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VASOCONSTRICTOR ACTIVITY IN THE RABBIT'S BLOOD AND PLASMA

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The following observations were undertaken primarily to obtain further information about the vasoconstrictor properties acquired by the circulating blood as the result of haemorrhage. They have led to a considerable clarification of this problem and to other results of interest. The methods devised seem to have many other applications.

In such work, a main difficulty is the ease and rapidity with which changes occur in shed blood, leading to the formation or release of vaso-active substances. This makes it uncertain that a constrictor substance detected in, or recovered from, shed blood or its products was actually free in the circulating blood. A further difficulty is to relate the concentration of the constrictor substance detected in the shed blood to the vascular state of the animal from which it was withdrawn. Both these difficulties are in large part overcome by using rabbits as both blood donor and pharmacological test object and by observing certain precautions in the handling of blood.

In the donor animal, the vessels are readily observable in the depilated ears. While the calibre of the vessels in a normal ear is determined mainly by the activity of the sympathetic nerves, that of the vessels in a chronically denervated ear depends mainly on the vaso-active properties of the circulating blood. In general, a greater or less constriction of the denervated ear vessels indicates a greater or less concentration of a circulating constrictor substance. But observation of the donor's ear vessels alone permits neither the estimation of the concentration of the circulating substance in terms of a known standard nor the assessment of how far the vascular state is influenced by changes in blood pressure. We have therefore devised a method for rapidly transferring at will, from any available vessel of the donor, a small volume of blood (0·1 ml.), through a system of non-wettable tubes and taps, to the central artery of the denervated ear of a test rabbit. Our hope that the rapidly transferred blood

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would be but little changed in vaso-active properties was in fact realized. In general, when the donor's denervated vessels are relaxed, the transferred blood causes little or no constriction of the test artery; when they are more or less constricted, the transferred blood provokes a correspondingly greater or less constriction of the test artery. In the same preparation, larger quantities of blood can be withdrawn from the donor and the separated plasma injected into the test artery. The constrictor activities of blood and plasma can thus be compared and assayed in terms of adrenaline or other standard.

METHODS

Animals and their preparation

Rabbits with half or three-quarter lop ears were used mainly; albinos for preference. The ear vessels are considerably larger than those of ordinary rabbits; this facilitates the introduction of catheters.

The rabbits are prepared as described by Armin & Grant (1953). Anaesthetized animals are kept warm and their rectal temperatures controlled.

Catheterization

For the withdrawal of blood a polythene catheter is inserted, under local anaesthesia, into the artery or vein most suitable for the experiment in view. To insert the catheter into an ear vein, it is not usually necessary to adopt the technique described by Armin, Grant, Pels & Reeve (1952, appendix) for short-eared rabbits. It suffices to pierce the marginal vein with a hypodermic needle and, after withdrawing the needle, to insert the catheter through the hole in the vein. Bleeding, controlled with the finger, is negligible. The catheter can often be passed from the ear to the heart and sometimes to the posterior vena cava. In some animals bifurcation or narrowing of the marginal vein at the base of the ear prevents the passage of the catheter.

It is important that blood should flow freely from the catheter. Therefore this should be as wide and as short as is feasible. For venous bleeding the tip is prepared as described by Armin *et al.* (1952, appendix); for arterial bleeding, a bevelled, unblocked tip is required. If more than a small volume of blood is to be withdrawn quickly, the catheter tip must lie in a large vessel.

When blood is to be withdrawn for the separation of plasma from an unanaesthetized rabbit, it is important, for reasons given later, that catheterization should be carried out smoothly without causing the animal to struggle. To secure this it is sometimes necessary to quieten a lively rabbit with a small dose of pentobarbitone sodium (Nembutal, about 0·25 ml./kg). An hour is then allowed to elapse so that the effects of the anaesthetic pass off before proceeding with the observations.

Apparatus

Fig. 1 shows the arrangement of polythene tubes, Perspex taps and syringes used. The apparatus was made in the laboratory by Mr J. Benson.

Tubing. All tubing is polythene; a suitable variety is that sold as 'Polyethylene' by Clay Adams and Co. Inc., New York. An end of this tubing held near a small flame forms a regular flare which makes a leak-proof junction with the tap nozzle (Fig. 3). The samples of polythene tubing made in this country that we have tested do not form a flare but a blob.

Tube *C*1 going to the test ear is as described by Armin & Grant (1953). The other tubes are wider, internal diameter 0·58 mm, external 0·965 mm (Clay Adams and Co., no. PE 50). The length of *C*2 is such that it contains a measured volume of 0·1 ml. (approximately 37 cm). *C*7 is short so that the needle of syringe *S*5 inserted into it reaches the tap. The length of the others is as short as is feasible. *C*6 is the catheter for insertion into the donor. The ends of the tubes to be joined to the taps are threaded through cap nuts and flared. To connect taps and syringes (except *S*5) short pieces of polythene tubing are fitted with cap nuts and flared at each end. One end is

screwed to the tap nozzle. The other end is screwed to a Perspex union piece (Fig. 2) which is fitted with a short piece of wider polythene tubing. The syringe nozzle fits snugly into this wider tube.

Taps. The small taps and their connectors are of Perspex (Fig. 3). Tap 1 is 4-way, the passages of the barrel being arranged so that any two adjacent nozzles can be connected. Taps *T*2 to *T*5 are of the usual 3-way type. The taps are mounted on Perspex bases for clamping to supports.

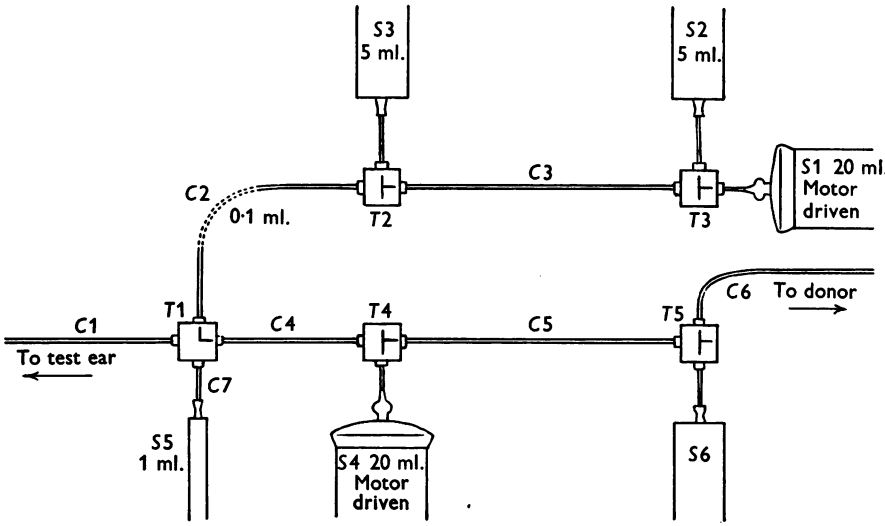

Fig. 1. Diagram (not to scale) of tubes (*C*), taps (*T*) and syringes (*S*) for transferring 0·1 ml. blood from donor to test ear and for bleeding donor. For description see text.

Syringes. Syringes *S*1 to *S*5 are glass, lubricated with silicone (M.S. 200/1000 CS; Hopkin and Williams, Ltd.); *S*1 and *S*4, 20 ml. capacity and motor driven (see Benson, appendix B in Armin & Grant, 1953); *S*2 and *S*3, 5 ml. and *S*5, 1 ml. capacity. Syringes *S*2 and 3 are arranged vertically. For bleeding, blood is withdrawn into 20 ml. glass syringes containing heparin and lubricated with silicone. When plasma is required, blood is withdrawn into a specially made Perspex syringe (4 ml. capacity) which also acts as a centrifuge tube (Fig. 4). A screw thread is cut in the base of the nozzle; a Perspex cap with a plastic washer in its base screws on to and seals the nozzle. The piston is pierced by a longitudinal hole into which is inserted a brass rod with a Perspex tip which seals the lower end of the hole. The syringe is lubricated with silicone and contains heparin (0·1 ml., 50 units heparin). The centrifuge bucket (3·5 × 9·25 cm) for use with the syringe contains a centrally-placed thin perforated metal sleeve into which the syringe fits snugly. The space between the sleeve and the wall of the bucket is filled with crushed ice. The prepared syringe and bucket are refrigerated (0–4° C) until required.

Preparation of solutions and apparatus

The NaCl, adrenaline and other solutions used are freshly prepared as described by Armin & Grant (1953). All apparatus must be clean. The Perspex taps should be dismantled and examined from time to time; the polythene tubing requires frequent renewal since deposits are apt to form from the blood.

The apparatus in use

The system of syringes, tubes and taps shown in Fig. 1 is filled with 0·9% (w/v) NaCl solution containing heparin (25 units/1 ml.), care being taken to exclude air bubbles and leaks. The free

end of the catheter $C6$ is inserted into the donor and a slow flow of saline (1–2 ml./hr) is started from $S4$ through $T4$, $C5$, $T5$ and $C6$. The needle attached to the free end of $C1$ is inserted into the central artery of the test ear and a slow flow of saline begun from syringe $S1$ through $T3$, $C3$, $T2$, $C2$, $T1$ and $C1$. The saline in $C4$, being out of either circuit, is stationary. The test ear is

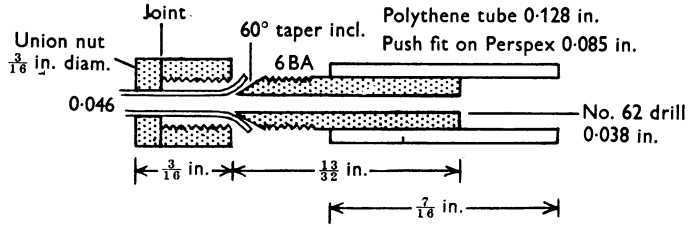

Fig. 2. Diagram of Perspex union piece; scale ×2·5.

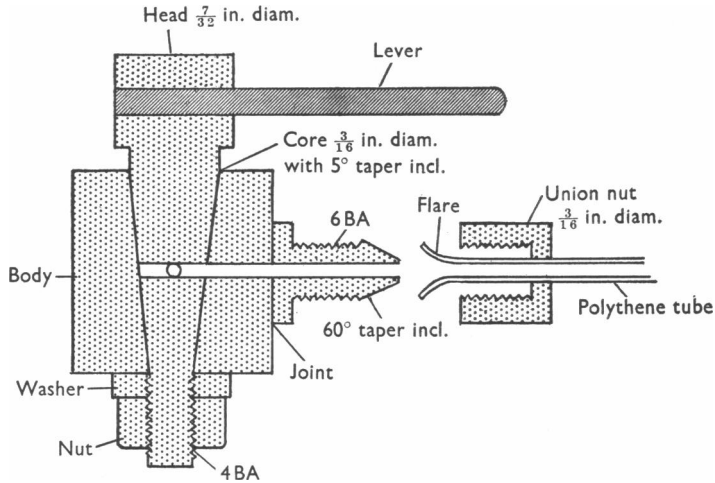

Fig. 3. Diagram of Perspex tap; scale ×2·5. All Perspex except washer, nut and lever.

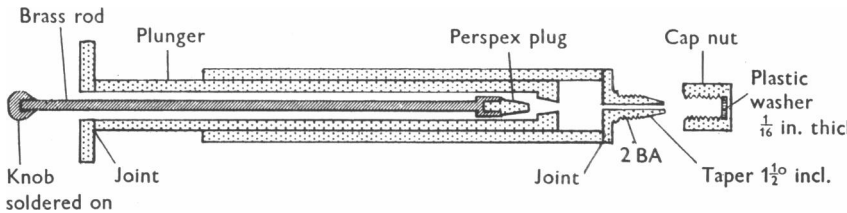

Fig. 4. Diagram of Perspex combined syringe and centrifuge tube; scale ×0·5.

arranged and observed microscopically as described by Armin & Grant (1953). Before transferring blood to the test ear, it is advisable to test the vasoconstrictor effect of saline alone.

The method of transferring blood is best followed from Fig. 1. An assistant first draws blood from the donor through the various tubes by the suction of syringe $S2$. This involves turning the taps

in the following order: $T1$, to connect $C4$ and $C2$, $T3$ to connect $C3$ and $S2$, $T4$ to connect $C4$ and $C5$. He then withdraws the piston of $S2$ and blood passes from the donor by $C6$, $T5$, $C5$, $T4$, $C4$, $T1$, $C2$, $T2$ and $C3$. As soon as blood unmixed with saline enters $C3$, withdrawal is stopped. The system is then arranged so that the 0·1 ml. blood in $C2$ can be injected into the test ear by means of syringe $S3$. The assistant turns $T1$, to connect $C1$ and $C2$, and $T2$ to connect $S3$ and $C2$. By pressing on the piston of $S3$ he injects the 0·1 ml. blood in $C2$ into the central artery of the test ear while this is obstructed centrally by the observer's finger. The observer sees saline followed by blood enter the artery. As soon as saline begins to follow the blood, the injection is stopped and the observer removes his finger. He watches the reaction of the test artery. As soon as injection is completed, the assistant turns $T1$ to connect $C4$ and $C2$, $T2$ to connect $C2$ and $C3$. By pressing on the piston of $S2$ he washes back into the donor the blood remaining in $C3$ and $C4$, $C5$ and $C6$. He then turns $T1$ and 3 to restore the saline flow from $S1$ to $C1$ and $T4$ to restore the flow from $S4$ to the donor.

The transfer of blood occupies less than 15 sec; 5 sec to withdraw, less than 5 sec to adjust the taps and 5 sec to inject the blood. Another 10–15 sec is required to wash out the blood and restore the saline flows. This timing should be repeated with each transfer. It is not necessary to stop the motor syringes during this brief period. During transfer, blood should not be allowed to enter syringes $S2$ and $S3$; this is liable to happen when $C6$ is in an artery and pressure is high. It is important that blood should not be allowed to enter and remain in the catheter $C6$ as is liable to happen when the catheter is in an artery and blood pressure varies. Blood lying in the catheter acquires a strong vasoconstrictor property. To ensure freedom from blood before an arterial sample is withdrawn, the catheter is flushed with saline.

To separate plasma, tap $T5$ is turned to connect $C6$ to $S6$ and 4 ml. blood is withdrawn into the chilled Perspex syringe. This occupies 10–15 sec for arterial and 15–30 sec for venous bleeding. The cap is then replaced, and withdrawal of the brass rod allows the piston to be removed without disturbing the blood. The syringe, now a centrifuge tube, is slipped into the bucket, quickly counterpoised and spun at 3500–4000 rev/min (radius 16 cm) for 5 min. Immediately after spinning, the plasma is pipetted off (siliconed Pasteur pipette) into a siliconed glass-stoppered tube and kept in crushed ice in a Dewar flask. Immediately after the withdrawal of blood from the donor, an assistant washes the blood from the tubes and tap and restores the saline flow. Plasma (about 2 ml.) is ready for test within 10 min of withdrawing blood.

To compare the activity of plasma with that of blood, 0·1 ml. of plasma is injected into the test artery from syringe $S5$ through $C7$, $T1$ and $C1$, the injection being made in the standard time of 5 sec. Constrictor activity of blood and plasma is assayed in terms of adrenaline or other constrictor substance injected into the test ear in the same way.

In comparing the constrictor activity of blood or plasma with that of adrenaline, an important point is the quality of the adrenaline used. At one time we became aware over a period of several months that the response of the test arteries to adrenaline gradually diminished. On comparing the stock adrenaline powder with a newly purchased sample, it was just detectably darker in colour and on testing solutions of the two powders on the denervated artery and the rat's uterus, the old sample was between 10 and 100 times less active than the new. The old sample had been in the laboratory for about a year, kept stoppered and in the dark. We have taken the precaution of frequently renewing the stock.

Adrenalectomy

The adrenal glands are removed with aseptic precautions by a one-stage operation (under Nembutal and local procaine) through flank incisions. The left gland is usually easily dissected out, ligated and removed. Occasionally it is attached to the left renal vein. The right gland is firmly attached to the posterior vena cava, and cannot be dissected free without tearing the caval wall. It is freed so far as possible by dissection round the margin and removed piecemeal. Remnants on the cava are cauterized. Sometimes the gland is crossed by a lumbar vein and for exposure of the gland this vein is double ligatured and cut. At operation, 10 mg cortisone (Cortisone acetate, Merck) is injected intramuscularly, and daily thereafter, 5 mg DOCA in oil. In each animal the

completeness of removal was ultimately checked at necropsy and a search was made for accessory adrenal bodies. In only one instance a small accessory adrenal was found on the posterior wall of the vena cava near the site of the excised adrenal gland.

The venous drainage of the adrenal glands varies. The right drains directly into the vena cava and often also into a lumbar vein. The left drains usually into a lumbar vein. The lumbar veins may open directly into the vena cava or the renal veins.

The response of test artery

The denervated ear artery is capable of detecting constrictor substances in high dilution (Armin & Grant, 1953) but, so far as our observations go, does not serve to identify them. The associated reactions of the minute vessels, however, provide useful indications. Adrenaline and noradrenaline are indistinguishable. In equal concentrations they cause arterial constrictions approximately equal in degree and duration. They also constrict the minute vessels. With concentrations of 10^{-9} and greater, this constriction is apparent to the naked eye as a paling of the ground tone in the injected area. 5-Hydroxytryptamine (5 HT) is approximately 5 times less effective (some variation in different samples) than adrenaline as an arterial constrictor but has little or no constrictor effect on the minute vessels. Histamine is 10 to 100 times less effective than adrenaline as an arterial constrictor, dilates the minute vessels and is liable to cause the artery to oscillate in diameter and to provoke oedema in the injected area. We have not so far found inhibitors which, when given in single intra-arterial injections of 0·1 ml. are specific for any of these substances. Procaine 10^{-3} potentiates not only adrenaline and noradrenaline but also 5 HT; mepyramine 10^{-3} and chlorpromazine (Largactil, 10^{-3}, May & Baker) inhibit adrenaline, noradrenaline, histamine and 5 HT. Dibenamine (Smith, Kline & French) and Piperoxane (933F, Fourneau) do not greatly inhibit the constriction to adrenaline. Lysergic acid diethylamide 10^{-6} (Sandoz) causes a long-lasting constriction of the artery; after this has passed off, the constrictor effect of 5 HT is not less than before that injection.

In most experiments we have first tested the response of the artery to injections of adrenaline in concentrations differing by a factor of 10, to provide an index for the adrenaline equivalent of subsequent injections of blood and plasma. In some instances a closer estimate of the adrenaline equivalent was made. Frequently, and particularly in experiments involving bleeding, injections of adrenaline have been repeated after transfers of blood or plasma. According to Shorr, Zweifach, Furchgott & Baez (1951) the vaso-excitor material released into the blood by haemorrhage is not itself a constrictor but potentiates the constrictor effect of adrenaline on the vessels of the rat's meso-appendix. We have not found that repeated injections of blood or plasma from the bled rabbit alter the responses of the test artery to subsequent injections of adrenaline.

In previous papers (Armin & Grant, 1953; Armin, Grant, Thompson & Tickner, 1953) the response of the test artery has been represented in the figures by a vertical line showing the duration of the reaction, headed by a fraction showing the initial diameter of the artery and its maximal change. Since it may be accepted that longer duration is usually associated with greater constriction, the fraction is now omitted to simplify the figures. For the drawing of the figures we are indebted to Miss S. Treadgold, Medical Illustrator at Guy's Hospital.

RESULTS

Preliminary observations to test the methods

Effect of blood transferred from various vessels

In the warm, resting, unanaesthetized or anaesthetized donor, whose normal and denervated ear vessels are relaxed, blood transferred from an artery, the right heart, jugular, iliac or femoral veins, anterior and lower posterior venae cavae, has only a slight and transient constrictor effect on the test artery, the

reaction passing off usually in 10–15 sec. Sometimes it may last as long as 20–25 sec and sometimes it has no appreciable effect, the artery quickly returning to, and remaining at, its initial diameter when the observer removes his finger. The reaction is usually indistinguishable from that to saline and regularly less than that to adrenaline 10^{-11}. Many successive transfers can be made with but little variation in the effect. Occasionally, as with adrenaline (Armin & Grant, 1953) larger variations occur, usually explicable by faulty technique.

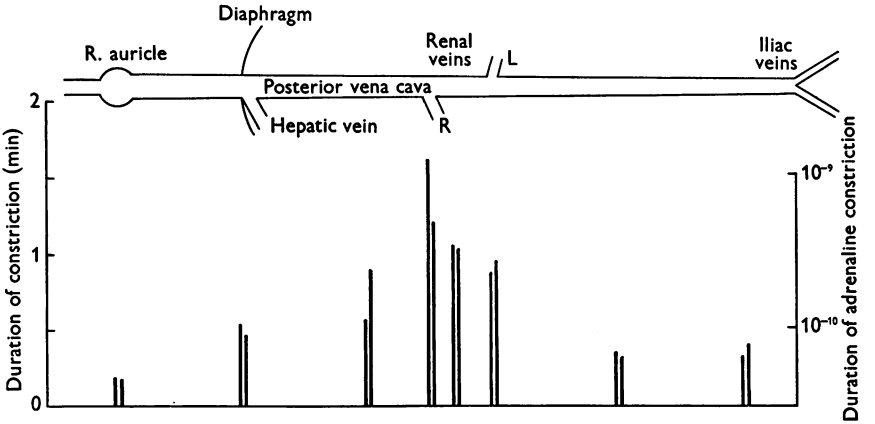

Fig. 5. Responses of test artery to blood transferred from posterior vena cava when catheter tip at sites shown. Responses to adrenaline 10^{-10} and 10^{-9} shown on right of figure.

When, in the anaesthetized animal, the catheter tip lies in the posterior vena cava, below the diaphragm but headwards of the entry of the renal veins, the constrictor activity of the transferred blood is greater and longer lasting, the adrenaline equivalent ranging from about 10^{-10} to 10^{-9}. Fig. 5 illustrates the findings in one of a series of six experiments. A marked catheter was inserted into a femoral vein, passed to the right auricle and subsequently withdrawn and re-inserted in steps. The observer of the test artery was unaware of the position of the catheter. At each step two blood samples were transferred. The rabbit was killed at the end of the experiment and the site of the catheter tip determined for each step. The greatest constrictor effect was provoked by blood withdrawn from close to the entry of the renal veins. In this instance it is nearly matched by adrenaline 10^{-9}.

The constrictor effect of caval blood is like that of adrenaline in that it constricts the minute vessels as well as the artery of the test ear; the arterial constriction is potentiated by procaine (Fig. 6) and inhibited by Largactil (Fig. 7). These tests, however, are not specific for adrenaline. But in rabbits deprived of both adrenal glands 3–5 days previously, caval blood has no greater effect than that from the auricle or iliac veins.

These findings strongly suggest that the constrictor effect of caval blood is due to adrenaline (perhaps also noradrenaline) entering the blood stream from the adrenal glands. It is presumably so diluted in its passage up the cava that it is undetectable in blood from the auricle. This secretion of adrenaline is independent of splanchnic nerve activity. In two rabbits in which both splanchnic nerves were cut (confirmed by necropsy) 7 and 14 days previously, the constrictor activity of caval blood was of the same order as in intact animals.

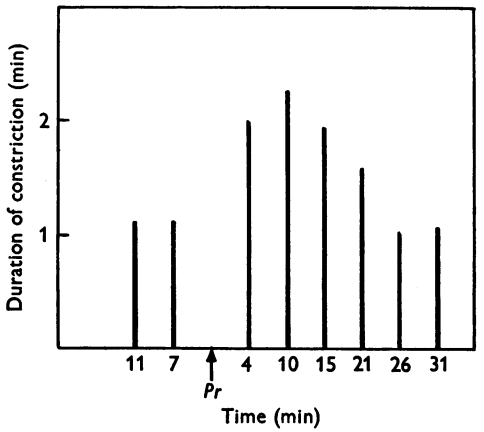

Fig. 6

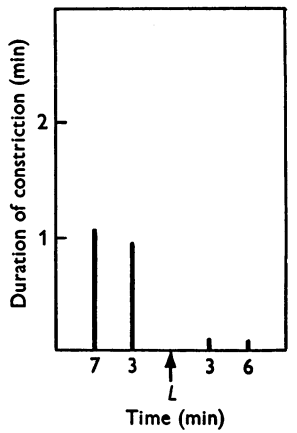

Fig. 7

Figs. 6 and 7. Responses of test arteries to transferred posterior caval blood (between diaphragm and right renal vein) at the minutes shown, before and after injection into the test artery of 0·1 ml. procaine 10^{-6} (*Pr*) Fig. 6; Largactil 10^{-3} (*L*) Fig. 7.

It is clear from these observations that arterial and peripheral venous blood, transferred from the warm, resting, unanaesthetized or lightly anaesthetized animal has a negligible constrictor effect on the test artery. This, together with the fact that the donor's denervated ear vessels are themselves relaxed, is strong evidence that the content of active adrenaline or other constrictor substance is extremely low. The observations also provide evidence that in the anaesthetized animal there is a continuous small release of adrenaline from the adrenal glands, which is independent of nervous activity.

Effects of plasma

We consider first plasma separated from arterial and peripheral venous blood. It is only here that the vaso-active properties of blood and plasma may be fairly compared. The 0·1 ml. blood transferred is blood from the immediate neighbourhood of the catheter tip. When 4 ml. blood is rapidly withdrawn, this volume must come from a wider area and, unless the blood is uniformly constituted, the constrictor activity of the 4 ml. sample may well differ from

that of the transferred 0·1 ml. sample. It is reasonable to assume that in the warm, resting animal, arterial and probably also peripheral venous blood is uniform in adrenaline content. But blood withdrawn from the vena cava may contain more or less blood from the adrenal veins with a higher adrenaline content depending on the position of the catheter tip in relation to these veins and on the conditions of flow, whether laminar or turbulent.

Plasma separated from blood withdrawn from an artery or peripheral vein of a warm, resting, unanaesthetized or anaesthetized animal, and tested immediately (i.e. about 10 min after withdrawal of the blood) has sometimes no, but usually a slightly greater and longer lasting constrictor effect than has transferred blood. The adrenaline equivalent is usually about 10^{-11} or rather greater, but less than 10^{-10}. Occasionally, however, the constrictor effect is much greater, the adrenaline equivalent ranging from more than 10^{-10} up to 5×10^{-8}. There is no corresponding constriction of the minute vessels even with a gross arterial constriction. This suggests that the constrictor substance is not adrenaline-like but rather like 5 HT. The plasma kept in ice remains unclotted and unchanged in constrictor activity for at least 8 hr.

The greater constrictor activity of plasma is not due to the rapid withdrawal increasing the activity of the blood itself. In a rabbit weighing 2–3 kg at least three 4 ml. samples may be withdrawn, allowing an interval of about 10 min between each, without provoking a significant change in the activity of transferred blood. The plasmas from successive samples usually have equal constrictor effects. For example, three 4 ml. samples were withdrawn from the jugular vein of a 2·6 kg rabbit at intervals of 11 min. The separated plasmas caused slight constriction of the test artery lasting 28, 31 and 30 sec respectively (mean of three injections each). Adrenaline 10^{-11} caused a transient constriction lasting 11 sec and adrenaline 10^{-10} a constriction lasting 73 sec (mean of three injections).

The constrictor activity develops during the centrifuging of the blood. This has been shown by withdrawing blood into the Perspex syringe which is surrounded by ice and left attached to the junction piece of tap 5 (Fig. 1). Tap 5 is turned to shut off the syringe and restore the saline flow to the donor. At intervals blood is then transferred to the test ear either from the donor or the syringe. Over a period of at least 30 min, the constrictor activity of the blood in the syringe remains unchanged and no greater than that of blood freshly transferred from the donor. It seems to be due to some change in the platelets and leucocytes, for a strong constrictor activity is associated with an abnormal appearance of the centrifuged blood. Usually the blood shows a well-defined white cell layer, 0·5–1 mm thick (total length of blood in syringe is about 55 mm) between the red cells and the supernatant plasma. The plasma is cloudy except for the top few mm where it is clear. The separated plasma has

little or no greater activity than transferred blood. Occasionally, however, the plasma is clear throughout and there is no white cell layer, the plasma abutting directly on the red cells, or else there is an intervening pinkish layer of mixed white and red cells. The plasma has a strong constrictor effect. Abnormal layering of the centrifuged blood and strong activity of the plasma seems to be almost restricted to the blood of a few rabbits. Their blood, sampled at intervals over a period of 2–3 months, has almost consistently displayed these unusual features. The reason for this is unknown. We have not detected any technical fault as responsible nor any other difference in life or after death between these rabbits and those whose centrifuged blood has almost always been normal in these respects. These changes are prevented by using sodium citrate as the anticoagulant (1 ml. 3·8% sodium citrate to 4 ml. blood). This, however, is unsuitable for our purpose since the citrate seems to inhibit the constrictor effect of adrenaline on the test artery.

In rabbits whose plasma from peripheral venous or arterial blood shows the usual findings, the plasma separated from posterior caval blood has a constrictor activity equal to, less or greater than that of transferred blood. This seems to be due to a varying admixture of adrenal vein blood. After excision of the adrenal glands, caval plasma is like that of peripheral vein blood and slightly greater in activity than transferred blood.

It seems, therefore, that the process of separation usually leads to an increase in the constrictor activity of plasma as compared with that of blood, and which is probably due to the release of a substance like 5 HT from the formed elements. In most animals, the increase is almost always small and for our purpose negligible. A plasma weakly constrictor for the test artery does not interfere with the assay of adrenaline added to the plasma by the rat's uterus. A strongly constrictor plasma, however, stimulates the uterus and augments the subsequent contraction to carbachol.

Effects of blood and plasma after adrenaline injection

It has been shown (Grant, 1935) that 1 μg adrenaline injected intravenously causes a moderate constriction of the denervated ear artery lasting about 4 min. Provided that transfer follows quickly after injection, adrenaline can be detected in the arterial blood for a brief period, as Fig. 8 exemplifies. In this experiment, a catheter was inserted into the normal ear artery of an anaesthetized rabbit and adrenaline, 1·0 μg in 0·1 ml. saline, was injected rapidly into the marginal vein of the opposite denervated ear. Six injections were made and each caused the expected constriction of the denervated central artery. Blood was transferred at the intervals after the injections shown in the figure. Blood transferred at 10 sec caused a constriction like that ordinarily provoked by adrenaline in a concentration of about 10^{-9} (but not tested on this occasion). Transferred at 30 sec the constrictor effect was much less and by 60 sec had

declined to the pre-injection level. Adrenaline is similarly detectable in the blood (though in higher dilution) after the injection of 0·5 μg but not after 0·1 μg. Plasma from blood withdrawn between 10 and 20 sec after the injection of 1·0 μg adrenaline also has a greater constrictor effect than has plasma from blood before the injection.

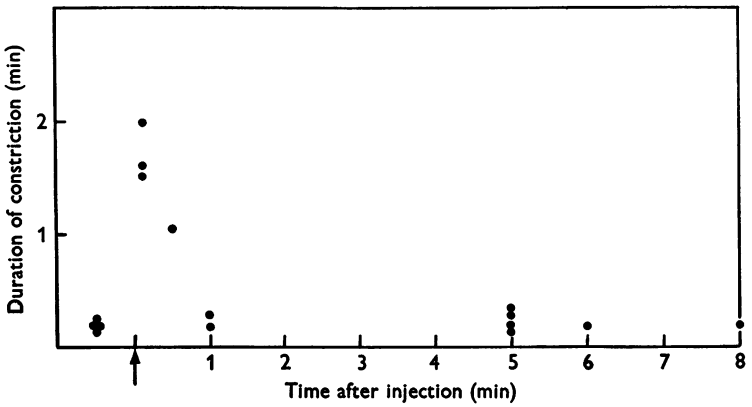

Fig. 8. Summary of responses of test artery to arterial blood transferred at the times shown after six intravenous injections of 1 μg adrenaline.

Effects of blood and plasma after stimulating the donor

In the rabbit, anaesthetized or not, cooling the body stimulates the sympathetic nerves and constricts the normal ear vessels; the vessels of the denervated ear remain relaxed (Grant, 1935). In two experiments, illustrated by Fig. 9, a catheter was inserted into the marginal vein at the base of a normal ear and its tip passed 2 cm distally. The anaesthetized rabbit, at first warm, was cooled, rewarmed and again cooled. The rectal temperature and state of the normal ear vessels are shown in the chart. When the normal ear vessels were constricted, blood transferred from the normal ear vein exerted a slightly greater effect on the test artery than when the vessels were dilated. The denervated ear vessels remained dilated throughout.

The slow flow through the constricted ear vessels does not allow the rapid withdrawal of a sufficient volume of blood for the separation of plasma.

It has been shown (Grant, 1935) that nervous and muscular activity causes constriction of the rabbit's denervated ear vessels due to the release of an adrenaline-like substance into the blood. This substance can be detected in transferred blood. In the experiment illustrated by Fig. 10, a catheter was inserted into the marginal vein of the normal ear and passed to about the heart. The rabbit was unanaesthetized and sat quietly on the bench. Transferred blood had little constrictor activity. Three times the rabbit was startled by making sudden noises and clapping it on the back. On the first occasion the animal

did not respond well and little constriction occurred in its denervated ear artery. On the other two occasions good constrictions were obtained. Owing to the difficulty of handling the animal, transfers could not be made so soon as

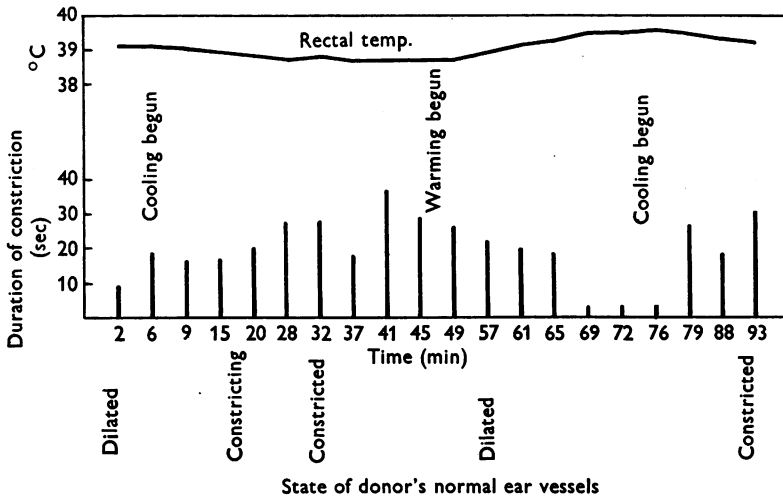

Fig. 9. Responses of test artery to normal ear vein blood transferred at min shown while donor is cooled, warmed and recooled. Changes in rectal temperature and donor's ear vessels also indicated.

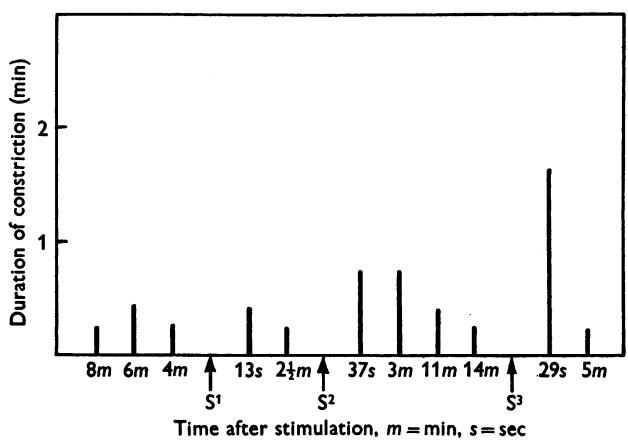

Fig. 10. Responses of test artery to arterial blood transferred at the min and sec shown before and after three stimuli (S^1, S^2 and S^3).

after adrenaline injections in the anaesthetized rabbit, but it is clear that after the second and third stimuli the blood temporarily acquired increased constrictor activity.

A gross constriction of the denervated ear vessels is more easily and regularly provoked by making the animal struggle for about 30 sec by, for example, holding a hind-leg. The degree of constriction seems to depend on how strongly the animal exerts itself. Not all rabbits struggle or struggle well when so held, and one animal may not struggle equally well on successive occasions. When the animal does struggle strongly, it is difficult to prevent displacement of the catheter. On a number of occasions, however, we have been able to transfer blood soon after a brief struggle which has resulted in a gross constriction in the denervated ear. Blood transferred within 30 sec of the end of the struggle has a greater constrictor effect on the test ear than control blood, the adrenaline equivalent ranging from 10^{-10} to 10^{-9} in different experiments. Successive transfers show that the constrictor activity quickly declines, returning to the control level within 5–10 min after the end of the struggle.

We can confirm the earlier observations (Grant, 1935) that after removal of both adrenal glands, struggle still causes constriction of the denervated ear vessels. In numerous observations we have not detected any regular difference in the degree of constriction from struggle before and after adrenalectomy. Moreover, in two rabbits, 4 days after bilateral adrenalectomy, the constrictor activity of blood transferred after a struggle was of the same order as in the intact animal, the adrenaline equivalent being between 10^{-10} and 10^{-9}.

The constrictor activity of transferred blood is like that of adrenaline in that it affects the minute vessels as well as the arteries. To test the effect of potentiators and inhibitors on the constrictor activity, we separated plasma from blood withdrawn before and after a struggle which resulted in a strong constriction of the donor's denervated vessels. The constrictor activity of after-struggle plasma is, however, not only much greater than that of control plasma but also greater than that of blood transferred after a struggle, the adrenaline equivalent ranging from 10^{-9} to 10^{-8}. This enhanced activity seems due to some addition to the constrictor properties of the plasma during the process of separation and is associated with the rapid clotting of the heparinized plasma brought about by the struggle. The increased constrictor activity is present before any visible signs of clotting; with clotting, the constrictor activity increases further, the adrenaline equivalent rising to between 10^{-8} to 10^{-7}.

While control plasma from the resting animal remains unclotted for at least 8 hr, after-struggle plasma clots within 1 or 2 hr of separation in spite of the 50 units of heparin in the Perspex syringe into which the 4 ml. blood is withdrawn. In one instance, the amount of heparin was doubled but in 2 hr the plasma had clotted to a jelly. Slight shaking of the after-struggle plasma from time to time hastens clotting, provoking the development of flocculi within about 30 min of separation. Repeated shaking sometimes, but not

always, causes a sparse granular precipitate to form in control plasma. In three rabbits the rapid clotting of after-struggle plasma was not prevented by bilateral adrenalectomy.

After-struggle plasma differs also from resting plasma in being slightly but distinctly browner in colour. This seems to be due to the presence of haemoglobin, as shown by the blue colour in the benzidine test (Ingham, 1932); spectroscopic examination reveals the absorption bands of oxyhaemoglobin in the green and yellow. We do not know if the pigment is derived from blood or muscle, the absorption bands being too faint for accurate observation

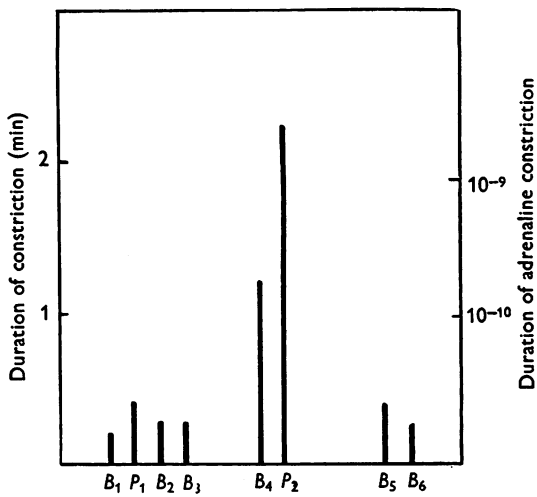

Fig. 11. Responses of test artery to blood and plasma withdrawn before and after a struggle. For explanation see text.

with the reversion spectroscope. In the three adrenalectomized rabbits mentioned above, although after-struggle plasma clotted quickly it did not differ in tint from resting plasma.

Fig. 11 exemplifies these observations on the constrictor activity of blood and plasma before and after a struggle. In the experiment which this figure illustrates, a catheter was passed from the central vein of the left ear to the left superior vena cava (site determined by necropsy) in an unanaesthetized rabbit 4 days after bilateral adrenalectomy. Three transfers of resting blood (B_1, B_2 and B_3) all caused negligible constrictor responses from the test ear. Between the first and second transfers, 4 ml. blood was withdrawn and the plasma separated (P_1). The rabbit then struggled for 20 sec. Blood (B_4) was transferred at 23 sec after the end of the struggle; at 1 min 22 sec, another 4 ml. blood sample was withdrawn. While the plasma (P_2) was being separated,

blood was again transferred at 4½ and 12 min and adrenaline 10^{-9} and 10^{-10} injected into the test ear. The plasmas were then injected, P_2 at 15 min and P_1 at 70 min after separation. The figure shows that the constrictor effects of resting blood (B_1, B_2 and B_3) and resting plasma (P_1) and of blood at 4½ and 12 min after struggle (B_5 and B_6) are of short duration, much less than of adrenaline 10^{-10}. The adrenaline equivalent of blood 23 sec after the struggle (B_4) is between 10^{-10} and 10^{-9}. The constrictor effect of after-struggle plasma (P_2) is considerably greater than that of B_4, the adrenaline equivalent being a little greater than 10^{-9}. Other observations have shown that the constrictor activity of blood transferred at this time after a struggle is no greater and usually less than that of blood transferred sooner after the struggle. The two plasmas were indistinguishable in colour; both gave a slowly developing trace of blue with the benzidine test; in both, oxyhaemoglobin bands were just detectable in the spectroscope. At the time of injection, neither plasma showed signs of clotting. Ten minutes later (30 min after separation from the blood) slight shaking caused a copious precipitate of coarse flocculi to appear in P_2. Considerable further shaking provoked a sparse granular precipitate in P_1 1½ hr after its separation.

We have not pursued these interesting effects of struggle on the coagulation of plasma. It is sufficient for the moment to recognize that struggling provokes such changes in the blood and that plasma separated from the blood of an active, or recently active, animal has a considerably greater constrictor effect that that of the circulating blood.

The effects of haemorrhage

The general pattern of the circulatory changes resulting from haemorrhage in the rabbit has been described elsewhere (Downman, Mackenzie & McSwiney, 1944; Armin & Grant, 1951). A further point of interest is the change in heart rate. Soon after beginning bleeding and before blood pressure falls, the heart rate rapidly increases, rising from its initial rate of between 200 and 250/min to between 300 and 350. As blood pressure falls steeply, however, the heart rate slows by a variable degree; it may return to its initial rate. Thereafter the rate gradually increases, ultimately reaching 350–400/min. Heart rate slows gradually after blood is replaced. Bleeding is associated with a rise of rectal temperature, probably due to diminished heat loss through peripheral vasoconstriction. In the rabbit, however, heart rate is independent of rectal temperature within the range 38–41° C.

Additional details of the changes in the ear vessels are required here since they supply evidence for the release of a constrictor substance into the circulating blood of the bled animal. In the unanaesthetized or lightly anaesthetized rabbit, the vasoconstrictor effect of haemorrhage on the normal ear vessels depends mainly on the state of bodily warmth and less on the amount

of blood withdrawn, whereas in the chronically denervated or sympathectomized ear, vasoconstriction is determined mainly by the amount of blood loss and is independent of bodily warmth. If the quiet animal is kept just warm enough to maintain the normal ear vessels dilated (rectal temperature usually about 39° C), then even a small blood loss (about 5 ml./kg) may cause a rapid and great constriction of the normal ear vessels. But if the animal is kept hot (rectal temperature over 40° C) a much larger haemorrhage (20–25 ml./kg, about one-third of the blood volume) sufficient to cause a steep fall of blood pressure causes only a slight and transient constriction of the central artery, though the ground tone of the ear pales a little.

In the denervated ear, independently of the rectal temperature, a small haemorrhage has no obvious effect on the calibre of the vessels. As bleeding proceeds, however, the ear pales and the central artery constricts. After 20–25 ml. blood/kg has been withdrawn, the ear is blanched and the central artery greatly constricted, to a quarter or less of its initial diameter. This constriction seems largely independent of mean blood pressure. If the blood is withdrawn slowly, the denervated artery may constrict and blood pressure rise before falling. Further, when blood pressure has fallen to low levels and subsequently rises, the denervated vessels remain constricted and relax little or not at all until the withdrawn blood is restored to the animal. Fig. 12 A illustrates the steep fall and speedy recovery of mean arterial blood pressure after the rapid withdrawal of 20 ml. blood/kg from an anaesthetized rabbit and the further rise of blood pressure when the shed blood is replaced. It shows also the rapid constriction of the denervated central artery with the fall of blood pressure, its persistence when blood pressure recovers and its gradual relaxation during blood replacement. In this example the shed blood was replaced after 15 min. In other instances we have waited 1 hr before replacing the blood; the constriction of the denervated artery persisted throughout the hour.

These observations on the donor rabbit provide strong evidence that as a result of the rapid loss of about one-third of the blood volume (Armin *et al.* 1952) the circulating blood acquires an increased constrictor activity that persists until the shed blood is replaced. By transferring blood and injecting plasma this increase is made evident in the test ear. Previous experience (Armin & Grant, 1951) showed us that large and rapid bleeding is apt to make unanaesthetized rabbits restless. Therefore, in view of what has been said about the effects of nervous and muscular activity we have used lightly anaesthetized animals for the further observations on the effects of haemorrhage. We have not detected any material difference between the vascular responses to haemorrhage in the lightly anaesthetized and those of the unanaesthetized donor. In the anaesthetized rabbit we have not observed increased coagulability or darkening of the colour of plasma by bleeding, nor

have we found the constrictor effect of after-bleeding plasma materially greater than that of blood transferred after bleeding. After-bleeding plasma is less cloudy than that separated before bleeding.

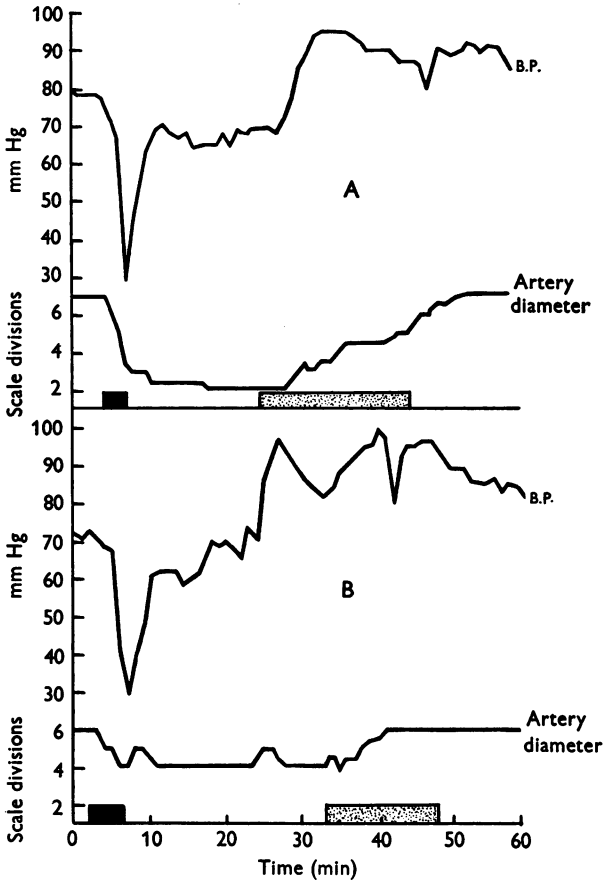

Fig. 12. Changes of mean arterial blood pressure (B.P.) and of diameter of denervated central artery resulting from bleeding (black rectangle) and restoring (open rectangle) 35 ml. blood (body weight 1·7 kg); A, 3 days before, and B, 4 days after, bilateral adrenalectomy. Blood pressure recorded by mercury manometer from femoral artery.

The increase in constrictor activity after bleeding is greatest in posterior caval blood drawn from headwards of the renal veins. Blood or plasma drawn from this region within 1 or 2 min of the end of bleeding and injected into the test artery exerts a strong constrictor effect obvious to the naked eye. The area of injection blanches, the smaller vessels disappear from view and the central artery narrows to a barely visible thread. The constriction passes off slowly, the central artery returning to its initial diameter after 5–15 min. The

adrenaline equivalent of such blood and plasma is usually between 10^{-8} and 5×10^{-8}, and on occasion it reaches 10^{-7}. Successive blood transfers show that this activity soon declines to persist at a lower level, with an adrenaline equivalent of 10^{-9} to 10^{-8}. When the shed blood is replaced the constrictor activity soon returns to about the pre-bleeding level, as Fig. 13 exemplifies.

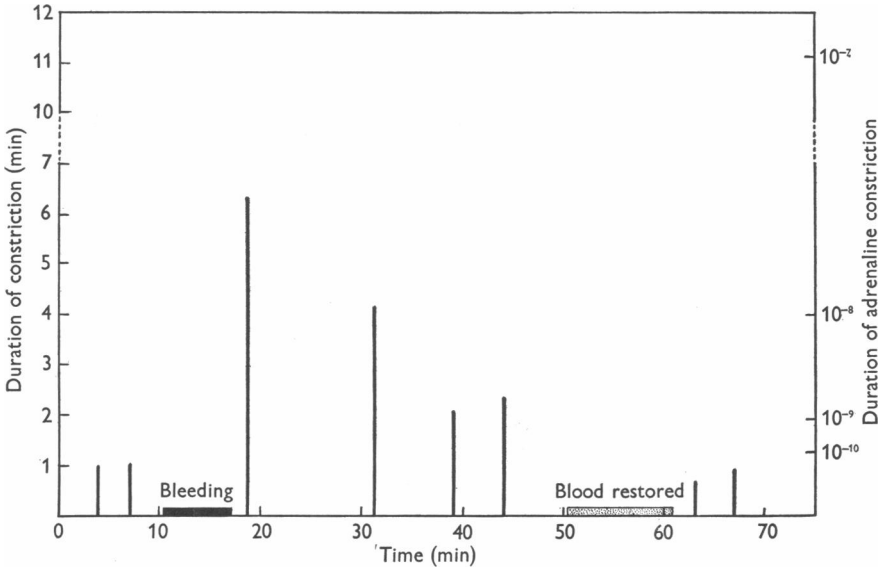

Fig. 13. Responses of test artery to blood transferred from posterior cava at the times shown before and after bleeding and after restoring 60 ml. blood. The responses of test artery to adrenaline 10^{-10} to 10^{-7} are shown on the right. Donor's body weight 2·7 kg. Catheter tip 1 cm headwards of right renal vein.

Arterial and peripheral venous blood and plasma have weaker constrictor effects and an initial peak is lacking, the adrenaline equivalent rising from the low initial level to persist usually about 10^{-9} though sometimes it is nearer to 10^{-10} and sometimes to 10^{-8}. The constrictor activity remains more or less uniform until the shed blood is replaced, when it returns to about the pre-bleeding level. Fig. 14 A exemplifies the effect of transferred arterial blood (adrenaline equivalent nearly 10^{-10}) and Fig. 15 that of jugular vein blood (adrenaline equivalent between 10^{-9} and 10^{-8}). Fig. 15 also shows that a further haemorrhage causes no material increase in constrictor activity.

The nature of the constrictor substance

The constrictor effect of after-bleeding blood or plasma on the test ear is indistinguishable from that of adrenaline. For example, Fig. 16 shows the details of the responses of the test artery to 0·1 ml. adrenaline 10^{-10}, 10^{-9} and

10^{-8} and, to plasma before and after bleeding and to 0·1 ml. pre-bleeding plasma containing adrenaline in a concentration of 10^{-8} made by adding 0·1 ml. adrenaline 10^{-7} to 0·9 ml. plasma.

The constrictor effect of plasma is also like adrenaline in that it is potentiated by procaine and is inhibited by Largactil (Fig. 17).

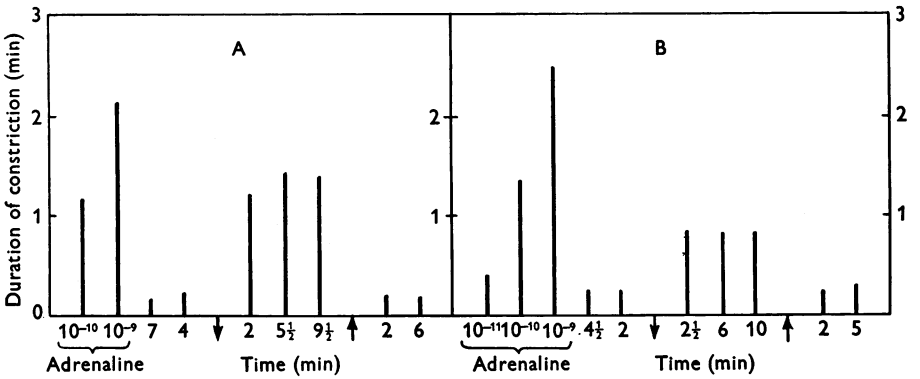

Fig. 14. Responses of test arteries to adrenaline and to arterial blood transferred at the min shown before and after bleeding (↓) and after restoring (↑) 40 ml. blood; A, 7 days before, and B, 3 days after, bilateral adrenalectomy. Donor's body weight 1·7 kg.

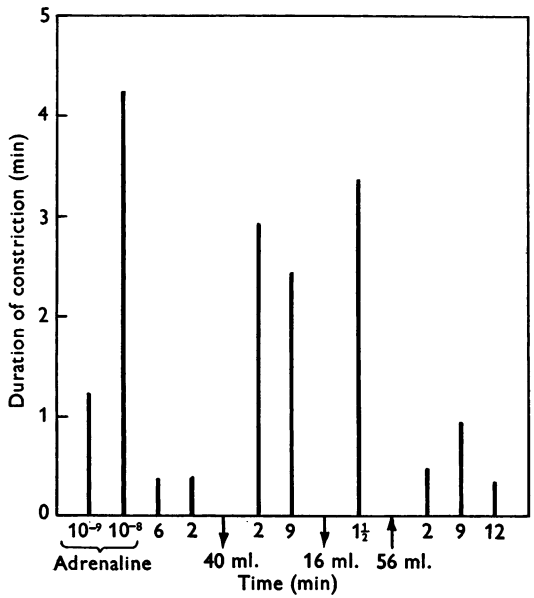

Fig. 15. Responses of test artery to adrenaline and to jugular vein blood transferred at the min shown before and after bleeding (↓) 56 ml. in two stages (40 and 16 ml.) and after replacing the blood (↑). Donor's body weight 2·9 kg.

When the adrenaline equivalent of plasma is, according to the test ear, about 10^{-8} or more, then the plasma in doses of 0·1 ml. added to the bath, inhibits the subsequent contraction of the rat's uterus to carbachol. It does not inhibit the contraction of the rat's colon to carbachol as does noradrenaline. In three instances the adrenaline equivalent of after-bleeding caval

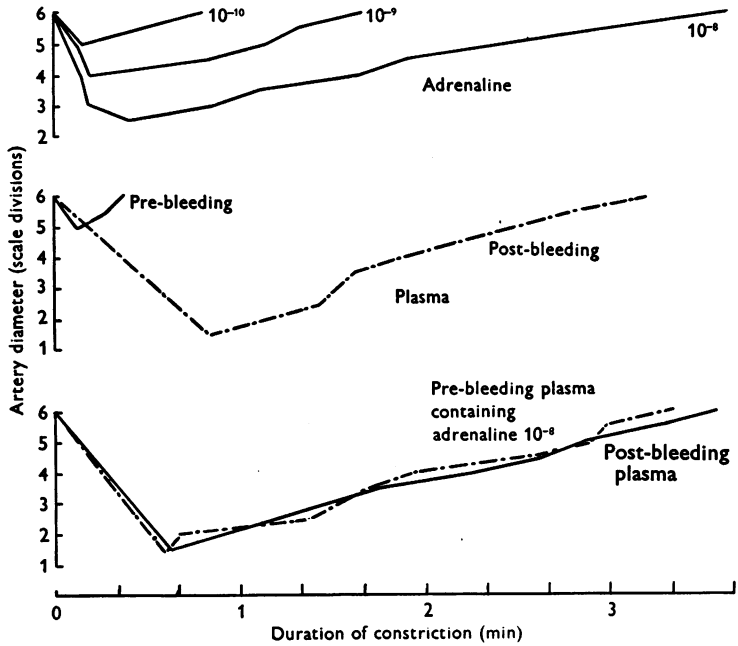

Fig. 16. Details of constrictor responses of test artery (*a*) to adrenaline 10^{-10}, 10^{-9} and 10^{-8}, (*b*) to pre- and post-bleeding plasma from heart region, and (*c*) pre-bleeding plasma to which has been added adrenaline to give a concentration of 10^{-8} and post-bleeding plasma.

plasma was determined both by the test ear and the rat's uterus with the results shown in Table 1. With the help of our colleague, Dr W. J. H. Butterfield, the estimations were made simultaneously. The results of the two methods seem to us to render it highly probable that after-bleeding plasma contains adrenaline in approximately the concentrations estimated, and that the noradrenaline content, if any, is unmeasurable by the rat's colon.

The source of the constrictor substance

The observations described above strongly suggest that the adrenaline-like substance entering the blood stream comes, at least in large part, from the adrenal glands. Proof of this source is provided by further observations on rabbits deprived of both adrenal glands.

Rabbits without their adrenal glands and treated as described remain apparently well. Their resting blood pressures and pulse rates are within normal limits and after bleeding show the same changes as in normal animals; in particular, there is no impairment of the quick recovery of blood pressure

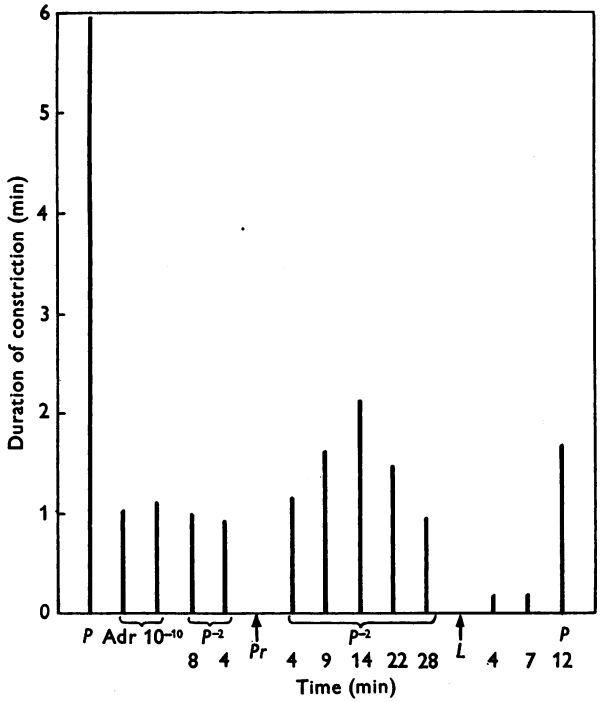

Fig. 17. Responses of test artery to after-bleeding plasma (*P*) from posterior cava, adrenaline 10^{-10} (Adr) and then (at the min shown) to plasma diluted 100 times with saline (P^{-2}) before and after 0·1 ml. procaine 10^{-6} (*Pr*) and after Largactil 0·1 ml. 10^{-3} (*L*) and lastly plasma (*P*) after Largactil.

TABLE 1. Adrenaline equivalent of after-bleeding caval plasma

	Denervated ear artery	Rat's uterus
(1)	$>10^{-8}$, $<2\times10^{-8}$	$=5\times10^{-8}$
(2)	$>0{\cdot}5\times10^{-8}$, $<10^{-8}$	$=0{\cdot}5\times10^{-8}$
(3)	$=10^{-8}$	$>10^{-8}$, $<2\times10^{-8}$

after a rapid haemorrhage of 20–25 ml./kg body weight. The denervated ear vessels also constrict, but more gradually and to a less degree than in the normal animal; the central artery is reduced only to about half its initial diameter instead of to a quarter. Fig. 12 A and B exemplifies the changes in blood pressure and ear artery diameter in response to bleeding the same animal 3 days before and 4 days after bilateral adrenalectomy. That the reduced constriction is not due to some effect of the first bleeding is shown by the fact

that adrenalectomized rabbits previously unbled display the same reduced constriction of their denervated vessels in comparison with other bled rabbits with intact adrenals.

To display this reduced constriction, it is important that the rabbit should remain quietly asleep. On several occasions, the rapid bleeding has caused the lightly anaesthetized animal to move restlessly; the movements were associated with a rise of blood pressure and an increase of the constriction of its denervated ear artery, reducing its diameter from about half to about a quarter its pre-bleeding calibre. When the movements ceased the additional constriction gradually passed off. Too rapid replacement of the shed blood may also cause movements associated with a temporarily increased arterial constriction. It has been seen that muscular activity causes constriction in the unbled adrenalectomized animal.

In the adrenalectomized animal the constrictor activity of arterial and peripheral venous blood is increased above the pre-bleeding level, but the increase is less by a factor of about 10 than in the normal rabbit. Moreover, caval blood does not differ significantly in activity from arterial blood. Thus, in four experiments on rabbits without adrenal glands, the adrenaline equivalents of arterial blood transferred after bleeding were in two a little less and in two a little greater than 10^{-10}. In three experiments, the equivalent of caval blood transferred was equal to, a little greater and a little less than adrenaline 10^{-10}. Fig. 14 exemplifies the activity of transferred arterial blood after haemorrhage: A, 7 days before, and B, 3 days after, bilateral adrenalectomy in the same animal.

This activity remaining after extirpation of the adrenals is also adrenaline-like since it is prolonged by procaine and inhibited by Largactil. We do not know its source, but it may well be released from endings of any of the sympathetic nerves stimulated by the bleeding. Granaat (1953) has suggested that vaso-active substances from the spleen may serve to regulate arterial blood pressure. We have found that excision of the spleen does not alter the constriction of the bled animal's denervated ear artery or the constrictor activity of its blood transferred either before or after excision of the adrenals. Shorr *et al.* (1951) attribute a renal origin for their vaso-excitor material (V.E.M.) present in the blood of the bled dog. In the rabbit, bilateral ligature of the renal artery and vein or excision of both kidneys is without effect on the responses to haemorrhage.

The discharge of the adrenaline-like substance from the adrenal glands is due to stimulation of the splanchnic nerves. In the two rabbits referred to earlier in which the splanchnic nerves had been cut 7 and 14 days previously, the constrictor activity of caval blood was equivalent to adrenaline 10^{-10} when the catheter tip was 1 and 2 cm respectively headward of the right renal gland. Rapid bleeding of 20 ml./kg increased the constrictor activity but slightly,

the adrenaline equivalent rising to a little greater than 10^{-10} but less than 10^{-9} and persisting at this level until the shed blood was replaced 15–20 min later, when it returned to about the pre-bleeding level. As in adrenalectomized rabbits, the donor's denervated ear artery constricted more slowly and to a less degree than that usually seen in intact animals.

DISCUSSION

The methods devised have several advantages for investigating the vasoactive properties of blood: (1) Evidence of constrictor activity is based on direct observation of the calibre of the blood vessels themselves and these are exposed to view without dissection. (2) The denervated ear vessels can detect vasoconstrictor substances in high dilution (Armin & Grant, 1953). (3) The use of the rabbit for both donor and test object avoids the difficulties that may arise from species differences. (4) Observation of the donor's denervated ear vessels, indicating the activity of the circulating blood, provides a check to the activity determined in the blood after withdrawal from the body. It has been seen, however, that a strong constriction of the donor's vessels does not necessarily mean a strong constrictor activity of the circulating blood at the moment of withdrawal. Thus, after the intravenous injection of 1 μg adrenaline into the donor, constrictor activity of transferred blood rapidly declines and becomes undetectable within 1 min of injection, yet the donor's artery remains constricted for several minutes. In the test ear also, the injection of 0·1 ml. adrenaline 10^{-7} provokes a constriction lasting for 10–15 min, although the adrenaline within the vessel must be washed away by the blood returning to the vessel as soon as the observer's obstructing finger is removed. It is already known that injected adrenaline disappears rapidly from the blood and that it may disappear at a time when some physiological effects are still visible (Bacq, 1949). (5) The fact that transfer requires only 0·1 ml. blood not only allows many observations to be made without materially depleting the donor's blood volume, but also allows the investigation of local concentrations, as the observations on caval and ear blood exemplify. The factor limiting the interval between successive transfers is the duration of the reaction in the test ear plus the 2 or 3 min we customarily allow after the observed end of the reaction in order to avoid possible cumulative effects (Armin & Grant, 1953). (6) Comparison of the effects of blood and plasma provides an index to the changes that may be produced in plasma by the process of separation. Provided there is no material difference between them, plasma can fairly be used to investigate the nature of a constrictor substance by methods for which whole blood is unsuitable.

The results indicate: (*a*) that the methods allow blood to be transferred from one animal to another in the same, or nearly the same condition (at least in respect of its constrictor action on ear vessels) as that in which it circulated in

the donor's body; and (*b*) that in most resting rabbits, plasma can be separated with but little further change. The precautions we have found to be necessary to accomplish this, are essentially those detailed by Code (1952) for obtaining low histamine values in rabbit plasma and by Gaddum, Peart & Vogt (1949) for preventing the appearance in cat's plasma of substances interfering with their pharmacological tests. These precautions are, speed, contact of the blood with only non-wettable surfaces, gentle handling, cooling, and heparin as the anticoagulant. During the rapid transfer, blood is in contact with only polythene and Perspex, except where it passes through the short length of stainless steel hypodermic needle inserted into the artery. Even this can be avoided by introducing the catheter through a wider needle inserted into the artery. This, however, does not alter the effect of transferred blood on the artery and has the disadvantage of causing greater local trauma during introduction and the subsequent escape of saline and blood at the site of insertion. The use of the combined Perspex syringe and the centrifuge tube not only ensures rapid withdrawal of blood even when the animal has been bled, but also avoids the transfer of blood from one container to another. Such transfer is liable to cause haemolysis; we know from previous experience that to avoid traces of haemolysis requires even greater care in handling rabbit than human blood. The precautions adopted are, however, insufficient to prevent the rapid development of a considerable constrictor activity in the separated plasma in (1) the muscularly active or recently active animal, and (2) a few animals even in the resting state. Gaddum *et al.* (1949) also found that sometimes, in spite of their precautions, interfering substances appeared in cat's plasma.

Judged by the test ear, arterial and peripheral venous blood from the warm and resting animal has a constrictor activity equivalent to adrenaline less than 10^{-11}, to histamine less than 10^{-9}; the activity of plasma is slightly greater. According to West (1947) rabbit's blood contains adrenaline (but not noradrenaline) in an average concentration of 0·09 μg/ml. (9×10^{-8}); he suggests that the majority of the adrenaline is carried in the red cells since the amount of adrenaline in plasma was unmeasurable by his tests (apparently less than 3×10^{-8}). For histamine in blood, the values quoted by Code (1952) range from 1 to 5 μg/ml. ($1 \times$ to 5×10^{-6}) and the lowest value for plasma is 0·05 μg/ml. (5×10^{-8}). Holgate (1953) finds even more histamine in rabbit plasma, 3–20 μg/ml. and also evidence for the presence of 5-hydroxytryptamine in a concentration of approximately 0·6 μg/ml.

It seems, if our results are accepted and if blood does contain these potent vasoconstrictors in such concentrations, that their usual effect on the sensitive denervated ear vessels of both donor and test rabbit must be in some way prevented. It is conceivable, but unlikely, that their constrictor effect is balanced by some opposite property of the circulating blood or that the vessels are habituated to their presence in the blood. Emmelin (1945) provides in-

direct evidence suggesting this latter possibility in the case of histamine. A more likely explanation is that they are in some way bound or held within the cellular elements and thus rendered inactive until released, or formed from precursors, during the handling of the blood. Emmelin (1945) points out that the more the methods have been improved, the smaller has become the part of the total blood histamine which can be found in plasma. Code (1952) concludes that normally histamine is held safely within the cells of the blood so that little or none is free in the plasma to produce physiologic effects. Recently, Humphrey & Jaques (1954) conclude that the histamine and serotonin found in the blood of various species (including rabbits) is almost all contributed by the platelets. When damage to platelets is minimized, the amounts of these substances found in the plasma are very small indeed, of the order of 0·002 μg/ml., a concentration of 2×10^{-9}. It is known that adrenaline added to blood is taken up by the cells (Bain, Gaunt & Suffolk, 1937). Our observations provide no evidence on the total amount of these substances to be found in the blood; they show only that blood and plasma from a resting rabbit exert a negligible constrictor effect on the denervated ear vessels except in the case of blood from the posterior vena cava near the entry of the renal veins and that here the constrictor effect is due to adrenaline, or noradrenaline or both, released from the adrenal glands.

The results also show that increased constrictor activity can be detected in the circulating blood as the result of nervous and muscular activity. Gaddum & Kwiatkowski (1938, 1939) found that in the perfused ear, stimulation of the sympathetic nerve causes vasoconstriction and the release of adrenaline into the perfusing fluid; according to Outschoorn (1952) the release of adrenaline is accompanied by larger amounts of noradrenaline. In the more natural conditions of our experiment, a slightly greater constrictor activity is detectable in the normal ear vein blood when the ear vessels are constricted by cooling the body than when they are dilated in the warm anaesthetized animal. The fact that the denervated ear vessels of the cooled rabbit show no constriction is evidence that the substance entering the blood stream is small in amount. It must soon be removed from the blood or diluted below an effective concentration. This increased activity is presumably due to a 'spill over' of adrenaline and noradrenaline released from the nerve endings in the vessel walls of the donor's normal ear. But it is uncertain whether or not this 'spill over' is greater when the vessels are constricted than when they are dilated. It might be that a constrictor substance is continuously released and that vasoconstriction, by reducing blood flow, merely raises its local concentration to a detectable level.

Cannon (1929) detected 'adrenaline' in defibrinated caval blood from the frightened cat, but not after the removal of the adrenal glands, though the animal still showed all the characteristic indications of sympathetic stimula-

tion. Grant (1935) reported that the vasoconstriction in the rabbit's denervated ear resulting from nervous and muscular activity persisted apparently unchanged after removal of the adrenal glands (confirmed by LeCompte, 1941) and pituitary glands. White, Okelberry & Whitelaw (1936), however, found that after removal of the left and denervation of the right adrenal gland, struggle resulted in a barely detectable narrowing of the denervated arteries. Our new observations show that adrenalectomy does not apparently diminish either the constriction in the donor's denervated ear after a struggle, or the adrenaline-like constrictor activity of transferred blood. The source of activity remaining after removal of the glands has not been determined. In view of the observations by von Euler & Hellner (1952) on the effects of muscular work in man, and by Gaddum & Kwiatkowski (1939) and Outschoorn (1952) on sympathetic stimulation in the rabbit, it may well be derived from an increased output of adrenaline and noradrenaline from adrenergic nerve endings. It has been seen, however, that further observations on the nature of the constrictor substance released by struggle are hindered by the changes occurring in the separated plasma. These changes lead to a rapid enhancement of the constrictor activity of plasma after withdrawal from the body, so that the constrictor effects of plasma after struggle cannot be taken as nearly the same as those of circulating blood. This enhancement is associated with rapid clotting of the heparinized plasma. We do not know the reason for this increased coagulability. Cannon and his associates (Cannon, 1929) have shown that in cats, strong emotion and also exercise shorten the coagulation time of blood, which effect they attribute to the release of adrenaline from the adrenal glands. Our observations refer, however, to heparinized plasma. The rapid clotting of after-struggle plasma is not prevented by adrenalectomy.

We have found no previous reference to the other change we have observed in after-struggle plasma, namely the altered colour due to the presence of haemoglobin or myoglobin, which is undetectable or barely detectable in control plasma. This addition of pigment is apparently prevented by adrenalectomy.

According to Gray & Lunt (1914) haemorrhage also causes in cats a decrease in the clotting time of blood, which is not prevented by adrenalectomy. According to our own findings, the heparinized plasma from bled unanaesthetized or anaesthetized rabbits does not clot sooner and is not darker in colour than control plasma. In anaesthetized animals, the heparinized plasma after bleeding has no materially greater constrictor effect on the test ear than has transferred blood.

Dealing with the mechanisms responsible for the vasoconstriction associated with 'oligemic shock' (under which term he includes the effects of haemorrhage) Page (1949) enumerates three possibilities: physical factors, active neurogenic constriction and humoral mechanisms. Various substances have

been described as present in the blood after haemorrhage and suggested as acting as humoral homoeostatic agents but, it seems to us, without satisfactory evidence that they were responsible for vasoconstriction in the animal from which the blood was withdrawn, e.g. angiotonin (Collins & Hamilton, 1944), V.E.M. (Shorr *et al.* 1951), possibly noradrenaline or serotonin (Page, 1943, 1949). It seems from our observations on the bled rabbit: (1) that the physical factor of reduced mean blood pressure plays little part in the constriction of the arteries of either the normal or denervated ears, (2) that the main factor responsible for arterial constriction in the normal ear is activity of the sympathetic nerves, and that this ordinarily conceals (3) a humoral factor which is clearly displayed by the constriction of the denervated ear artery of both donor and test rabbit. The observations provide strong evidence that adrenaline is the substance chiefly responsible for the constriction and is derived mainly, though not entirely, from the adrenal glands. Saito (1928), by collecting adrenal vein blood from a caval pocket, demonstrated an increased output of 'adrenaline' after haemorrhage in dogs. He showed that in unanaesthetized animals the rate of secretion is increased as much as 10 to 30 times the control level, and that this increase persists for a considerable time. We, however, cannot exclude an admixture of noradrenaline; our preparation does not distinguish between them. The initial quickening of the heart rate followed by slowing and then by a gradual increase suggests the participation of both adrenaline (quickening) and noradrenaline (slowing). Schuler & Heinrich (1949) find both adrenaline and noradrenaline in the rabbit's adrenal gland, noradrenaline constituting 20–50% of the total amount, but according to Holtz & Schümann (1950) the gland contains only adrenaline. West (1950) finds very little noradrenaline in gland extracts but when the splanchnic nerve is stimulated, noradrenaline appears in the venous effluent, though for only a brief period.

The release of adrenaline after haemorrhage results from a stimulation of the splanchnic nerves, but our observations do not show how haemorrhage excites or replacement of blood inhibits nervous activity. It is apparently not through a fall and rise of mean arterial pressure; it may be effected by changes in venous pressure (see McDowall, 1938).

Soon after the end of bleeding the constrictor activity of caval blood reaches a peak which is not long maintained and which is absent from the peripheral blood. We suggest that the initial high output of adrenaline (possibly preformed and stored in the gland) which this peak indicates, serves rapidly to raise its concentration in the peripheral blood; the subsequent lower output (possibly of newly formed adrenaline) suffices to maintain the peripheral concentration by balancing the rapid removal of adrenaline from the blood and its destruction by amine oxidase. Our observations do not disclose what function is served by the increased constrictor activity of the blood. They show, however,

that removal of the adrenals, though considerably lessening the constriction in the donor's denervated ear and reducing the constrictor activity of transferred blood by a factor of 10, does not impair the recovery of blood pressure or alter the heart rate changes after the loss of a third of the blood volume. This is in agreement with the work of Swingle, Pfiffner, Vars & Parkins (1934) who found that adrenalectomized dogs maintained in good health by cortical hormone, dilute their blood and recover blood pressure after haemorrhage as do normal dogs.

The source of the constrictor substance remaining after extirpation of the adrenal glands remains undetermined. It is adrenaline-like and may enter the blood by a 'spill over' from widespread adrenergic nervous activity. It apparently does not come mainly from either spleen or kidneys; it is possible that some of the activity may be derived from these sources and that our methods are inadequate to detect the small changes caused by removal of these organs.

SUMMARY

1. Methods are described for (*a*) transferring 0·1 ml. samples of blood from any available vessel of a donor rabbit to the central artery of the denervated ear of another rabbit, and (*b*) quickly withdrawing larger volumes of blood and separating the plasma for injection into the test ear. The constrictor activity of blood and plasma is assayed in terms of adrenaline or other constrictor substance.

2. Observation of the vessels of the donor's denervated ear provide an indication of the constrictor activity of the circulating blood for comparison with the constrictor activity of blood and plasma injected into the test ear.

3. Arterial and peripheral venous blood from the warm resting animal whose denervated ear vessels are relaxed has a negligible constrictor effect on the test ear, the adrenaline equivalent being less than 10^{-11}. Blood from the posterior vena cava has greater activity, the adrenaline equivalent ranging from 10^{-10} to 10^{-9}; this greater activity disappears after removal of the adrenal glands, though it persists after bilateral section of the splanchnic nerves.

4. Plasma has usually a slightly greater constrictor activity than has blood, the adrenaline equivalent of plasma from peripheral blood being 10^{-11} or slightly greater; occasionally the constrictor activity is much greater. This greater activity is due to the release of a substance like 5-hydroxytryptamine during the handling of the blood.

5. Adrenaline injected intravenously in doses of 1·0 or 0·5 μg can be detected in the arterial blood for a brief period. Its constrictor effect in the donor outlasts its presence in the blood.

6. Normal ear vein blood has a slightly greater constrictor effect when the vessels are constricted by cooling the body than when they are dilated in the

warm animal. This is thought to be due to a 'spill over' of adrenaline and noradrenaline released from sympathetic nerve endings in the vessel walls.

7. Nervous and muscular activity in the intact or adrenalectomized rabbit constricts the donor's denervated ear vessels and increases the constrictor activity of blood on the test ear. After a brief struggle the adrenaline equivalent of peripheral blood rises to between 10^{-10} and 10^{-9}. The constrictor effect is adrenaline-like. The source of the adrenaline-like activity is undetermined.

8. Plasma separated from blood after a struggle has a greater constrictor effect on the test ear than transferred blood. This enhancement of activity is thought to be due to some addition to the constrictor properties of the plasma during the process of separation and is associated with rapid coagulation of the heparinized plasma. After-struggle plasma is browner in colour than control plasma due to the presence of haemo- or myoglobin. This addition of pigment is, but the rapid coagulation is not, prevented by adrenalectomy. The reason for these changes is unknown.

9. Rapid bleeding of one-third of the blood volume constricts the donor's denervated ear vessels and increases the constrictor activity of blood and plasma, the adrenaline equivalent rising to between 10^{-8} to 10^{-7} in caval blood and to 10^{-10} to 10^{-9} in peripheral blood. Adrenalectomy considerably reduces, but does not abolish, the constriction of the donor's denervated vessels and the constrictor activity of blood and plasma. The extra-glandular constrictor activity is also adrenaline-like; its source is undetermined; it may come from widespread sympathetic nerve endings; the spleen and kidneys supply no major contribution.

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