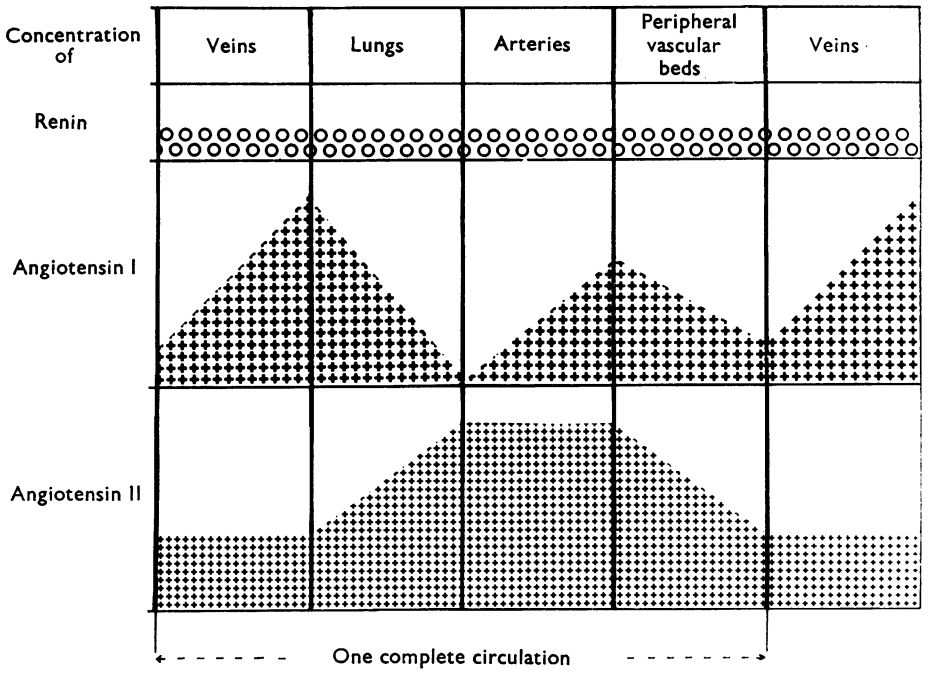

FIG. 10. Changes in concentration in the circulation of the components of the renin angiotensin system. Renin has a long half-life (>15 min) and the concentration will change little in one circulation (15–30 sec). Angiotensin I will be generated in veins and arteries : that generated on the venous side will be converted to angiotensin II in the lungs ; that generated on the arterial side will be removed (65%) without conversion in peripheral vascular beds. The concentration of angiotensin II will increase in the lungs because of generation from angiotensin I ; it will decrease by 65% as it passes through peripheral vascular beds.

Fate of angiotensin II. The half-life of angiotensin II in circulating blood varies between 100 and 200 sec according to the structure of the angiotensin II studied (Hodge, Ng & Vane, 1967). Destruction in blood is therefore relatively unimportant as an inactivation mechanism. The lungs do not inactivate or remove angiotensin II, in striking contrast to the disappearance of angiotensin I and bradykinin. However, about 50–70% of an infusion of angiotensin II disappears in peripheral vascular beds, such as the liver, the kidneys and the hindlegs (Hodge, Ng & Vane, 1967). Figure 10 represents the changes in concentration of the individual components of the renin-angiotensin system as they progress around the circulation.

Several conclusions can be drawn. First, the localization of converting enzyme in the lungs, but not in other tissues, makes untenable hypotheses (Thurau, 1964; Thurau, Schnermann, Nagel, Horster & Wahl, 1967; Leyssac, 1967; Britton, 1968) which propose a wholly intra-renal function for the renin-angiotensin system. Secondly, the generation of angiotensin II in the pulmonary circulation makes it likely that the renin-angiotensin system is designed as a circulating hormone system. Indeed, the positioning of converting enzyme in the pulmonary vascular bed rather than in blood might be a mechanism for protecting the afferent arteriole of the kidney from the high concentrations of angiotensin II that would otherwise be generated by the high local concentration of freshly-secreted renin.

The renin-angiotensin system provides further examples of the dangers of extrapolating *in vitro* results to the physiological situation. For instance, it was thought for many years that both converting enzyme and angiotensinase in blood were important enzymes in the renin-angiotensin system, but it now appears that neither has physiological importance. Angiotensin II is removed in peripheral vascular beds and not by inactivation in blood. The β-aspartyl analogue of angiotensin II resists the actions of aminopeptidases in blood, but disappears in peripheral vascular beds to the same extent as α-aspartyl angiotensin II (Biron, Meyer & Pannisett, 1968); thus, if the tissue removal process for both forms is the same, it is not the action of an aminopeptidase. Another example is afforded by experiments on homogenized lung tissue (Bakhle, 1968). Despite the fact that angiotensin II passes through the lungs *in vivo* without loss, homogenized lung tissue rapidly destroys angiotensin II, suggesting that peptidases which inactivate angiotensin II have been released from inside cells by the homogenization. These examples show that great caution is necessary in interpreting the results of enzyme activities demonstrated *in vitro*. Studies of enzymes in blood- or saline-perfused whole organs are needed to reinforce or invalidate such interpretations. For instance, converting enzyme activity cannot be detected in blood-perfused (Ng & Vane, 1968b) or saline-perfused (Bakhle, Reynard & Vane, unpublished) organs. Even so, the presence of "converting enzyme" activity in homogenized tissues has been used to substantiate the conclusion (Biron & Higgins, 1968) that appreciable conversion of angiotensin I to II takes place *in vivo* in peripheral vascular beds.

Release of other peptides by renin. Fasciolo, Halvorsen, Itoiz & Paladini (1958) showed that when renin was incubated with plasma protein *in vitro*, not only angiotensin but also a potent vasodilator substance similar to bradykinin was generated. The extracted incubation mixture caused a rise in blood pressure when given intravenously, but a fall in pressure when given intra-arterially. Ng (1969) has now shown that this paradoxical effect was probably due to the simultaneous generation of both angiotensin I and a kinin. Renin, prepared by the method of Brown, Davies,

Lever, Robertson & Tree (1964), was infused into a stream of blood and 10–15 sec contact was sufficient to generate a substance which strongly contracted the cat jejunum. The contraction was potentiated by " bradykinin potentiating factor " (Ferreira, 1965), a substance which inhibits bradykininase in blood (Ferreira & Vane, 1967a). The effect was not due to contamination of the renin extract with kininogenase or plasmin.

A simultaneous generation of angiotensin I and a kinin by renin extracts explains well the differences which Fasciolo *et al.* (1958) observed between an intravenous injection of the incubation mixture (which gave vasoconstriction) and an intra-arterial injection (which gave vasodilatation). The intravenous route would give rise to vasoconstriction because of the predominance of the effects of angiotensin II derived from angiotensin I together with the simultaneous removal of the kinin in the lungs. The intra-arterial injection would allow the vasodilator effect of the kinin to predominate over the weak pressor effects of angiotensin I.

The generation of a kinin by renin extracts also explains the findings of Van Armen (1955), who pre-treated plasma with renin and was then unable to release bradykinin with trypsin, and those of Feldberg & Lewis (1965), who found that injected renin made the cat adrenal gland refractory to the catecholamine-releasing effects not only of angiotensin but also of bradykinin.

(c) Vasopressin

Release. The rôle of osmotic pressure of extracellular fluid in the control of vasopressin release was shown by Verney (1947). Jewell & Verney (1957) located the osmoreceptors to a region of the anterior hypothalamus. Other stimuli for the release of vasopressin include emotional stress, reduction in blood volume and hypotension (see Ginsburg, 1968). After vagotomy, which in itself may cause release of vasopressin, occlusion of the carotid arteries causes a further release (see Share, 1967). Thus many of the stimuli which induce secretion of renin also induce secretion of vasopressin.

The blood-bathed rabbit rectum is relaxed by vasopressin in low concentrations. Oxytocin is only 1/30th as potent and the relaxant effects of catecholamines can be abolished by intraluminal perfusion with pronethalol, making the tissue relatively

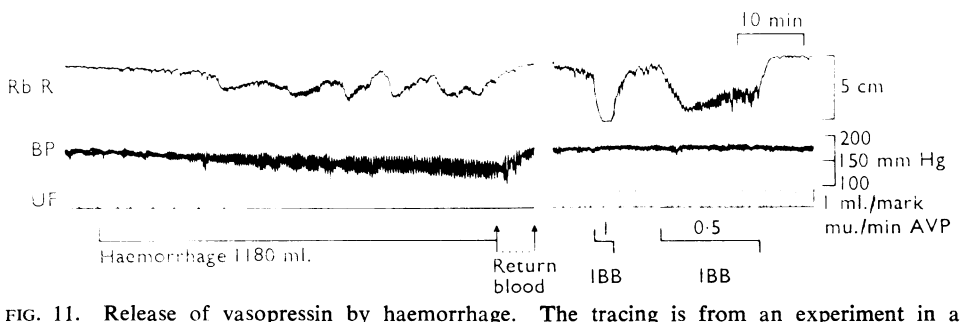

FIG. 11. Release of vasopressin by haemorrhage. The tracing is from an experiment in a 26 kg male dog. A rabbit rectum (RbR) was superfused at 10 ml./min with arterial blood; mean blood pressure (BP) and urine flow from both ureters (UF) are also shown. The animal was bled at 20 ml./min for 59 min, during which there was a gradual fall in blood pressure. The rabbit rectum showed periodic relaxations during the haemorrhage, sometimes reaching the equivalent of an increased vasopressin concentration in the blood of 0.05 mu./ml., as shown by the calibrating infusions directly into the blood (IBB) bathing the rabbit rectum. Time, 10 min: vertical scales 5 cm and mm Hg. (From Gilmore, 1968.)

specific for vasopressin. Figure 11 illustrates an experiment in which vasopressin was released by haemorrhage. An interesting feature of the release was its phasic nature.

Fate. Vasopressin is stable in blood and passes through the pulmonary circulation without loss (Gilmore, 1968). The half-life of vasopressin in the circulation of the dog is quoted at 5–7 min (Lauson & Bocanegra, 1961 ; Share, 1962 ; Czaczkes & Kleeman, 1964). These figures were determined after intravenous infusions of vasopressin lasting for 2–3 hr and may be complicated by a leakage back into the plasma of vasopressin which had saturated the extra-vascular fluid.

We have infused vasopressin into the ascending aorta with the coaxial catheter described earlier and measured the amount of vasopressin removed in one circulation through the total peripheral vascular beds. The infusions were given for 5–10 min and the average removal was 30%. With this technique, the half-life of vasopressin in the circulation is therefore less than 3 circulation times, or about 1 min. The main site for removal of vasopressin from the circulation is the kidney and it has been calculated that 50–70% of the disappearance is due to inactivation or excretion in this organ (see Ginsburg, 1968).

Prostaglandins

Release. Prostaglandins may be released into the circulation under a variety of conditions. When nerves are stimulated one or more of the prostaglandins are released from such diverse sites as the cerebellum (Coceani & Wolfe, 1965), the cerebral cortex (Ramwell & Shaw, 1966), the spinal cord (Ramwell, Shaw & Jessup, 1966), the adrenal glands (Ramwell, Shaw, Douglas & Poisner, 1966), adipose tissue (Shaw, 1966), diaphragm (Ramwell, Shaw & Kucharski, 1965), stomach (Bennett, Friedmann & Vane, 1967 ; Coceani, Pace-Asciak, Volta & Wolfe, 1967) and the spleen (Davies, Horton & Withrington, 1967, 1968 ; Ferreira & Vane, 1967d ; Gil-

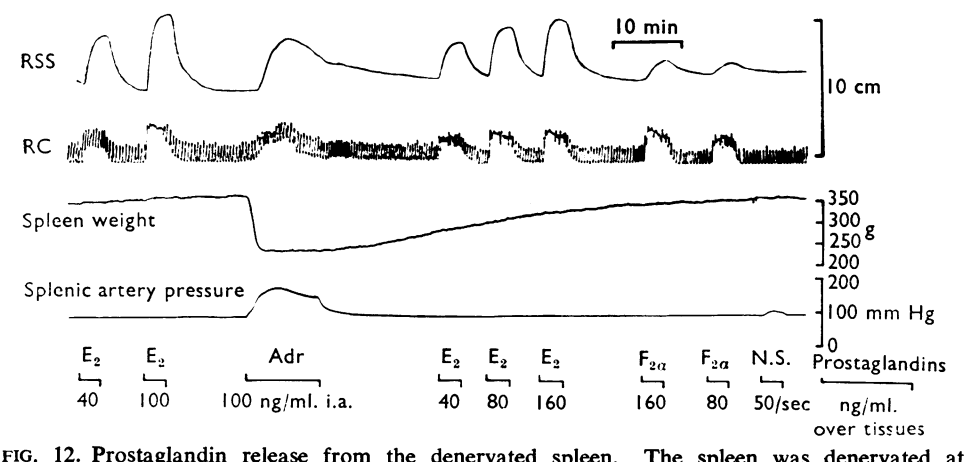

FIG. 12. Prostaglandin release from the denervated spleen. The spleen was denervated at aseptic surgical operation 9 days before the experiment. It was then perfused with Krebs-dextran solution as described in Gilmore, Vane & Wyllie (1968). Infusion of adrenaline (100 ng/ml. intra-arterially for 10 min) caused a contraction of the spleen and an output of prostaglandin indicated by contractions of a rat stomach strip (RSS) and a rat colon (RC) superfused by the effluent from the spleen. Stimulation of the degenerate splenic nerves (together with the splenic artery) at 50 shocks/sec (0.5 msec duration and 40V) for 3 min produced only a slight rise in arterial pressure. Time scale 10 min: vertical scales, 10 cm, spleen weight in grammes and splenic artery pressure in mm Hg. (From Gilmore, Vane & Wyllie, 1968.)

more, Vane & Wyllie, 1968). Although many of these experiments were with isolated tissues, it is reasonable to assume that *in vivo* the prostaglandin would find its way into the venous effluent. Certainly as far as the spleen is concerned, the concentration of prostaglandins (assayed as prostaglandin E_2) in splenic venous blood after nerve stimulation can be as high as 200 ng/ml. After stimulation of the spleen either by the nerves or by adrenaline, both prostaglandin E_2 and $F_{2\alpha}$ emerge in the venous effluent (Gilmore, Vane & Wyllie, 1968). The released prostaglandins did not come from nerve fibres because contraction of the spleen caused by adrenaline was accompanied by a release of prostaglandin. Furthermore, chronic denervation of the spleen did not prevent release of prostaglandins by adrenaline infusion (Fig. 12). Antagonists of α-receptors for adrenaline prevented splenic contraction and also prevented release of prostaglandin in response to nerve stimulation or to catecholamine infusion (Davies, Horton & Withrington, 1967, 1968; Ferreira & Vane, 1967d). Thus, prostaglandin release was clearly associated with splenic contraction. Since the spleen expels blood when it contracts, the prostaglandins might be derived from decaying blood cells or from the plasma trapped in the splenic sinusoids. Repeated stimulation of the Krebs perfused spleen eventually cleared the blood from the sinusoids but there was still an output of prostaglandin with each stimulation; thus the prostaglandin did not arise either from blood cells or from plasma stored in the sinusoids.

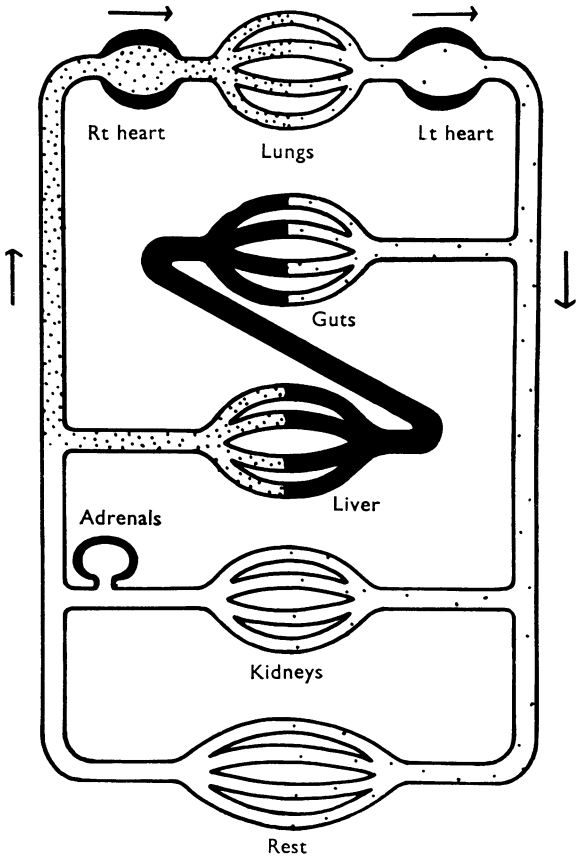

FIG. 13. Fate of prostaglandins in the circulation. The liver removes about 90% and so does the pulmonary circulation, leaving little or none to reach the arterial circulation.

The prostaglandin might have come from contracting smooth muscle cells; if so, it was probably the trabecular and not the vascular smooth muscle, because infusions of vasopressin which produced rises in perfusion pressure of up to 40 mm Hg did not release prostaglandins. We have recently also been able to cause prostaglandin release from the spleen without an associated contraction (Gilmore, Vane & Wyllie, 1969). The spleen contains an abundance of reticulo-endothelial cells and we wondered whether these could be a source of synthesis and release of prostaglandins. Infusions of various colloids, including insulin zinc suspension, iron dextran and saccharated iron oxide all released a mixture of prostaglandins E_2 and $F_{2\alpha}$ from dog isolated perfused spleens. Thus the process of phagocytosis may also release prostaglandins into the circulation.

It has been known for many years that anaphylaxis leads to the release of several vaso-active hormones (see Collier, 1968). When isolated lungs from sensitized guinea-pigs are challenged the release of histamine (Bartosch, Feldberg & Nagel, 1933), slow reacting substance in anaphylaxis (SRS-A) (Kellaway & Trethewie, 1940) and kallikrein (Jonasson & Becker, 1966) has been detected. We have now found that prostaglandins are also released (Piper & Vane, 1969). Lungs isolated from sensitized guinea-pigs were perfused via the pulmonary artery with Krebs solution at 37° C and the prostaglandins in the effluent were assayed by isolated organs both by immediate superfusion and after extraction of the acidified effluent with ethylacetate (Gilmore, Vane & Wyllie, 1968). These procedures, together with thin-layer chromatography, showed that prostaglandins E_2 and $F_{2\alpha}$ (up to 20 ng/ml.) were released into the perfusate for 10–20 min after the lungs were challenged. A similar release of prostaglandins could be induced by injections of SRS-A into unsensitized lungs. Thus prostaglandin release in anaphylaxis may in part be mediated by the initial release of SRS-A.

Fate. Since they have strong actions on vascular and intestinal smooth muscle, it is important to know what happens to prostaglandins which appear in the circulation. In cats, dogs and rabbits, prostaglandins E_1, E_2 and $F_{2\alpha}$ are stable in blood but the liver removes more than 80% of an infusion and the lungs remove more than 95% (Ferreira & Vane, 1967d). Thus little of the prostaglandins liberated from intestine or spleen will pass the liver and less than 5% of that left will pass the pulmonary circulation. In the ewe (Horton, Main & Thompson, 1965) as well as in dogs (Carlson & Orö, 1966), cats and rabbits (Ferreira & Vane, 1967d) the hypotensive effects of prostaglandins are greater with intra-aortic than with intravenous injections. The removal of prostaglandins in the pulmonary circulation therefore takes place in several species. An enzyme which metabolizes prostaglandins can be extracted from the lungs of many species (Anggård & Samuelsson, 1964; 1966; 1967) suggesting that the pulmonary removal of prostaglandins may be by enzymic inactivation. Figure 13 shows the fate of prostaglandins in the circulation. It can be concluded, as for acetylcholine, 5-HT and bradykinin, that the body has a very efficient mechanism to prevent prostaglandins from reaching the arterial circulation. The prostaglandins released during the anaphylactic response of lungs (Piper & Vane, 1969) will, however, reach the arterial circulation and this may be one situation in which they have a potent cardiovascular effect.

Conclusions

(1) Different vaso-active hormones are removed from the circulation in different ways (Fig. 14). Contrary to popular belief, blood is relatively ineffective as an

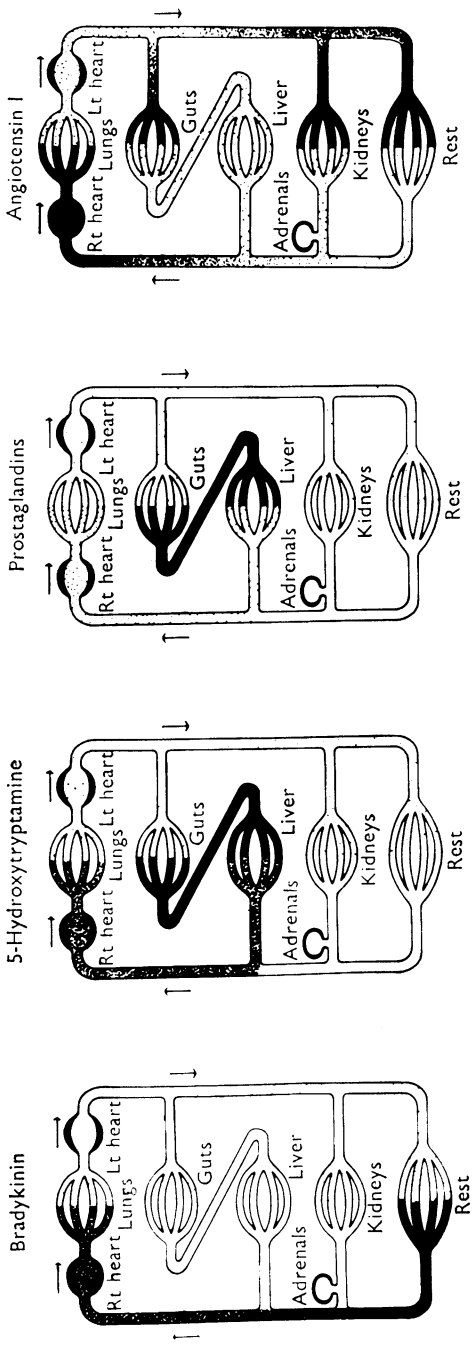

FIG. 14. Different patterns formed by the release, activation and inactivation of hormones in the circulation.

inactivating agent, except for acetylcholine. The lungs are most important for removal of 5-HT, angiotensin I, bradykinin and the prostaglandins. The lungs probably remove 5-HT by uptake and storage in septal macrophages. Uptake of many other basic drugs (see Vane, 1968b) may also be a facet of the same process. The removal process shows high affinity for 5-HT, but noradrenaline is only partially removed and adrenaline and histamine are not removed at all. If storage sites in the lungs are available to different bases the ones with a high affinity might displace others of lower affinity. Since affinity for the storage site may not parallel pharmacological activity a relatively inactive substance may displace a much more active substance directly into the pulmonary venous blood and thence into the arterial circulation. The release of histamine into the blood perfusing isolated lungs by injections of 5-HT (Moore, Normell & Eiseman, 1963) may be an example for such an action.

(2) The pulmonary circulation inactivates peptides by enzymic action. The enzyme(s) cleave bradykinin and angiotensin I but do not attack eledoisin, polesteskinin, vasopressin or angiotensin II. The peptidase in the lungs which inactivates bradykinin is presumably different from the peptidase which converts angiotensin I to angiotensin II. The location of the latter in the lungs offers a new area for pharmacological attack on the renin-angiotensin system.

(3) The pulmonary removal process for prostaglandins is probably also enzymic, although physiological evidence for this is lacking.

(4) Since 5-HT, angiotensin I, bradykinin and prostaglandins disappear in the pulmonary circulation, they are unlikely to reach target organs through the arterial circulation. However, the inactivation mechanism of the lungs may sometimes be by-passed or overwhelmed. For instance, there may be a continuous release of bradykinin by a circulating enzyme and of 5-HT from platelets or from the lungs; angiotensin I may also be released on the arterial side of the circulation by renin. The release of substances by the lungs themselves is also an important consideration. These results lead to the suggestion that defects in the protective function of the pulmonary circulation may lead to disease states.

(5) The efficiency of the various metabolic processes associated with the pulmonary circulation suggests that vaso-active hormones can be divided into at least two types—" local " and " circulating " hormones. (*a*) The local hormones (bradykinin, 5-HT and prostaglandins) are those which are effectively removed by the lungs and if they have a physiological function, it is probably localized at or near to the site of release. It is intriguing to think that venous blood may be full of noxious, as yet unidentified, chemicals released from peripheral vascular beds, but removed by the lungs before they can cause effects in the arterial circulation. One such substance has already been described (Hall & Sackner, 1966), although the authors did not seem to have considered that the unidentified substance may be a prostaglandin. (*b*) The circulating hormones are those which pass through the lungs either unchanged (adrenaline, histamine, vasopressin) or with an actual increase in activity (angiotensin I to II).

(6) The liver is less efficient than the lungs in removing the vaso-active hormones studied. This, coupled with the fact that the portal circulation receives only part of the cardiac output, suggests that the liver is of secondary importance in the inactivation of many vaso-active substances. The lungs, therefore, may well be a more useful source of inactivating enzymes than the liver and possibly than the kidneys or blood. This would be especially true if enzymes isolated, for instance from

blood, have different specificities from those in the lungs, even though they may share one or two substrates.

(7) Peripheral vascular beds remove circulating hormones from the blood. Thus, catecholamines, angiotensin I and II all disappear as they pass through the peripheral vascular beds. The inactivation of these substances may be by enzymic breakdown or by some other process.

(8) The selective removal of some substances by the pulmonary circulation and of others by the peripheral vascular beds means that venous and arterial concentrations of any one substance will be substantially different. For example, the concentration of noradrenaline in peripheral venous blood will give little indication of the concentration in arterial blood, since it will be the sum of the small fraction of the arterial concentration which passes through the peripheral vascular beds added to that which may be escaping from nerve endings. Indeed, even the concentration of noradrenaline in central venous blood (which includes the adrenal medullary secretion) will be different from that in arterial blood because of uptake in the lungs. This example illustrates the importance of stating which blood concentration is being measured and of choosing a site for sampling blood which will have some relevance to the problem under investigation.

(9) Several examples have arisen which illustrate that the isolation of an activating or inactivating enzyme from a particular tissue or fluid does not necessarily mean that the enzyme has that function *in vivo*. Monoamine oxidase isolated from various tissues will destroy 5-HT, but the immediate process of inactivation of 5-HT *in vivo* is probably one of uptake and storage in the lungs. The enzymes in blood which convert angiotensin I to II and which destroy angiotensin II do not seem to be of physiological importance. Similarly, the enzymes which are released when lungs are homogenized and which inactivate angiotensin II and histamine probably have little importance in the removal of these substances from the circulation *in vivo*.

(10) Many of the naturally occurring substances which have been discussed are active on venous vasculature, on pulmonary vasculature and on airways resistance. Another interpretation of the results is that those substances which are inactivated by the lungs have a selective hormonal function on the smooth muscle in the venous or pulmonary circulation. The general description of a substance as a hormone may therefore have to be further qualified to " local " hormone, " venous " hormone, " pulmonary " hormone or " arterial " hormone, depending on the site of their release or formation and on the target organ.

I would like to thank all my colleagues who made the work described here possible. I would also like to thank the Athlone Press for permission to reproduce Figs. 3, 4, 6, 8 and 13, which first appeared in Vane (1968a) and Macmillan (Journals) Ltd. for permission to reproduce Figs. 5, 9 and 12.

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