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THE ACTION OF ADRENALINE, NORADRENALINE, ACETYLCHOLINE AND HISTAMINE ON THE PERFUSED LIVER OF THE MONKEY, CAT AND RABBIT

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In an earlier paper (Andrews, Hecker, Maegraith & Ritchie, 1955) we described the actions of adrenaline, noradrenaline, acetylcholine and histamine on the perfused canine liver. In this paper we record briefly a study of the action of the same substances on the livers of the monkey, cat and rabbit. The work has been carried out in order to assess differences in the reactions of the hepatic vessels of these mammals. We were especially interested in the part played by the hepatic veins in the control of liver blood flow.

METHODS

Apparatus

The apparatus used was based on that described by Andrews (1953), but was modified to work with an extra-hepatic fluid volume of 150 ml. It is illustrated in the text diagram and is described below.

From the hepatic veins the blood was conducted via an outflow recorder (Andrews, 1952) and a nylon filter to the main reservoir. From here it was pumped to a Hooker oxygenator, at whose base was a small chamber which acted as an upper constant-level reservoir. The excess of blood drained back via an outflow tube to the main reservoir. The upper reservoir was approximately 40 cm above the level of the liver, and blood flowed from it to the liver via a water-bath which was placed between the hindlegs of the animal. In the water-bath the blood stream was warmed to body heat and then passed to either the portal or arterial systems.

The pressure within the portal vein was measured by a manometer, and was adjusted to physiological levels by means of a screw clamp. Variations of pressure were recorded by an inverted piston volume recorder, the kymographic tracings indicating changes in rate of flow. The majority of experiments were performed with a portal pressure of 7–10 cm blood. It was found necessary to include a bubble-trap in the circuit. Both glass and metal portal cannulae were used: for small animals metal was more suitable, and shortened, wide-bore, stainless-steel aspiration needles proved excellent.

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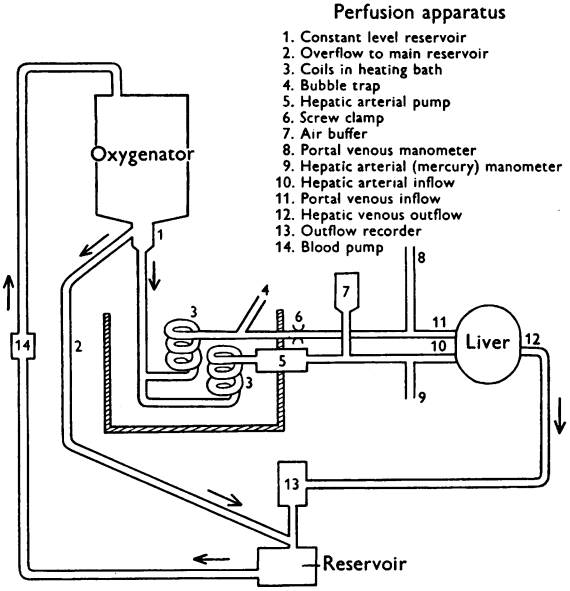
The arterial pump was based on that described by Vane (1953). An air buffer was included, with a screw clamp to regulate pulse pressure. Arterial pressures were of the order of 80/90 mm Hg (monkey and rabbit) and 110/120 mm Hg (cat) and were recorded with a mercury manometer. Any small bubbles entering the arterial system rose into the air buffer.

Liver volumes were measured both by the 'funnel and balloon' method, described by Andrews (1953), and by total plethysmography. The validity of records obtained by the former method is discussed elsewhere (Andrews *et al.* 1955).



Foaming was prevented by coating the main reservoir with 'Antifoam A' (Hopkin and Williams, Ltd.) which we found to be most effective. The main reservoir was a glass jar, the neck being loosely plugged with cotton-wool to prevent gaseous interchange with the atmosphere. Heparin was used as the anti-coagulant.

The water-bath and, when used, the plethysmograph were heated by circulating water through a larger, heated tank. The temperature was kept constant, and the temperature of the liver, when covered with cotton-wool, was always within one degree of that of the bath.

Operative technique

Sodium thiopentone, given intravenously, was used to induce unconsciousness in monkeys and rabbits, and ether then given to produce and maintain anaesthesia. Cats received intraperitoneal sodium pentobarbitone (14 mg/kg body weight) and 15 min later were anaesthetized with ether.

The surgical procedure was basically the same as that described elsewhere (Andrews, 1953), but owing to the small size of the animals the yield of blood was increased by adding warm Ringer-Locke solution (NaCl, 0·85 g, KCl, 0·042 g, $CaCl_2$, 0·025 g, $NaHCO_3$, 0·02 g, distilled water to 100 ml.) to the general circulation via a jugular vein during the period of bleeding. The animal having been anaesthetized, one operator opened the abdomen with a mid-line incision and freed the portal vein from peritoneum and other structures, whilst a second operator cannulated a jugular vein and the trachea. Artificial respiration was begun and the thorax opened by splitting the sternum; if there was any bleeding the internal mammary arteries were ligated. The thoracic

inferior vena cava was now cannulated, blood being drained into the main reservoir, and immediately warm Ringer-Locke solution was allowed to flow into the jugular vein; the rate of flow was sufficient to maintain a good heart beat and was regulated by an assistant.

The superior mesenteric artery was next clamped, and the portal vein cannulated. Portal flow was started as soon as there was sufficient blood in the reservoir, this being usually within 2 or 3 min.

When the main reservoir contained enough fluid the inflow of Ringer-Locke solution was stopped, the inferior vena cava was ligated below the liver and the hepatic artery was cannulated. In the cat the artery itself was cannulated and anastomosing branches tied. We found that the most convenient method in monkeys and rabbits was to ligate and divide the oesophagus with its vessels and then, after turning the stomach caudally, to approach the hepatic artery via the aorta and coeliac arteries. By placing a loose ligature as a guide around the hepatic artery whilst the heart was beating and the aorta intact, the task of cannulating the correct vessel was considerably simplified. Arterial branches to viscera other than the liver were ligated.

It was seldom possible to complete the surgical procedure in less than 20–30 min., but in almost every case the portal vein was cannulated and inflow begun while the natural arterial flow consisted mainly of blood. Cannulation of the artery of rabbits proved especially difficult owing to the fragility of the vessel walls. Haemoglobin values of the perfusion fluid seldom exceeded 8 g/100 ml. Our results on canine livers suggest that, although the metabolism of the liver may be depressed by haemodilution, the vascular reactions are not greatly altered during a period of at least half an hour.

RESULTS

In a previous communication (Andrews *et al.* 1955) we have indicated some of the difficulties of perfusion of the canine liver; unless the operative technique is impeccable, within relatively few minutes of beginning the perfusion the liver becomes swollen and congested, the hepatic blood flow is greatly diminished, and the reactions to drugs vary considerably. The livers of monkeys, rabbits and cats were much less affected by imperfections of technique, and we were able in almost all cases to perfuse at physiological pressures for hours without encountering significant deterioration of the flow rates. A few livers, especially of cats, became somewhat swollen as perfusion continued, but a typical 'blue liver' was not seen in this series. The results described were obtained from a study of the livers of eighteen monkeys, eleven cats, and ten rabbits.

Adrenaline and noradrenaline

The reactions to these substances appeared identical, and did not differ significantly in cat, rabbit, or monkey. These animals are therefore considered together. The usual amounts given varied from 1·0 to 5·0μg, but responses were occasionally obtained with as little as 0·1μg.

Adrenaline injected into the hepatic artery. The arterial and portal pressures were increased (i.e. a decrease of flow). The liver volume decreased. The hepatic venous outflow usually showed a short preliminary increase (sometimes absent) followed by a decrease. With amounts of 1–5μg the reaction usually lasted 20–120 sec; the duration of increase of outflow was seldom greater than 20 sec.

Adrenaline injected into the portal vein. The response was similar to that of an equivalent amount given into the artery, but the rise of arterial pressure was smaller and the rise of portal pressure greater after portal administration. The outflow was almost always temporarily increased, but when large amounts were given the increase was followed by a decrease, a response which was occasionally seen in rabbits after moderate dosage. The volume of the liver invariably decreased. When the arterial flow was stopped the response was similar to that obtained with the circulation reversed (see below).

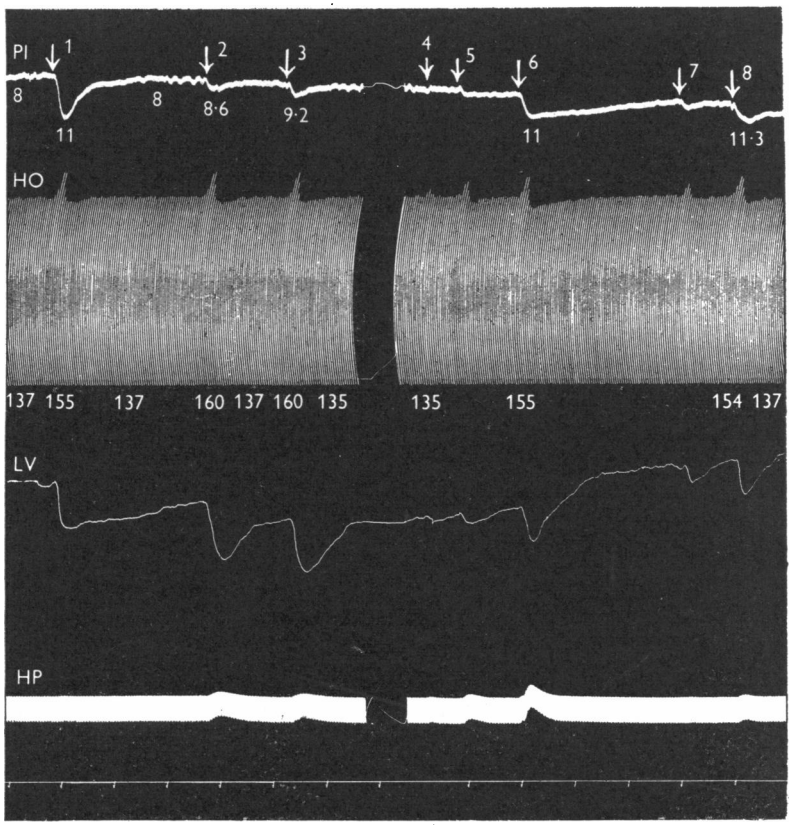


Fig. 1. Cat. Adrenaline 1 μg injected into portal vein (1) and into hepatic artery (2 and 3). ACh injected into hepatic artery (4, 1 μg; 5, 5 μg; 6, 25 μg) and into portal vein (7, 5 μg; 8, 25 μg). PI, portal venous inflow (the fall in the recording indicates a decrease of flow and an increase of pressure); HO, hepatic venous outflow; LV, liver volume; HP, hepatic arterial pressure. Time signal in min. At the start of the tracing portal venous pressure = 9 cm water, and hepatic arterial pressure = 120/110 mm Hg. The numbers under PI indicate the pressure in cm of the portal venous inflow. Those under HO indicate the hepatic venous outflow in ml./min.

Adrenaline injected into the hepatic vein. The direction of the circulation was reversed, the inflow being through the hepatic veins and the outflow from the portal vein: the artery was clamped. The inflow was decreased, the outflow temporarily increased and the liver volume decreased. Sometimes the increase of outflow was followed by a decrease, especially when large amounts of drug were given.

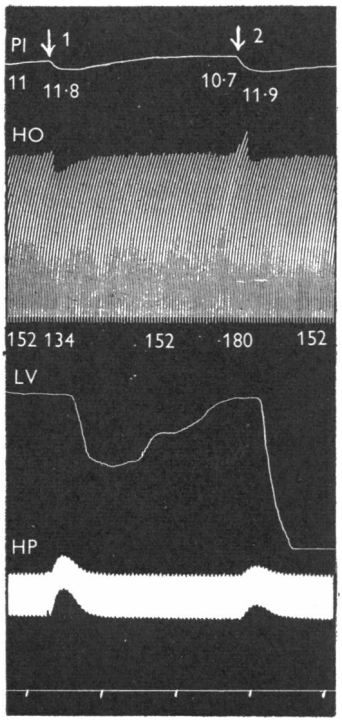


Fig. 2

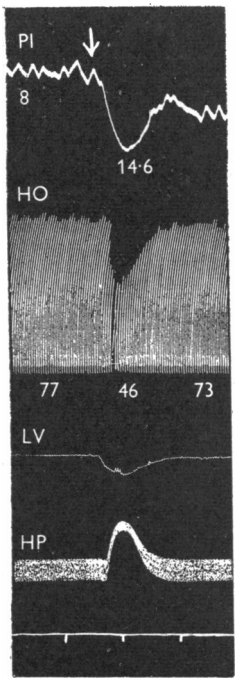


Fig. 3

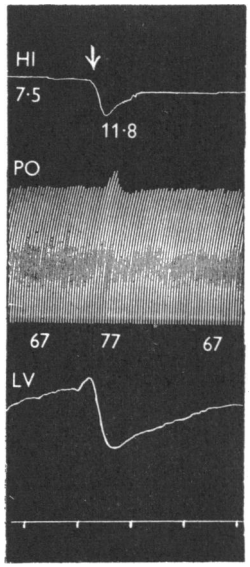
Fig. 2. Cat. (1) ACh 20μg and (2) adrenaline 3μg injected into the hepatic artery. The abbreviations and numbers have the same significance as in Fig. 1. Diastolic arterial pressure varied from 110 to 124 mm Hg.

Fig. 3. Rabbit. Noradrenaline 2μg injected into hepatic artery. The arterial pressure rose from 100 to 120 mm Hg systolic.

Acetylcholine

The vascular responses to ACh of the livers under study showed considerable variation not only from species to species, and from animal to animal of the same species, but also from time to time in the same animal. Thus, during the course of a perfusion the action of ACh on the artery in all species sometimes altered from constrictor to dilator, and in one cat and one rabbit liver this change occurred between two successive injections spaced at about

2 min. In general, however, fairly consistent results were obtained within each species. The amounts of ACh required to produce an effect varied from 1·0 to 10μg.



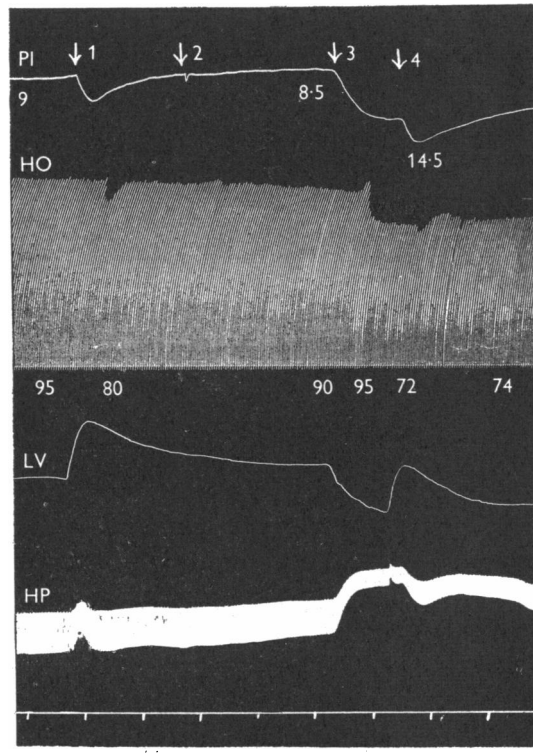


Fig. 4 Fig. 5

Fig. 4. Monkey. Adrenaline 1μg into the hepatic vein. Circulation reversed and artery clamped. HI, hepatic venous inflow; PO, portal venous outflow; LV, liver volume.

Fig. 5. Monkey. ACh 25μg was injected into the hepatic artery (1) and portal vein (2). After adrenaline 25μg was added to the perfusion fluid (3), ACh 25μg was injected again into the hepatic artery (4).

Acetylcholine injected into the hepatic artery. In cats arterial resistance was usually increased by ACh. The portal resistance was invariably increased; the volume diminished, except on one occasion when it increased, and the outflow usually showed a slight increase, followed by a decrease. In monkeys hepatic arterial and portal venous resistances were usually raised, the outflow usually decreased and, despite the reduction in inflow, the volume was almost always increased. In rabbits there was usually a diminution of inflow, volume and outflow. In two animals, however, there was an increase of out-

flow and arterial flow. In all species the reaction to ACh was modified by the presence of circulating adrenaline and noradrenaline, arterial resistance then being diminished by 1–5μg of ACh, though the portal resistance was further increased.

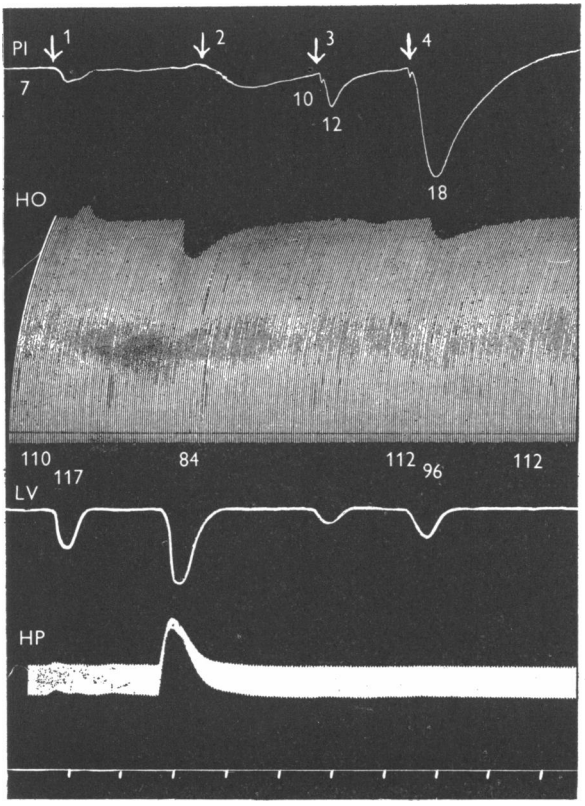


Fig. 6. Rabbit. Liver enclosed in a plethysmograph. The recorded volume changes have been magnified for ease of observation. Adrenaline 2μg injected into portal vein (1) and hepatic artery (2). ACh 5μg injected into hepatic artery (3) and portal vein (4).

Acetylcholine injected into the portal vein. In the monkey huge amounts, e.g. up to 500μg, were sometimes required before an effect was seen. In all experiments a vastly greater amount of the drug was required to give a reaction after portal venous than after arterial administration. The response was an increase of volume, and decrease of outflow, the portal venous inflow being hardly affected. In the cat and rabbit the response to intra-portal ACh was similar to that obtained after intra-arterial injections, but the effect on the portal vein was greater and on the hepatic artery was less. The usual effect in these two animals was an increase followed by a decrease of out-

flow, and a decrease of volume, arterial pressure and portal inflow: the volume occasionally increased when both inflow and outflow were reduced.

Acetylcholine injected into the hepatic vein. In the monkey ACh in large amounts sometimes produced an increased resistance to hepatic venous inflow with a fall in liver volume. No reaction was noted in the few experiments performed on the other species.

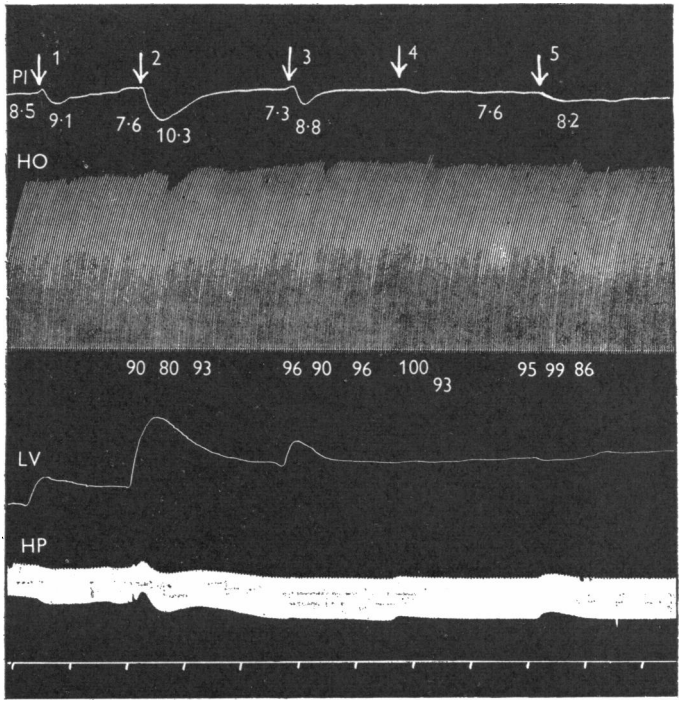


Fig. 7. Monkey. ACh injected into hepatic artery (1, 2 and 3) and portal vein (4 and 5). (1 and 3) 1μg, (2) 25μg, (4) 100μg, (5) 500μg.

Histamine

This drug was given in amounts of 5–25μg, but occasionally huge amounts, e.g. 100μg, were injected.

Histamine injected into the hepatic artery. The response in the monkey was similar but less marked than that in the dog. The arterial flow either remained steady or increased slightly, the portal venous inflow slightly decreased, as did the outflow, and the volume increased. This response was obtained in three-quarters of the livers perfused. When the artery was constricted with adrenaline, histamine produced an increase of flow.

In the rabbit a similar effect was obtained on two occasions, but a common

reaction was a diminution of portal venous inflow, hepatic venous outflow and liver volume, arterial flow being unchanged. The artery, when constricted by adrenaline, was dilated by histamine. In the cat there was very little effect even with amounts of 100μg, except for slight arterial constrictor action; when the artery had been constricted with adrenaline, histamine produced dilatation. In one preparation, however, the outflow was reduced and the volume increased, the portal and arterial flows remaining unchanged when 10μg were injected.

Histamine injected into the portal vein. The response in the monkey was similar, though less well marked than after intra-arterial injection; after the arterial flow had been stopped histamine still produced a rise in volume and fall in outflow. In the rabbit the usual effect was to decrease portal inflow and hepatic venous outflow, the volume decreasing, but in two preparations in which the arterial flow had been stopped there was a rise in volume. In another preparation histamine produced a rise in portal pressure and a fall in volume, an effect also obtained in this liver by injection of compound 48/80. The liver of cats appeared to be very insensitive to histamine, but in one preparation where the arterial flow had been stopped, 10μg produced a great increase in volume, together with a decrease in both inflow and outflow. This reaction was also obtained in another preparation with compound 48/80, though this compound frequently was without effect.

DISCUSSION

In a previous paper (Andrews *et al.* 1955) we described in detail the responses of the perfused canine liver and their interpretation. The experiments described in this paper were performed in order to find whether livers of other species perfused, using our technique, showed any fundamental differences of reaction. Also, in view of a paper published from this department in 1947 (Maegraith, Andrews & Gall) we wished for further information on the power of constriction of the hepatic venous tree. The results were not obtained in great detail, but they do indicate species differences in hepatic vascular reactions, and they suggest that the differences between the reactions of canine and macaque (and therefore possibly human) livers are largely a matter of degree, whilst the reactions of cat and rabbit livers may be considerably different in type.

Adrenaline and noradrenaline. Our original interpretation of reactions was that adrenaline constricted both the hepatic artery and the portal vein, but that the temporarily increased outflow through the hepatic vein was accounted for by dilatation of the hepatic vein. A decrease in liver volume accorded with this hypothesis. We expected therefore that adrenaline introduced into the hepatic vein in the reverse direction would temporarily increase inflow, reduce outflow and raise liver volume. Instead, we have observed the same

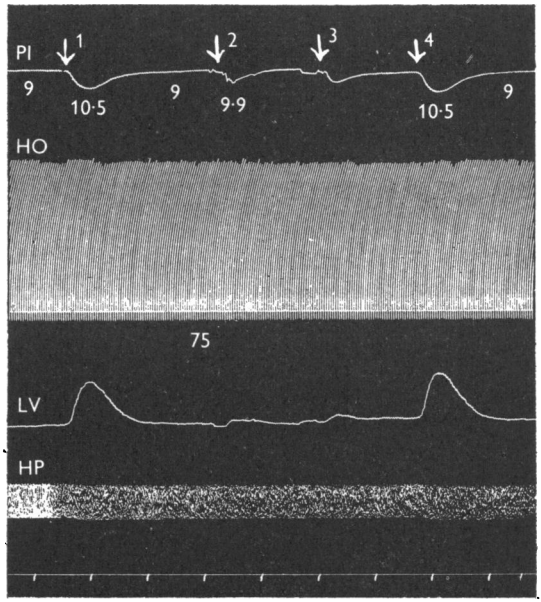


Fig. 8. Monkey. Histamine 10 μg injected into hepatic artery (1 and 4) and into portal vein (2 and 3).

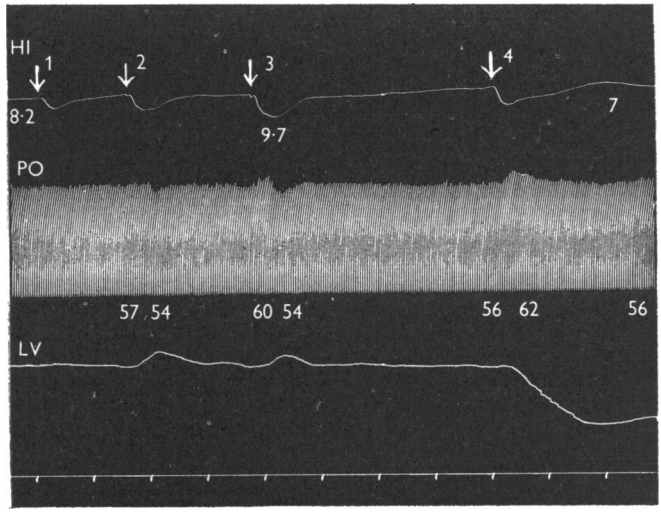


Fig. 9. Monkey. Circulation reversed and artery clamped. Histamine injected into hepatic vein: (1) 5 μg, (2) 10 μg, (3) 25 μg. (4) Noradrenaline 1 μg injected.

changes in inflow, outflow and volume as occurred in the normal direction with a clamped hepatic artery. This has led us to suspect that our original interpretation of dilatation of the hepatic veins was incorrect, and that the increased outflow is probably due to squeezing of blood out of the liver by contraction of vessels.

It appears highly probable that adrenaline constricts the hepatic artery and the portal vein, but it is difficult to see how contraction of hepatic arterial and portal venous radicles would have any effect on blood outflow other than causing a reduction. The sinusoids appear to be incapable of active contraction, but distended hepatic veins occupy a considerable volume in the liver and their contraction alone may well explain the increased outflow.

As the same flow changes are observed after reversing the circulation it is almost certain that adrenaline has the same action on both portal vein and hepatic vein. The observed changes suggest that the reaction of these two vessels to a given dose is of the same magnitude. Unpublished observations indicate that, despite popular acceptance to the contrary, the hepatic venous tree of monkey, cat and rabbit, as well as other mammals (including man), contains considerable amounts of smooth muscle. Thomas & Essex (1949) produce evidence of the contractile power of the hepatic vein in species other than the dog.

Increased outflow from 'squeezing' of the liver can only be temporary, lasting at most a few seconds. Bauer, Dale, Poulsson & Richards (1932) have shown, however, that one dose of adrenaline (100μg) may increase the outflow and maintain it above its original level for as long as 16 min. We have confirmed this finding, though we used smaller amounts of adrenaline, and are convinced that adrenaline can produce dilatation of the hepatic vein under certain circumstances in the dog. In the cat, monkey or rabbit increased outflow never lasted more than 20 sec and was never very great.

We believe, therefore, that the main effect of adrenaline on the hepatic vessels in monkey, rabbit and cat is constrictor, and the responses which we have obtained may be explained on this basis. Arterial flow is readily affected by adrenaline and has been virtually stopped by 20μg, yet the arterial flow forms but a small part of the total hepatic flow. Under the conditions of our experiments portal venous flow is less affected than in the dog, but in one experiment (on a monkey) we found that 50μg adrenaline in 250 ml. perfusing fluid reduced the recorded outflow from 140 to 15 ml./min, the portal pressure being kept constant. The hepatic vessels themselves, therefore, possess considerable powers of constriction, and it is possible that the technical difficulties of working with small animals have prevented us from obtaining really sensitive preparations.

Acetylcholine. The action of ACh on the hepatic circulation has not been extensively studied. Using a transillumination technique, Wakim (1944)

reported that ACh had no direct action on the liver vessels in rats. Seneviratne (1949), however, reported some direct action, but he used huge injections of 200μg each. Bauer *et al.* (1932) reported dilatation of the artery in cats and constriction in goats. McMichael (1933) reported a constrictor effect of ACh on the portal vein of cats with amounts of 1–200μg. Ginsburg & Grayson (1954) have shown that administration of atropine abolishes hepatic vasodilator reflex responses, but this does not necessarily indicate that the only action of ACh is to dilate hepatic vessels. Deysach (1941) reported constriction of hepatic 'sluices' in the cat with 25μg of ACh.

Our results show considerable variation, but certain responses appear to be consistent. The action of ACh on the portal vein was, when present, constrictor, and this response was independent of the presence of circulating adrenaline. The action on the artery in the presence of adrenaline was dilator. In monkeys ACh usually produced well-marked constriction of the hepatic veins. There is evidence also that the drug can produce a constriction of the hepatic veins of other animals.

Histamine. In all species histamine reduced arterial resistance which had been raised by adrenaline. The other responses to histamine were irregular, though hepatic venous constriction was usually produced in monkeys, occasionally in rabbits, and once in cats. The majority of cats' livers appeared to be markedly insensitive to histamine.

Adrenaline and ACh are able to regulate over a wide range the blood flow through the livers of all species so far perfused. From the kymographic records it would appear that the outflow does not alter very greatly, but in order to measure changes of portal flow by our method it was necessary to allow the portal pressure to rise. When the portal pressure was kept constant greater changes in outflow were recorded.

Under the conditions of our experiments it appeared that the reactions of the hepatic vessels of the cat and rabbit were very similar, although the rabbit proved more sensitive to both ACh and histamine. The hepatic vessels of the monkey differed in that the hepatic venous tree was far more sensitive to both ACh and histamine. The hepatic venous tree of the dog appeared to be far more physiologically active than that of the other species so far examined, but there is a definite similarity in the reactions of the hepatic vessels of dogs and monkeys.

SUMMARY

1. In perfused livers adrenaline and noradrenaline produced constriction of the hepatic artery, the portal vein and hepatic vein in monkey, cat and rabbit.

2. Acetylcholine produced constriction of the portal vein in rabbits and cats; in these animals some constriction of the hepatic veins apparently

occurred, but was not marked. In the monkey hepatic venous constriction, leading to an increase of volume, usually occurred. In all species the action on the artery varied, but in the presence of circulating adrenaline acetylcholine produced arterial dilatation.

3. Histamine produced hepatic venous constriction in most monkeys, occasionally in rabbits and once in a cat. The action on other vessels varied but, in the presence of circulating adrenaline, histamine always produced arterial dilatation.

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