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Effects of histamine on hepatic volume (outflow block) in anaesthetized dogs

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Summary

1. Hepatic volume was recorded by plethysmography in dogs anaesthetized with sodium pentobarbitone. Histamine infusions into the hepatic artery or portal vein increased hepatic volume while hepatic sympathetic nerve stimulation decreased the volume. Simultaneous nerve stimulation and histamine infusion decreased hepatic volume.

2. The hepatic volume responses to histamine and nerve stimulation could not be explained on the basis of differences in the responses of the larger hepatic veins and the possibility of a histamine-sensitive sphincter at the junction of the hepatic vein with the inferior vena cava was excluded. Differences in the responses of the hepatic arterial bed contributed to but did not explain the different hepatic volume responses.

3. It is suggested that histamine produced an intense and specific constriction of the sublobular veins with passive distension of post-sinusoidal venules, sinusoids and portal venules while hepatic nerve stimulation produced a uniform and generalized constriction of both pre- and post-sinusoidal vessels.

4. Intravenous infusions of histamine produced a marked hypotension but small variable effects on hepatic volume. It is concluded that hepatic pooling is not the cause of the hypotension produced by intravenous histamine and that significant hepatic pooling will only be produced by mechanisms which release endogenous histamine within the liver.

Introduction

Histamine and agents which release endogenous histamine cause the liver to distend with blood in dogs but not in cats. Ever since Mautner & Pick described this phenomenon in 1915, it has been the subject of two controversies which have been summarized in an excellent review by Rocha e Silva (1966). The first controversy concerns the relative roles of hepatic pooling and peripheral vasodilatation in the hypotension produced by intravenous infusions of histamine. Although general opinion now favours peripheral vasodilatation as the major cause, the hepatic pooling has never been studied quantitatively and the problem remains unsettled. The second controversy concerns the mechanism of the hepatic pooling. Earlier workers did not agree on whether the response was due to generalized hepatic venous constriction or to contraction of a sphincter mechanism at the junction of the hepatic veins and the inferior vena cava (Bauer, Dale, Poulsson & Richards, 1932 ; Arey, 1941 ; Thomas & Essex, 1949 ; Walker, MacDonald & Pickard, 1960). The histological studies by Elias (1953) suggested that histamine contracted the sublobular veins. Stimulation of the hepatic sympathetic nerves caused a decrease in hepatic

blood volume and since up to 50% of the hepatic blood volume could be expelled, this response was attributed to widespread contraction of the intrahepatic vessels (Greenway, Stark & Lautt, 1969; Greenway & Oshiro, 1972). Thus we have the interesting situation where constriction of the hepatic venous bed induced by sympathetic nerve stimulation results in a decreased hepatic blood volume while that induced by histamine results in an increased volume. Clearly the sites of action of histamine and of noradrenaline released by the hepatic nerves must be different.

We have attempted to clarify and quantitate the response to histamine in the canine liver and to resolve these two controversies.

Methods

Dogs were anaesthetized by intravenous injection of sodium pentobarbitone (Abbott Laboratories, 30 mg/kg body weight). When reflex limb and swallowing movements returned, additional doses (2 mg/kg) were given through a cannula in a forelimb cutaneous vein. The trachea was intubated with a cuffed endotracheal tube and mean arterial pressure was recorded from a femoral artery. The abdomen was opened along the mid-line and mean portal pressure was recorded from a cannula inserted through a small branch of the splenic vein. All recordings were made on a polygraph (Grass Instrument Co.). The gastroduodenal branch of the hepatic artery was cannulated to allow infusions of histamine into the hepatic artery. The hepatic nerves were dissected from round the hepatic artery, ligated and the distal end was inserted into a ring electrode for stimulation (Greenway & Oshiro, 1972).

Hepatic volume was recorded by the method described previously (Greenway, Stark & Lautt, 1969; Greenway & Oshiro, 1972). The ligaments connecting the central and left lobes of the liver to the diaphragm and mediastinum were ligated and cut and the liver with the exception of the right lateral and caudate lobes, was inserted into a Perspex plethysmograph. To obtain sufficient space for the large plethysmograph required for the dog, it was necessary to institute artificial positive pressure ventilation and to split the lower end of the sternum for about 10 cm. The artificial ventilation was adjusted so that spontaneous diaphragmatic movements were almost suppressed. The vessels to and from the liver remained intact and passed through a 5 cm diameter aperture which was sealed with a plasticized hydrocarbon gel (Plastibase, Squibb). The plethysmograph was filled with Ringer-Locke solution at 37° C and connected to a float recorder which operated an isotonic transducer (Harvard Apparatus Co. Model 356). The pressure within the plethysmograph was adjusted to zero relative to the hilum of the liver.

In other experiments, hepatic venous pressure within the liver was recorded. A catheter was introduced through the left jugular vein and passed down until its tip could be felt within the inferior vena cava at the diaphragm. The tip was then guided into a hepatic vein for a variable distance and mean pressure was recorded. At the end of each experiment, the exact position of the catheter tip was determined relative to the junction of the hepatic vein and the inferior vena cava. The vein was always much larger than the catheter (external diameter 1·2 mm) and the pressures recorded were not wedged pressures. In other experiments, hepatic arterial flow was recorded by placing a non-cannulating flow probe from an electromagnetic flowmeter (Nycotron, Oslo) on the common trunk of the hepatic and gastroduodenal arteries after ligation and cannulation of the gastroduodenal branch

(see above). Frequent zero checks were made and calibration was carried out as described previously (Greenway & Oshiro, 1972).

In experiments on isolated strips of portal and hepatic veins, the dogs were anaesthetized with sodium pentobarbitone and the veins were removed into Krebs–Henseleit solution at 4° C. Uniform circular strips (10 mm long by 4 mm wide) were cut and suspended in a 10 ml organ bath containing Krebs–Henseleit solution at 37° C bubbled with 95% O_2:5% CO_2. The composition of the solution was, in mM/l.: NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, KH_2PO_4 1·1, $NaHCO_3$ 25, glucose 11. Isotonic recordings under 1 g tension were made on a kymograph by a lever with a magnification of 10; results are expressed as actual shortening of the strips.

Histamine acid phosphate (British Drug Houses) and (−)-noradrenaline bitartrate (Winthrop Laboratories) were dissolved in 0·9% w/v NaCl solution immediately before use. All doses are expressed as the free base.

Results

In the dogs (7·2 ± 0·2 kg body weight), the mean femoral arterial pressure was 114 ± 4 mmHg (mean ± S.E.) before and 103 ± 6 mmHg after the reported observations were made. Mean portal pressure was 7·3 ± 0·5 mmHg before and 9·0 ± 1·3 mmHg after. The mean weight of the liver was 37 g/kg body weight and of this 69 ± 1·5% was included in the plethysmograph.

Hepatic volume responses to arterial or portal infusions of histamine

The hepatic volume response to intra-arterial or intra-portal infusions of histamine were studied in 9 dogs. The responses to each dose by either route of administration did not differ significantly and the data were pooled. Dose-response curves were obtained over the range (1–20 μg histamine/min)/kg. At each dose level, the infusion was continued for 5 min and 20 min elapsed between each infusion. The responses to infusion of histamine and to stimulation of the hepatic nerves in one animal are shown in Figure 1. Histamine infusion caused an increase in hepatic volume while nerve stimulation caused a decrease. The mean dose-response curve for histamine in all the animals is shown in Figure 2. With small doses of histamine, the response was reproducible and the volume recovered

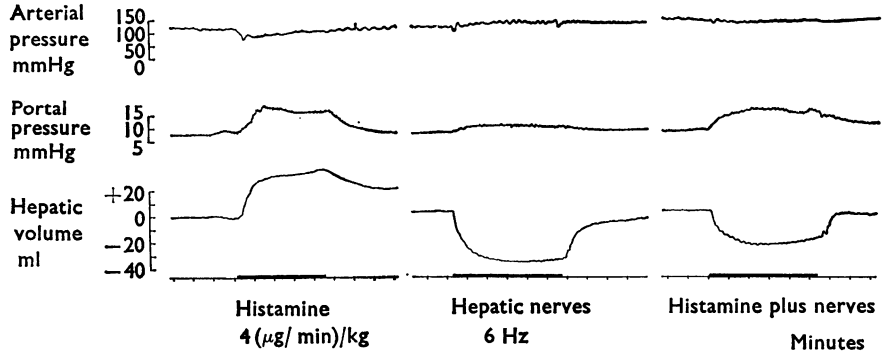

FIG. 1. The responses of arterial and portal pressures and hepatic volume in one dog to infusion of histamine into the hepatic artery, stimulation of the hepatic nerves and simultaneous infusion and nerve stimulation.

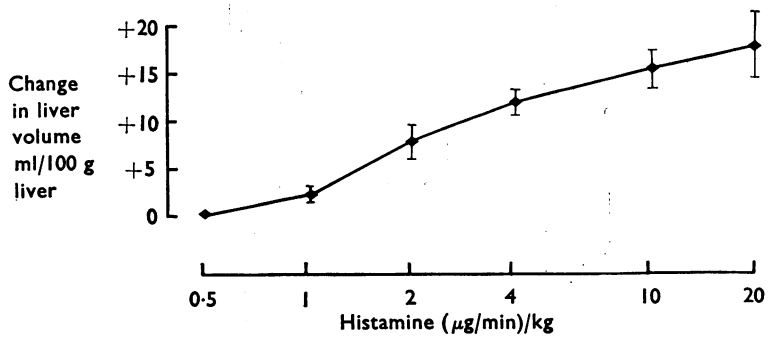

FIG. 2. Dose-response curve for intra-arterial infusions of histamine on hepatic volume. Each point represents the mean ± S.E. of results in 9 dogs.

on cessation of the infusion. With large doses, the recovery was prolonged (Fig. 1) and repeated infusions of the same dose gave progressively smaller responses. Also, the responses to larger doses were accompanied by decreases in arterial pressure.

In 4 dogs, control responses to intra-arterial infusions of histamine (4 (μg/min)/kg) and stimulation of the hepatic nerves (6 Hz) were first obtained, and then nerve stimulation and histamine infusion were carried out simultaneously. The results in one experiment are shown in Figure 1. Hepatic volume always decreased during the simultaneous procedure but the decrease was smaller than when the nerves were stimulated alone. In all the experiments, the mean decrease in hepatic volume during simultaneous stimulation and infusion was 58 ± 4% of the decrease during nerve stimulation alone. Arterial pressure did not decrease during simultaneous infusion and stimulation.

Hepatic venous pressure responses

Nine experiments were carried out to exclude the possibility that the increase in hepatic volume produced by histamine was due to contraction of a sphincter in the large hepatic veins. Hepatic venous pressure was recorded at various distances from the junction of the hepatic vein and inferior vena cava. The pressure recorded during the control period was greater when the catheter tip lay further within the liver. During intra-arterial infusions of histamine (2 (μg/min)/kg) and during hepatic nerve stimulation (6 Hz), hepatic venous pressure did not increase significantly ($P > 0{\cdot}1$, paired t test) when the catheter lay 0–4 cm from the junction of the hepatic vein and inferior vena cava but when the catheter was 4–8 cm within the hepatic vein, the increases were significant ($P < 0{\cdot}01$, paired t test). The results are shown in Figure 3. The increases in hepatic venous pressure were very similar for both histamine and hepatic nerve stimulation.

Isolated organ experiments

To obtain further data on whether the responses of hepatic venous smooth muscle to histamine and noradrenaline were different, isolated strips of canine hepatic veins were studied *in vitro*. Four strips from each of 6 dogs were prepared. For 2 strips, cumulative dose-response curves were obtained for histamine and 2 h later for noradrenaline. For the other 2 strips the order of the drugs was reversed. The results are shown in Figure 4. The dose-response curves to histamine and noradrenaline were not significantly different.

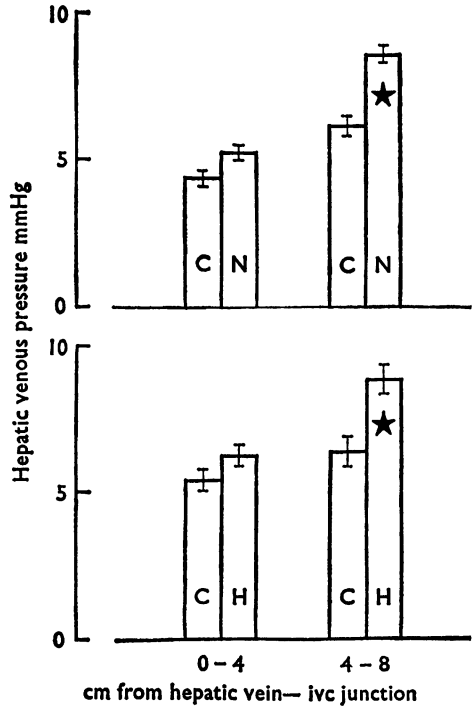

FIG. 3. The hepatic venous pressures recorded from catheters 0–4 or 4–8 cm from the junction of the hepatic vein and inferior vena cava before (C) and during hepatic nerve stimulation (N) or histamine infusion (H). Each value represents the mean ± S.E. of 9 experiments and the asterisks denote $P<0{\cdot}01$, paired t test.

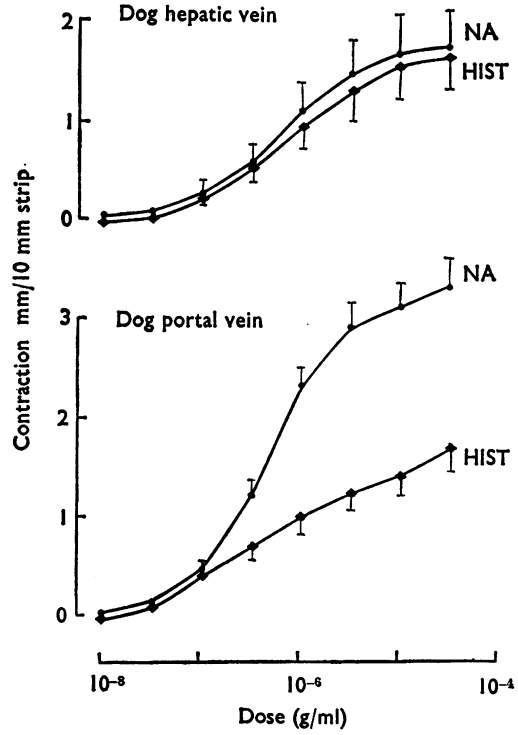

FIG. 4. Dose-response curves to histamine (HIST) and noradrenaline (NA) on hepatic venous and portal strips. The response is the actual contraction by the 10 mm strips and each point is the mean ± S.E. of 24 strips from 6 dogs.

Similar experiments were carried out with strips from the portal veins of 6 dogs and the results are shown in Figure 4. The minimal doses of histamine and noradrenaline required to elicit a response were similar but at higher doses, the response to histamine was smaller than that to noradrenaline.

Hepatic arterial flow

Hepatic arterial flow responses to intra-arterial infusions of histamine were recorded in 7 dogs. Histamine caused hepatic arterial vasodilatation and maximal responses were obtained at 1 (μg histamine/min)/kg when the flow increased to $150 \pm 5\%$ control. With large doses of histamine (>10 (μg/min)/kg) the increase in flow was smaller but these doses caused a fall in arterial pressure. The decrease in hepatic resistance (pressure gradient divided by flow) was the same at all doses above 1 (μg/min)/kg.

To determine whether an increase in arterial flow was necessary for histamine to increase hepatic volume, the responses to infusions of histamine into the portal vein were recorded before and during complete occlusion of the hepatic artery in 6 dogs. The increase in hepatic volume during occlusion was $54 \pm 9\%$ of the increase before occlusion.

Effects of intravenous infusions of histamine

Intravenous infusions of histamine (2–20 (μg/min)/kg) were given in 6 dogs. In 3 dogs, all doses caused a decrease in hepatic volume, in 1 dog all doses caused an increase and in 2 dogs the hepatic volume decreased at low doses and increased at 20 (μg/min)/kg. In all 6 dogs, histamine caused a dose-dependent fall in arterial pressure. The mean results are shown in Table 1. It is clear that the fall in arterial pressure was not caused by pooling of blood in the liver. On 8 occasions in 3 dogs where histamine increased hepatic volume at least in high doses, the histamine infusion was repeated while the hepatic volume increase was prevented by simultaneous stimulation of the hepatic nerves. The fall in arterial pressure was not significantly altered by prevention of the increase in hepatic volume.

TABLE 1. *Effects of intravenous infusions of histamine on arterial pressure and hepatic volume (means ± S.E.) in 6 dogs*

Dose (μg/min)/kg	Arterial pressure (mmHg) Before	During	(Paired S.E.)	Change in hepatic volume (ml/100 g)
2	111	99*	(2·3)	$-0{\cdot}2 \pm 2{\cdot}0$
4	103	80*	(4·0)	$-0{\cdot}8 \pm 3{\cdot}8$
10	106	65*	(4·9)	$-2{\cdot}6 \pm 2{\cdot}5$
20	109	48*	(5·3)	$6{\cdot}3 \pm 5{\cdot}9$

* $P < 0{\cdot}05$ paired t test.

Discussion

Although the occurrence of outflow block in the dog liver is well known, the mechanism has been difficult to study due to the problem of recording hepatic volume quantitatively and continuously. In the dog, the plethysmographic technique has the disadvantage that the chest must be opened but the responses to hepatic nerve stimulation were very similar to those in cats breathing spontaneously (Greenway *et al.*, 1969 ; Greenway & Oshiro, 1972).

Histamine infusions into the hepatic artery or portal vein produced a dose-dependent increase in hepatic volume in dogs. This response was not seen in cats (Greenway & Lautt, 1972). The fall in arterial pressure which accompanied the response in the dogs appeared to be secondary to the pooling of blood in the liver and not due to recirculation of the histamine, since it was absent when the pooling was prevented by simultaneous stimulation of the hepatic nerves (Fig. 1). The intense congestion during histamine infusion cannot be explained on the basis of relaxation of the venous bed and the response must involve active constriction of some part of the vascular smooth muscle. Clearly the sites of action of histamine and sympathetic nerve stimulation must be different since the nerves decrease hepatic volume and antagonize outflow block. Several possible sites of action of histamine may be considered.

The possibility of a sphincter at the junction of the hepatic vein and inferior vena cava has never been conclusively disproved. The existence of sphincter-like smooth muscle bands in the dog but not the cat is well known (Arey, 1941; Thomas & Essex, 1949; Elias & Sherrick, 1969) but it has never been shown that this smooth muscle responds to histamine but not to hepatic nerve stimulation. Fluorescence studies have demonstrated that this smooth muscle has a rich adrenergic innervation which is unusual in that the nerve fibres penetrate the media of the vessels (Ungváry & Donáth, 1969). In our experiments, the control hepatic venous pressure and the pressure during histamine infusions progressively increased as the catheter was introduced up the hepatic vein but it was clear that both histamine and hepatic nerve stimulation produced qualitatively and quantitatively similar responses. Thus we cannot account for the different effects on hepatic volume on the basis of responses in the large hepatic veins. The similarity of the responses of isolated hepatic vein strips to histamine and noradrenaline supports this conclusion. The absence of any significant increases in hepatic venous pressure when the cannula was 0–4 cm within the hepatic veins excluded the possibility of a sphincter at the junction of the hepatic vein and inferior vena cava.

It has been clearly shown that the site of action of histamine is post-sinusoidal. Mahfouz & Geumei (1967) perfused the canine liver through the hepatic artery and allowed the effluent to drain through both portal and hepatic veins. Histamine caused the proportion draining through the portal vein to increase markedly while noradrenaline did not alter this proportion. This suggested that histamine caused a marked increase in post-sinusoidal resistance while noradrenaline had almost equal effects on pre- and post-sinusoidal venules. The responses of the isolated portal strips confirm that portal smooth muscle is relatively insensitive to histamine. Since we have excluded the large hepatic veins as a specific site for histamine, it is reasonable to suggest that the constriction occurs in the smaller sublobular veins as shown in histological studies by Elias (1953). If histamine also constricted the small hepatic and portal venules, congestion of the liver would not occur. Thus our data, with that in the literature, suggest that histamine causes a specific and intense constriction of the sublobular veins with resultant congestion of the vessels up-stream, while the sympathetic nerves cause a uniform constriction of both hepatic and portal venules with expulsion of blood from the liver. If histamine infusion and nerve stimulation are applied simultaneously, the passive congestion is prevented except possibly in the sinusoids which are non-contractile and the hepatic volume response is similar but somewhat smaller than that during nerve stimulation alone. Further support for this hypothesis would be the demonstration that hist-

amine causes trans-sinusoidal fluid filtration due to the raised sinusoidal hydrostatic pressure. Certainly hepatic volume tends to increase steadily after the initial rapid increase during histamine infusion and this slow increase is also seen during simultaneous histamine infusion and nerve stimulation (Fig. 1). However, we have not yet been able to prove conclusively that these slow increases in volume are due to trans-sinusoidal filtration rather than delayed compliance of the venous bed. The difficulties of such studies have been discussed previously (Greenway & Lautt, 1970).

The hepatic volume responses to intravenous infusions of histamine were small and variable even though arterial pressure decreased by up to 50 mmHg. If any hepatic volume increase was prevented by simultaneous nerve stimulation, the hypotension was not significantly altered. The decreases in hepatic volume which were often seen may have been responses to release of adrenal medullary hormones during histamine infusion (Staszewska-Barczak & Vane, 1965). Thus it seems clear that hepatic pooling plays no significant part in the hypotension due to intravenous infusions of histamine. Conversely, it follows that circulating histamine is unlikely to be a cause of hepatic outflow block which occurs in dogs under a variety of conditions such as endotoxin administration, anaphylactic reactions and hypoxia (reviewed by Greenway & Stark, 1971). Since histamine is the only vasoactive substance known to produce outflow block and since the liver of the dog contains large amounts of histamine (Ojers, Holmes & Dragstedt, 1941; Riley & West, 1953), the hypothesis that histamine release within the liver is the final common mechanism for outflow block remains attractive but not proved.

In these experiments, hepatic volume increased by up to 17 ml/100 g liver during histamine infusion. This represents a 50% increase over the normal blood content of 31 ml/100 g (Greenway & Oshiro, 1972), and is about 8% of the total blood volume of the dog. It is as difficult for us as it was for Sir Henry Dale in 1929 to avoid the temptation to attribute a physiological significance to this mechanism in the control of blood volume distribution and venous return, but no evidence to support this has been produced in the intervening years. Perhaps one contribution of this paper is to describe a method for quantitative measurement of liver volume which allows these problems to be studied.

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REFERENCES

AREY, L. B. (1941). Throttling veins in the livers of certain mammals. *Anat. Rec.*, **81**, 21–33.

BAUER, W., DALE, H. H., POULSSON, L. T. & RICHARDS, D. W. (1932). The control of circulation through the liver. *J. Physiol., Lond.*, **74**, 343–375.

DALE, H. H. (1929). Local vasodilator reactions. *Lancet*, **i**, 1233–1237.

ELIAS, H. (1953). Functional morphology of the liver. *Res. Serv. Med.*, **37**, 26–51.

ELIAS, H. & SHERRICK, J. C. (1969). *Morphology of the liver*, New York: Academic Press.

GREENWAY, C. V. & LAUTT, W. W. (1970). Effects of hepatic venous pressure on transsinusoidal fluid transfer in the liver of the anesthetized cat. *Circulation Res.*, **26**, 697–703.

GREENWAY, C. V. & LAUTT, W. W. (1972). Effects of infusions of catecholamines, angiotensin, vasopressin and histamine on hepatic blood volume in the anaesthetized cat. *Br. J. Pharmac.*, **44**, 177–184.

GREENWAY, C. V. & OSHIRO, G. (1972). Comparison of the effects of hepatic nerve stimulation on arterial flow, distribution of arterial and portal flows and blood content in the livers of anaesthetized cats and dogs. *J. Physiol., Lond.* (in press).

GREENWAY, C. V. & STARK, R. D. (1971). Hepatic vascular bed. *Physiol. Rev.*, **51**, 23–65.

GREENWAY, C. V., STARK, R. D. & LAUTT, W. W. (1969). Capacitance responses and fluid exchange in the cat liver during stimulation of the hepatic nerves. *Circulation Res.*, **25**, 277–284.

MAHFOUZ, M. & GEUMEI, A. (1967). Pharmacodynamic of intrahepatic circulation in shock. *Surgery*, **61**, 755–762.

OJERS, G., HOLMES, C. A. & DRAGSTEDT, C. A. (1941). The relation of the liver histamine to anaphylactic shock in dogs. *J. Pharmac. exp. Ther.*, **73**, 33–38.

RILEY, J. F. & WEST, G. B. (1953). The presence of histamine in tissue mast cells. *J. Physiol., Lond.*, **120**, 528–537.

ROCHA E SILVA, M. (1966). Action of histamine upon the circulatory apparatus. *Handbook of Experimental Pharmacology*, **18**, 238–293.

STASZEWSKA-BARCZAK, J. & VANE, J. R. (1965). The release of catecholamines from the adrenal medulla by histamine. *Br. J. Pharmac. Chemother.*, **25**, 728–742.

THOMAS, W. D. & ESSEX, H. E. (1949). Observations on the hepatic venous circulation with special reference to the sphincteric mechanism. *Am. J. Physiol.*, **158**, 303–310.

UNGVÁRY, G. & DONÁTH, T. (1969). On the monoaminergic innervation of the liver. *Acta anat.*, **72**, 446–459.

WALKER, W. F., MACDONALD, J. S. & PICKARD, C. (1960). Hepatic vein sphincter mechanism in the dog. *Br. J. Surg.*, **48**, 218–220.

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