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VASCULAR RESPONSES OF THE SPLEEN TO NERVE STIMULATION DURING NORMAL AND REDUCED BLOOD FLOW

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SUMMARY

1. The splenic artery flow, the splenic weight and the arterial blood pressure were recorded in cats anaesthetized with sodium pentobarbitone.

2. Oscillations in splenic artery flow and splenic weight were observed. Following occlusion and release of the splenic artery, there was a brief increase in flow to above the pre-occlusion level and the oscillations in flow were greatly increased in amplitude. It is suggested that the brief increase is a consequence of the reduction of arterial pressure and that the oscillations are due to synchronization of rhythmic activity of smooth muscle within the spleen.

3. Stimulation of the splenic nerves resulted in decreases in splenic artery flow and splenic weight. The size of the responses varied with the frequency of stimulation and maximum responses in both flow and weight were obtained at about 3 impulses/sec.

4. After stimulation for 10 min, the splenic weight response was maintained while the flow response showed some recovery towards the control level.

5. When the splenic artery flow was reduced to about half the control level for periods up to 2 hr, the flow and weight responses to stimulation of the splenic nerves remained unchanged; the significance of this after a haemorrhage is discussed.

6. Intravenous administration of atropine or propranolol did not affect the responses to nerve stimulation. After phenoxybenzamine, nerve stimulation caused a smaller decrease in splenic weight, while the splenic artery flow increased to above the control level. This increase was unaffected by atropine but abolished by propranolol.

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INTRODUCTION

In recent years several workers have studied quantitatively the resistance and capacitance responses in various vascular beds (e.g. Mellander, 1960; Folkow, Lewis, Lundgren, Mellander & Wallentin, 1964; Greenway, Lawson & Mellander, 1967), but the spleen has not been studied in this way. It appears to have an important capacitance function in many species since it shows a marked decrease in size after haemorrhage (Barcroft & Stephens, 1927; Lewis, Werle & Wiggers, 1943). Although changes in splenic volume in response to nerve stimulation have been studied in a semi-quantitative way (Celander, 1954), the blood flow and volume changes have not been studied simultaneously in the spleen with an uncannulated arterial supply. Recording of the spleen volume with an oncometer is not easy since the venous drainage is readily obstructed. The capacitance function of the spleen can be measured more easily and accurately by recording its weight with an isometric transducer (Stish, MacLean & Visscher, 1956).

The aim of the present experiments was to examine the splenic flow and weight responses to stimulation of the splenic nerves at various frequencies and to investigate possible impairment of these responses during periods of reduced splenic blood flow. The effects of adrenergic blocking agents and some incidental observations on oscillations in splenic artery flow are also described.

METHODS

Cats were anaesthetized by intraperitoneal injection of sodium pentobarbitone (30 mg/kg, Abbott Laboratories) and, when reflex limb and ear movements returned, additional doses of 15 mg sodium pentobarbitone were given through a cannula in a forearm vein. The trachea was cannulated, the abdomen opened by a mid line incision and the spleen exposed. The vessels in the gastro-splenic ligament and the inferior part of the lieno-renal ligament were tied and the ligaments divided to allow mobilization of the spleen. The vessels to the body and tail of the pancreas were tied.

The splenic blood flow was recorded by a method similar to that used for hepatic blood flow (Greenway *et al.* 1967). The coeliac artery was exposed at its origin from the aorta and the left gastric and hepatic branches tied. A 2 mm diameter electromagnetic flowmeter probe and a micrometer-controlled artery clamp were placed round the coeliac artery and carefully positioned to avoid obstruction to the flow. The clamp had three functions: it steadied the flow probe, provided a means for checking the flowmeter zero and was used when necessary to control the splenic artery pressure. The flow probe was connected to a Nycotron blood flow meter and the output from this was recorded continuously on a Devices pen recorder. The system was calibrated at the end of each experiment as previously described.

A 5 mm length of the splenic nerves was separated from the splenic artery, tied and divided. The peripheral end of the nerves was inserted through a 2 mm diameter ring electrode of Perspex, within which circles of platinum wire formed the bipolar electrodes (Greenway *et al.* 1967). Thus the majority of the nerve fibres to the spleen were divided. No attempt was made to ensure complete denervation and the adventitia of the splenic artery was not removed.

The spleen was lifted through the abdominal incision, wrapped in gauze moistened with Ringer–Locke solution and surrounded by thin polythene sheet. It was placed on a cradle of Tubegauz (Scholl Mfg Co. Ltd.) stretched on to polythene-covered wire which was bent to the shape of the spleen and indented to accommodate, and prevent undue tension on, the vascular pedicle. The cradle was suspended just above the abdominal wall from a strain gauge transducer (Statham G1-8-350) connected to the Devices pen recorder. A thermometer lying between the spleen and covering gauze recorded the surface temperature, which did not vary by more than 2° C from the rectal temperature. At the end of each experiment the pedicle was tied and cut. Any difference in recorded weight caused by this procedure was due to tension on the pedicle and this weight (0–17 g, mean 8 g) was subtracted from the values obtained during the experiment. It was assumed that the pedicle tension remained constant during each experiment. This assumption seems reasonable since, if the pedicle was tied but not cut, spleen weight remained constant over several hours. On rare occasions, when an accidental disturbance which appeared likely to alter the pedicle tension occurred, the weight responses obtained before this disturbance were discarded. The system was calibrated by adding weights to the empty cradle. The stability of the weighing system was tested by leaving weights on the cradle for periods up to 8 hr while continuously recording the response. The pen deflexion did not vary by more than 3 %.

Mean arterial pressure was recorded through a cannula in the femoral artery by a Devices/C.E.C. pressure transducer connected to the pen recorder. A cannula in the proximal end of the hepatic artery was used to record the splenic artery pressure distal to the arterial clamp. In some experiments, portal vein pressure was obtained by catheterization of a small branch of the superior mesenteric vein in such a way that the cannula tip lay in the portal vein close to the entry of the splenic vein. All pressures were referred to the level of the middle of the right atrium determined post mortem.

Propranolol HCl (1 mg/kg, I.C.I.), atropine sulphate (1 mg/kg, British Drug Houses), phenoxybenzamine (10 mg/kg, Smith, Kline & French) and promethazine HCl (2 mg/kg, May & Baker) were dissolved in 0·9 % (w/v) sodium chloride solution.

RESULTS

Control values

Experiments were carried out on thirty-two cats (weights 2·1–4·5 kg, mean 2·9 kg). The mean arterial pressures at the beginning of the experiments ranged from 120 to 185 mm Hg (mean 141 mm Hg). In experiments in which no drugs were given, the arterial pressure usually changed little over 3 hr; the largest changes were a rise of 15 mm Hg and a fall of 30 mm Hg. Portal pressure ranged from 3·5 to 11 mm Hg (mean 6 mm Hg).

Splenic weight. The weights of the denervated spleens at the beginning of the experiments ranged from 12 to 56 g (mean 28 g). This represents a range of 5·8–19 g/kg body wt. (mean 11 g/kg). During the course of the experiments the weight decreased by 0–9 g (mean 5·2 g) over 3 hr.

Splenic artery flow. Flow in the splenic artery at the beginning of the experiments varied from 12 to 88 ml./min (mean 29 ml./min). This represents a range of 4·6–32 ml./min. kg body wt. (mean 10 ml./min. kg). The flow can also be expressed in terms of spleen weight and this gives a range of 41–163 ml./min. 100 g spleen (mean 88 ml./min. 100 g). In experi-

ments in which no drugs were given, the flow changed little over 3 hr; the greatest changes were an increase of 2 ml./min and a decrease of 13 ml./min and the mean value of the variations in all the animals was a decrease of 5·1 ml./min.

Oscillations in flow and weight

During these experiments, large, slow oscillations in the splenic artery flow and splenic weight were observed. They were most obvious following occlusion and release of the coeliac artery (thirty-two cats) as shown in Fig. 1. They also occurred after splenic nerve stimulation (twenty-eight

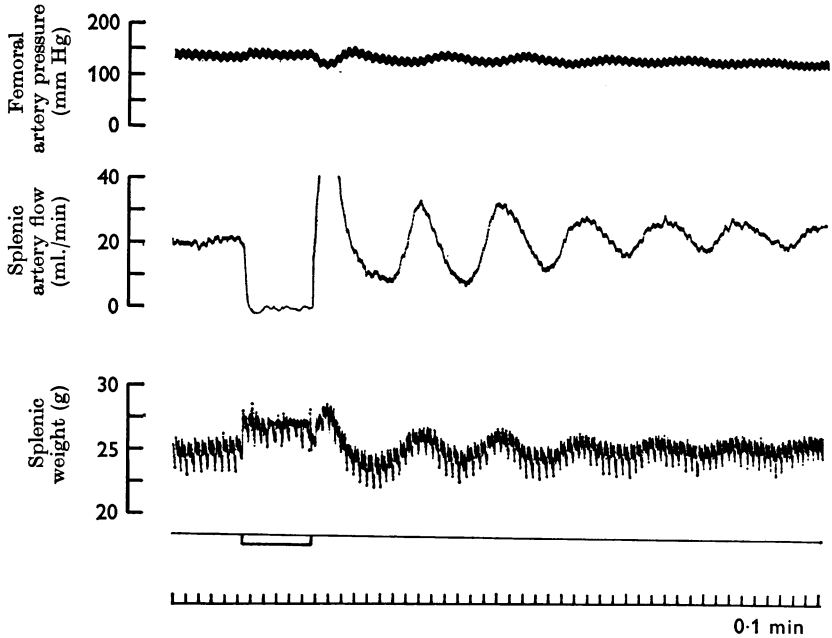

Fig. 1. Cat, wt. 2·6 kg. The response to occlusion and release of the splenic artery. Ordinates, femoral artery pressure, splenic artery flow and splenic weight. Abscissa, time, 10 intervals/min. The splenic artery was occluded during the period shown by the signal. The change in splenic weight during the occlusion is an artifact due to the clamping procedure.

cats, Fig. 3), on injection of blood (three cats) and after occlusion and release of the carotid arteries (five cats). These oscillations persisted after intravenous injection of atropine (four cats), propranolol (six cats), promethazine (three cats) and phenoxybenzamine (eight cats).

Following occlusion and release of the coeliac artery, the first oscillation was always the greatest in amplitude and thereafter the size progressively decreased over 4–10 min. The wave-length of the oscillations (excluding the first peak) ranged from 30 to 43 sec (mean 35 sec). The

oscillations after an arterial occlusion of 30–60 sec were studied quantitatively in five cats; the first peak flow was 224–392 % (mean 312 %) of the pre-occlusion level, while the first trough was 17–81 % (mean 47 %) of the control. In two cats, when the period of occlusion was varied between 5 and 120 sec, the first peak flow increased progressively with the duration of occlusion until it became maximal with occlusions of 30 sec or longer.

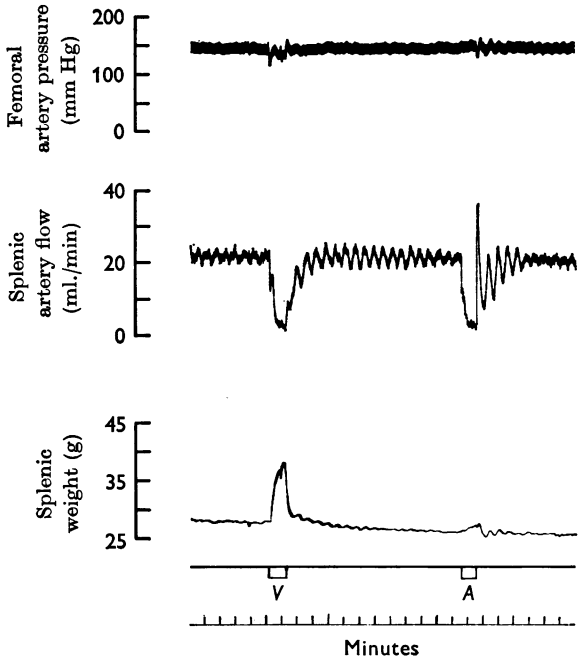

Fig. 2. Cat, wt. 2·6 kg. The responses to occlusions of the splenic vein and artery. Ordinates, femoral artery pressure, splenic artery flow and splenic weight. Abscissa, time in min. When the splenic vein was occluded for the period *V*, the blood flow fell to a low level. This same blood flow was then produced for the period *A* by partly occluding the splenic artery.

The wave-length of the subsequent oscillations was independent of the duration of the occlusion. In two cats, the effects of reducing the flow by occlusion of either the splenic vein or the artery were compared. Owing to the presence of small venous anastomoses, occlusion of the splenic vein did not completely stop flow in the splenic artery. The same flow was then produced by partially occluding the splenic artery. Figure 2 shows one such experiment. The first peak in the flow was completely absent after release of the occluded splenic vein while the oscillations, although smaller, were still present.

In many experiments, oscillations were present in the splenic weight

and these were synchronous with the oscillations in the flow. They were usually much smaller in amplitude; the peak was less than 120 %, and the trough more than 88 %, of the control weight (Figs. 1, 2 and 3).

Stimulation of the splenic nerves

In eighteen cats, the splenic nerves were stimulated for 3-min periods at frequencies between 0·5 and 5 impulses/sec with supramaximal stimuli (15 V, 1 msec duration, see below). Figure 3 shows that splenic artery flow and splenic weight decreased during stimulation and that the size of these responses varied with the frequency of stimulation. After cessation

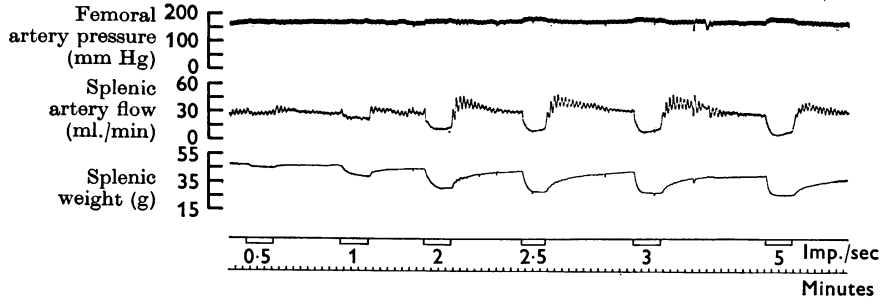

Fig. 3. Cat, wt. 3·7 kg. The response to stimulation of the splenic nerves. Ordinates, femoral artery pressure, splenic artery flow and splenic weight. Abscissa, time in min. The splenic nerves were stimulated (15 V, 1 msec duration) during the periods shown by the signals at the frequencies given by the numbers below each period.

of stimulation, the splenic artery flow rapidly recovered to the control level; large oscillations in flow occurred and then slowly decreased in amplitude. The splenic weight recovered much more slowly. During stimulation, mean splenic arterial pressure rose by 0–25 mm Hg. The portal vein pressure rose 1–2 mm Hg at the onset of stimulation and then returned to the control level.

In twelve cats an attempt was made to dissociate the flow and weight responses by slowly increasing the voltage of stimulation but this proved impossible. The reduction in flow and weight began at the same voltage and reached a maximum with a stimulus of 10 V, 1 msec duration.

In all the eighteen cats, maximum responses were obtained with stimuli of 15 V, 1 msec duration, at a frequency of 2–5 impulses/sec. The splenic artery flow decreased to 11–47 % (mean 25 %) of the control level, while the splenic weight decreased to 56–93 % (mean 74 %) of the control. The increase in vascular resistance ranged from 200 to 1071 % (mean 460 %) of the control.

In each individual experiment, the sizes of the changes in flow and weight were related to the rate of stimulation. Figure 4 shows the frequency–response curves for splenic weight and splenic artery flow in the eight experiments in which the complete range of frequencies was tested. The maximum decrease in each series of stimulations was taken as 100 % and the decreases at other frequencies were expressed as percentage of this.

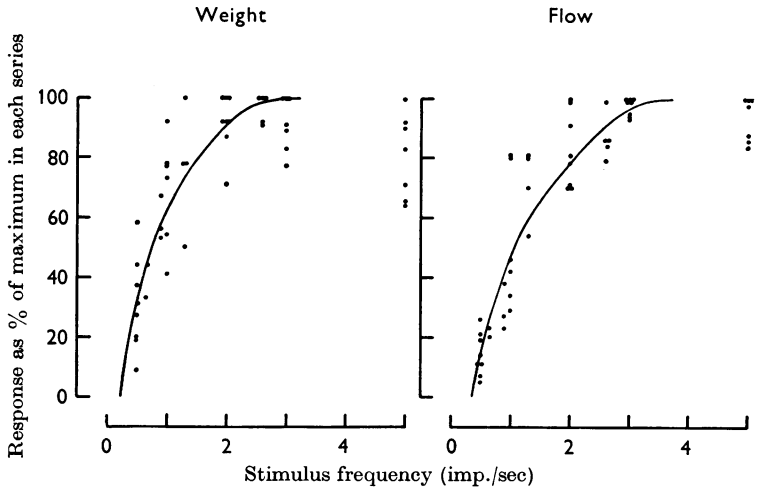

Fig. 4. The changes in splenic weight and splenic artery flow at various frequencies of stimulation of the splenic nerves. Ordinate, in each series of stimulations at frequencies of 0·5–5 impulses/sec, the largest response was taken as 100 % and the responses at the other frequencies were expressed as percentages of this. Abscissa, frequency of stimulation.

When the mean value at each frequency was plotted against the logarithm of the frequency, the points lay very close to a straight line. The smooth curves were constructed from these lines. The response at a stimulus frequency of 2–3 impulses/sec was usually larger than that at 5 impulses/sec, as shown by the points below 100 % at 5 impulses/sec. The flow changes are underestimated as the splenic arterial pressure rose by 0–25 mm Hg. Since the pressure changes were greatest with the highest frequencies of stimulation when the flow decreased to very low levels, the error is probably small. The shape of the pressure–flow curve during nerve stimulation is unknown and it is difficult to correct for this error but the flow points should lie to the left of those shown in Fig. 4.

In six cats, the splenic nerves were stimulated at 3 impulses/sec (15 V, 1 msec duration) for periods of over 10 min. One such experiment is shown in Fig. 5. The splenic artery flow tended to recover towards the control level and after stimulation for 10 min 21–58 % (mean 37 %) recovery had

occurred. In contrast, the splenic weight did not recover but remained steady during the period of stimulation.

After cessation of stimulation, the splenic artery flow recovered rapidly and oscillations occurred. In half the experiments the mean flow rose briefly to above the control level. In contrast, the splenic weight recovered very slowly; after large responses it was usually 8–10 min before the weight returned to the control level.

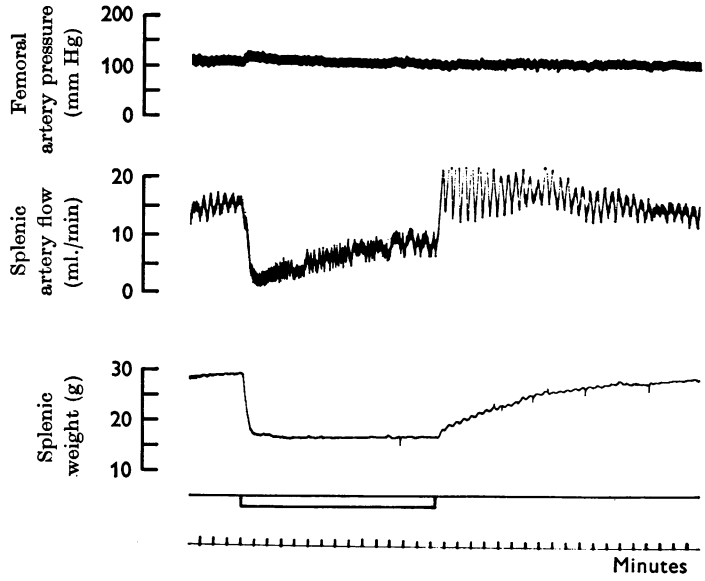

Fig. 5. Cat, wt. 3·0 kg. The response to stimulation of the splenic nerves for a 14-min period. Ordinates, femoral artery pressure, splenic artery flow and splenic weight. Abscissa, time in min. The splenic nerves were stimulated at a frequency of 3 impulses/sec (15 V, 1 msec duration) during the period shown by the signal.

Response to nerve stimulation after reduction of splenic artery flow

In five cats, after control responses to stimulation of the splenic nerves had been obtained, the splenic artery flow was reduced to between one half and one third of the control level, by means of the clamp on the coeliac artery, and was maintained at that level for 95–125 min. Splenic artery pressure fell to 62–80 mm Hg (mean 74 mm Hg). The response to stimulation of the splenic nerves was tested at intervals and one such experiment is shown in Fig. 6. The last response to nerve stimulation before restoration of the flow did not differ from the first response after reduction of the flow by more than 0–23 % (mean 8 %) in the case of the splenic artery flow and 4–17 % (mean 7 %) in the case of the splenic

weight. The weight responses during the period of reduced flow were very similar to the control responses before reduction and after restoration of the flow. The flow responses before reduction and after restoration of the flow were very similar, but during the period of flow reduction they were reduced in size since the control flow was reduced.

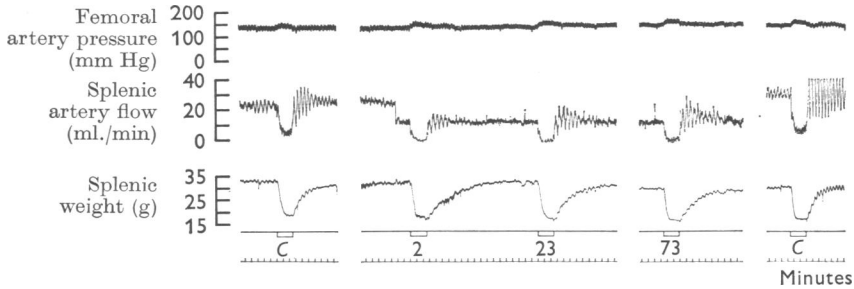

Fig. 6. Cat, wt. 2·1 kg. The responses to stimulation of the splenic nerves when the splenic artery flow was reduced to one half by partial clamping of the splenic artery. The splenic artery pressure fell from 140 to 80 mm Hg. Ordinates, femoral artery pressure, splenic artery flow and splenic weight. Abscissa, time in min. The splenic nerves were stimulated at a frequency of 3 impulses/sec (15 V, 1 msec duration) during the periods shown by the signals. Control responses (*C*) were obtained 23 min before reduction and 22 min after restoration of the flow. The flow was reduced for 95 min and the nerves were stimulated at the times shown by the numbers beneath the signals.

Effect of blocking agents

In three cats, a series of responses to stimulation of the splenic nerves at frequencies of 0·5–5 impulses/sec was obtained before and after intravenous administration of atropine (1 mg/kg). No modification of the responses was apparent. Similar experiments were carried out before and after intravenous administration of propranolol (1 mg/kg). However, in these doses, propranolol reduced the arterial pressure and the splenic artery flow, thus making comparison of the responses before and after its administration unsatisfactory. These effects could be greatly reduced by prior administration of promethazine (2 mg/kg). In three further cats, therefore, promethazine was administered and a control series of nerve stimulations obtained. Propranolol was then given and the series repeated. No modification of the responses was apparent.

In eight cats, splenic nerve stimulation was carried out before and after the intravenous administration of phenoxybenzamine. To obtain the effects described below, it was necessary to administer a dose of 10 mg/kg and wait 1 hr before repeating the nerve stimulations. Under these conditions, stimulation of the splenic nerves produced either no effect or a

small decrease in splenic weight, while the flow response was reversed—a marked vasodilatation being obtained. One such response is shown in Fig. 7. The size of the vasodilatation varied with the frequency of stimulation, a maximum response being obtained at 2–3 impulses/sec. The increase in flow obtained in this way was unaffected by intravenous administration of atropine (1 mg/kg) but was blocked by propranolol (1 mg/kg) (Fig. 7).

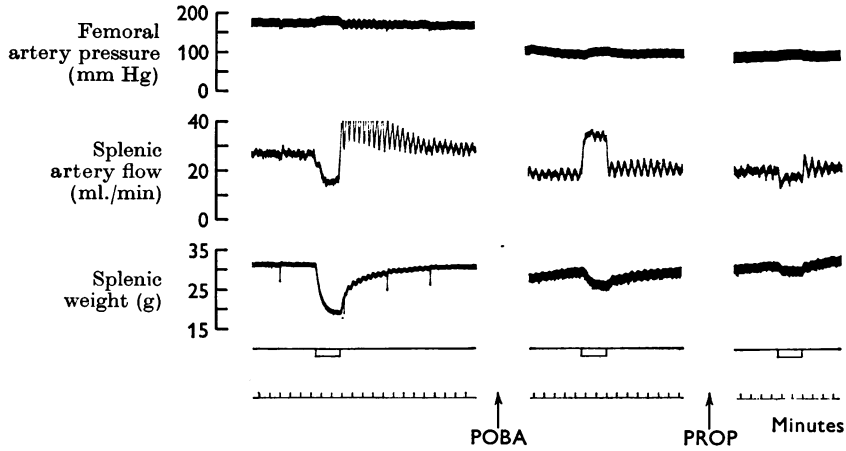

Fig. 7. Cat, wt. 3·3 kg. The response to stimulation of the splenic nerves after phenoxybenzamine. Ordinates, femoral artery pressure, splenic artery flow and splenic weight. Abscissa, time in min. The splenic nerves were stimulated at a frequency of 2 impulses/sec (15 V, 1 msec duration) during the periods shown by the signal. One hour elapsed after the intravenous administration of phenoxybenzamine (POBA, 10 mg/kg) and 15 min after propranolol (PROP, 1 mg/kg).

DISCUSSION

The literature on the spleen has been reviewed by Grayson & Mendel (1965). While a splenic capacitance function is well established in some species, other functions are not clear. The arterial flow in the denervated spleen of the anaesthetized cat is about 10 ml./min.kg or 7% of the cardiac output (Greenway & Lawson, 1966*a*, *b*). In view of the large and variable blood content, values expressed for unit spleen weight may be unreliable but the mean value of 88 ml./min.100 g is larger than that obtained for the hepatic artery flow (Greenway *et al.* 1967) and about 20 times that for resting skeletal muscle (Mellander, 1960). This suggests either that the spleen has a very high metabolic rate or, more likely, that a large part of this flow is not nutritive. The histology of the vascular bed is complex (Knisely, 1936; MacKenzie, Whipple & Wintersteiner, 1941)

and the observed vascular responses cannot be interpreted as fully as in the case of skeletal muscle.

Occlusion and release of the splenic artery was followed by a brief flow increase to above the pre-occlusion level but this was not seen after occlusion of the splenic vein for a similar period. This may be a myogenic response of the smooth muscle to a lowering of the pressure within the vessels; such responses are well known (Folkow, 1962). The occurrence of a flow over-shoot is at variance with the observations of Green, Ottis & Kitchen (1960). The reaction of the canine spleen may differ from that of the cat, but perhaps a more likely explanation lies in the technique used since an arterial long-circuit is known to disturb vascular reactivity (Folkow, 1952; Dresel & Wallentin, 1966; Greenway *et al.* 1967).

Oscillations in splenic flow and volume (Barcroft, Khanna & Nisimaru, 1932; Grindlay, Herrick & Baldes, 1939) and microscopic evidence of cyclical blood flow (Knisley, 1936; MacKenzie *et al.* 1941) have been reported previously. In our experiments, the weight oscillations were of small amplitude and it is likely that they were caused by the flow oscillations. It seems probable that the rhythmic activity of the arteriolar smooth muscle is normally out of phase in different parts of the spleen (Knisely, 1936; MacKenzie *et al.* 1941) but becomes temporarily synchronized following procedures which increase the amplitude of the oscillations. The function of this rhythmic activity is still unknown.

During maximal stimulation of the splenic nerves, the flow can decrease to one tenth and up to 5 ml. blood/kg body wt. can be discharged into the splenic vein. Owing to the possibility of damage to, or deterioration of, the nerve fibres, the largest responses obtained in our experiments are likely to be closest to the maximum possible in the intact animal. These responses occur at lower rates of discharge in the sympathetic nerves than do those in many other vascular beds (Celander, 1954; Mellander, 1960; Folkow *et al.* 1964; Greenway *et al.* 1967). In the cat, such responses probably play an important role after haemorrhage, when they can be maintained for at least 2 hr. They are not impaired by the reduction in blood flow resulting from the lowered arterial pressure, as is the case in the vascular beds of skeletal muscle (Lewis & Mellander, 1962) and intestine (Folkow *et al.* 1964). This absence of impairment might be expected if the splenic flow is greater than that required for nutrition of the splenic tissue, as suggested above.

Comparison of the frequency–response curves for flow and weight (Fig. 4) suggests that stimulation of the sympathetic nerve fibres to the spleen produces flow and capacitance responses which are approximately the same proportion of the maximum possible responses. Thus, in the spleen, the sympathetic nerve fibres do not have a relatively more pronounced

effect on the capacitance responses as they do in skeletal muscle (Mellander, 1960). However, the capacitance responses are not truly comparable since the splenic capacitance response involves contraction of the trabeculae and capsule.

It is perhaps not surprising that no slow changes in weight were observed during prolonged periods of stimulation (Fig. 5). Reabsorption of substantial amounts of interstitial fluid does not appear to occur in the spleen. During such prolonged periods of stimulation the flow shows some recovery towards the control level (Fig. 5). This is similar to, but much less marked than, that seen in the hepatic artery vascular bed (Greenway *et al.* 1967).

No evidence was found for any cholinergic innervation of the spleen. The smooth muscle controlling splenic artery flow appears to possess α- and β-adrenergic receptors, both of which are innervated. This is suggested by the reversal of the flow response to nerve stimulation produced by phenoxybenzamine and blocked by propranolol. These conclusions are in agreement with results obtained in the dog by Green *et al.* (1960). Since nerve stimulation reduced splenic artery flow to near zero and propranolol had no effect on this, the response involving stimulation of the α-receptors does not appear to compete with the response involving the β-receptors but to abolish it completely. The smooth muscle controlling splenic weight appears to possess innervated α-receptors but no innervated β-receptors, since the response can be partially or completely blocked by phenoxybenzamine and is unaffected by propranolol.

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