J. Physiol. (1953) 122, 209–219

THE SPLEEN IN THE REGULATION OF THE ARTERIAL BLOOD PRESSURE

By D. GRANAAT

From the Physiological Laboratory, University of Amsterdam

(*Received* 11 *December* 1952)

Contraction of the spleen is known to be associated with a rise in arterial blood pressure which is explained by the discharge into the circulation of the blood stored in the dilated spleen (Roy, 1881; Schäfer & Moore, 1896; Barcroft & Nisimaru, 1932). The finding that splenectomized dogs are less resistant to the development of shock than normal dogs is explained on similar lines (Kendrick & Uihlein, 1942; Lewis, Werle & Wiggers, 1942). There is so far, however, no direct evidence that the injection into the portal vein of a volume of blood corresponding to that discharged by contraction of a dilated spleen would cause a significant rise in arterial blood pressure. According to Roy (1881) and Barcroft & Nisimaru (1932) the quantity of blood discharged by the spleen during rhythmic contraction is relatively small and such a small quantity of blood would hardly affect the arterial blood pressure (Guyton, Batson & Smith, 1951; Guyton, Batson, Smith & Armstrong, 1951). According to various other authors, the fully dilated spleen of the dog may, on the other hand, contain and discharge as much as 130 and over 200 ml. of blood (cf. Feldberg & Lewin, 1928).

There is another possibility, which has not been considered so far, of explaining the observed rise in arterial blood pressure: i.e. a discharge of a pressor principle into the circulation from the contracting spleen. This possibility is tested by the experiments described in this paper.

The spleen contains relatively large amounts of noradrenaline (Euler, 1946, 1949; Bacq, 1947), the presence of which seems to a large extent to be dependent on its sympathetic innervation (Euler & Purkhold, 1951). Furthermore, noradrenaline appears in the venous blood of the spleen on stimulation of its sympathetic nerves (Peart, 1949; West, 1950; Mann & West, 1950; Cicardo, 1952). Although the spleen-contracting effect of noradrenaline is weaker than that of adrenaline (Schümann, 1949), noradrenaline would have a more pronounced pressor effect than adrenaline if it were discharged from

the contracting spleen, because adrenaline is more quickly inactivated in the liver than noradrenaline. When given intraportally, the dose of adrenaline has to be increased about 5-fold, that of noradrenaline only 2–2½-fold, in order to produce the same pressor effect as on intravenous injection (Dawes, 1946; West, 1948). West also obtained some evidence suggesting that adrenaline injected into the spleen caused the release of histamine. In cats, a small dose of adrenaline injected into the branch of the splenic artery supplying the caudal portion of the spleen resulted 'in a small rise, followed by a large fall in blood pressure' which was eliminated by benadryl. Noradrenaline injected in this way produced a pressor effect only.

METHODS

Most experiments were done on dogs, a few on cats. The animals were anaesthetized by intraperitoneal injection of either thiopentone sodium (2 ml./kg of a 2% solution of pentothal), or of pentobarbitone sodium (0·4 ml./kg nembutal). If it became necessary during the course of an experiment to deepen the anaesthesia, 0·5–1 ml. of the pentothal solution were injected intravenously. The blood was rendered incoagulable by intravenous injection of 10 mg/kg body weight heparin.

In order to remove splenic blood or to inject various substances either through the splenic artery into the spleen or through the splenic vein intraportally, the main splenic vessels supplying the right pole of the spleen were divided and reconnected with metal cannulae and rubber tubing. For this purpose artery and vein were each clamped at two points, 3–4 cm apart from each other, so that the clamped portion could be slit open longitudinally for a distance of about 1½–2 cm and metal cannulae filled with heparinized Ringer's solution inserted at both ends. The vessels were then divided between the cannulae which were connected with a piece of soft rubber catheter. When the clamps were then removed, blood could be aspirated or drugs injected through the rubber connexion by means of a fine injection needle. As a result of these manipulations the spleen always became somewhat contracted. In some experiments the injections into the splenic artery were only made into the more or less fully contracted spleen. For this purpose the splenic nerves were stimulated with faradic current. In later experiments, instead of dividing a main splenic vein and reconnecting it with metal cannulae, a branch of the mesenteric vein was tied and cannulated with a polythene cannula. This modification reduced the length of the operation and ensured a more effective discharge from the spleen.

In most experiments the collateral vessels to and from the spleen were left intact, since they did not interfere with the injection experiments. Between each injection time was allowed for the blood pressure to have returned to pre-injection level for at least 2 min.

The following substances diluted with Ringer solution were used for injection: adrenaline hydrochloride (Brocapharm), noradrenaline bitartrate (Philips-Roxane Ltd.), 10% solution sympathol (Sympatol, Boehringer; L-1-*o*-hydroxyphenyl-2-methylaminoethanol; Synephrine), heparin (Thromboliquine; Organon Ltd.), splenic extracts prepared according to the method of Euler (1946).

The arterial blood pressure was recorded from the femoral artery, and for intravenous injections a polythene cannula was tied into the femoral vein.

RESULTS

Changes in the blood volume of the portal vein by injection of 10–80 ml. blood into the splenic vein of a dog or a few millilitres into that of a cat have scarcely any effect on the arterial blood pressure; cats are slightly more

sensitive than dogs but, as shown in Fig. 1, even in this species slow or rapid injections of 2·5 ml. of blood into the splenic vein merely result in very slight and transient rises of arterial blood pressure. A different effect is obtained when splenic contractions are produced by injection of sympathomimetic substances into the splenic artery.

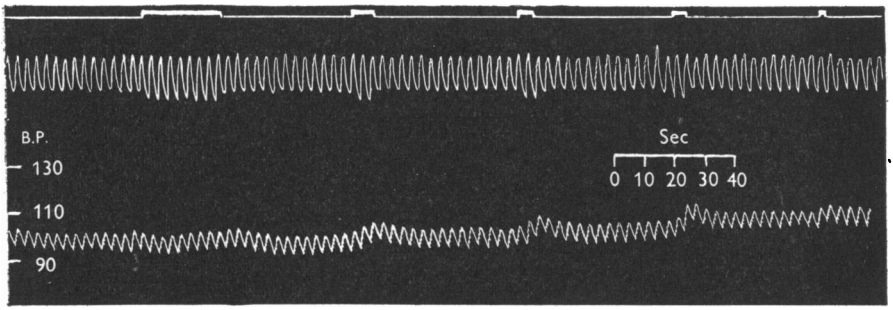

Fig. 1. Respiration (upper tracing) and arterial blood pressure (lower tracing) from 3 kg cat in thiopentone anaesthesia. At the signals, injections of 2·5 ml. blood into the splenic veins. Speed of injection increased each time. Time in 10 sec.

Adrenaline. An injection of 0·2 μg/kg into the splenic artery of a dog led to a gradual rise of blood pressure of about 10 mm lasting for about 3 min. With 4 μg/kg adrenaline the effect was more pronounced and more complicated. For instance, in a typical experiment on a 9·5 kg dog, such an injection produced, after a latency of 16 sec, a brief rise from 155 to 170 mm Hg, followed by a fall of 12 mm lasting for 8 sec. When the blood pressure then rose again, the heart rate decreased and consequently the pulse pressure became increasingly greater; 90 sec later it was about 30 mm Hg. Simultaneously, respiration slowed and became more shallow, whilst the blood pressure continued to rise and reached a level of 215 mm Hg about $3\frac{1}{2}$ min after the injection. The splenic contraction occurred much more quickly; islands appeared on the spleen surface 5 sec after the injection, and within 45 sec the organ had paled completely. Within 12 min arterial blood pressure had returned to the pre-injection level, but the spleen was still strongly contracted and firm. A similar injection of adrenaline into the splenic vein caused either no effect on the blood pressure or at most a rise of 10–15 mm lasting for about a minute.

The more pronounced and more prolonged pressor effect of adrenaline, when injected into the splenic artery instead of into the splenic vein, could scarcely be explained by a combined effect of adrenaline with the mechanical effect of the discharge of blood into the circulation, because the amounts of blood discharged were relatively small. The spleens were usually in more or less

contracted condition when the injections were made, and the injections of small amounts of blood into the portal circulation were shown to be ineffective. Further, practically the same result was obtained when the injections of adrenaline into the splenic artery were repeated some minutes after the blood pressure had returned to its pre-injection level but whilst the spleen was still contracted from the previous injection, or when the injections were made into a spleen which had previously been made to contract by stimulation of the splenic nerves. In these experiments the difference, however, was not always as pronounced as in the experiments in which the splenic nerves had not been stimulated before the injection. The strong, prolonged rise in blood pressure on injection of adrenaline into the splenic artery was always associated with bradycardia and depression of respiration; rate and amplitude of respiration decreased. These cardiac and respiratory changes are probably the result of the rise in arterial blood pressure and were therefore less pronounced, or absent, when the same dose of adrenaline was injected into the splenic vein.

The injection of adrenaline into the splenic artery of dogs never produced a fall of arterial blood pressure, as observed by West (1948) in cats, but an interruption of the rise by a small, transient fall was sometimes observed.

When the same dose of adrenaline was once injected into the splenic artery and once into the femoral vein, the pressor response to the arterial injection was always more prolonged, even if the actual rise was not as great as after the intravenous injection. Sometimes, however, the injection into the splenic artery produced also a greater rise than the intravenous injection, although the rise obtained with an intravenous injection was always greater than that with an intraportal injection through the splenic vein. The rise in pressure following an intravenous injection of adrenaline was often followed by a fall below the pre-injection level; this was never observed when the adrenaline was injected into the splenic artery. On the contrary, the pressure often remained 10–20 mm Hg higher than the pre-injection level for periods up to 15 min and longer. This was particularly so in dogs with low initial blood pressure.

Noradrenaline. The results resembled those obtained with adrenaline. For instance, in one experiment on a 16 kg dog with a low blood pressure of 55 mm Hg, 4 μg injected into the splenic artery caused a rise of 30 mm Hg lasting for 4 min, whereas the same amount injected intravenously caused a rise of 25 mm Hg lasting for 40 sec only. A similar experiment in a dog with a higher blood pressure is illustrated in Fig. 2 *A–C*. Again, the injection of noradrenaline into the splenic artery produced a more pronounced and more prolonged pressor effect than the injection into the splenic vein.

From a comparison of Fig. 2 *A* and *C*, it will be seen that the pressor response on injection of noradrenaline into the splenic artery develops more gradually and persists for a longer time than the pressor response following

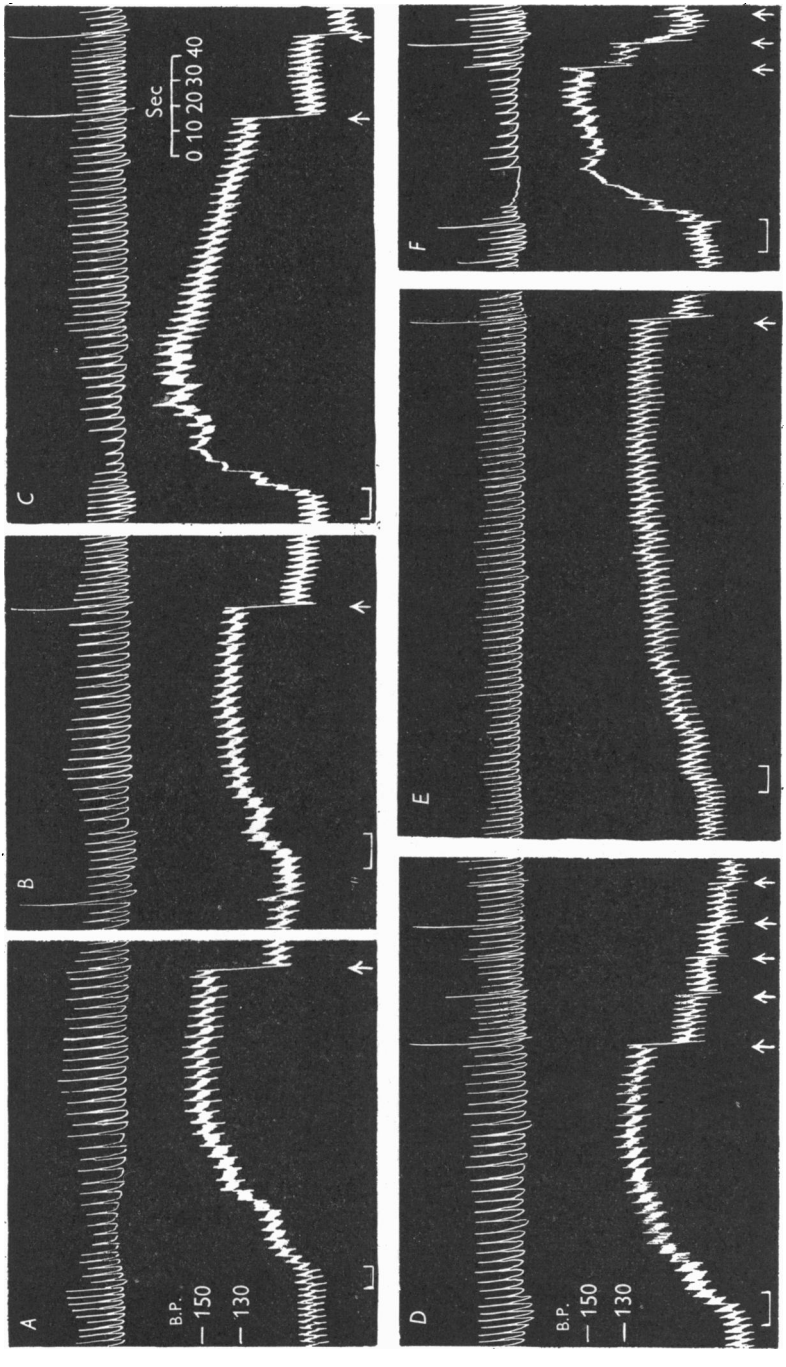

Fig. 2. Respiration (upper tracing) and arterial blood pressure (lower tracing) from 20 kg dog in thiopentone–pentobarbitone sodium anaesthesia. Injections of 4 μg noradrenaline/kg at *A* into the splenic artery, at *B* into the splenic vein, at *C* into the femoral vein. Injections of 4 ml. spleen extract at *D* into the splenic artery, at *E* into the splenic vein and at *F* into the femoral vein. Before the first injection, contraction of spleen was produced by stimulation of splenic nerves. At the arrows drum stopped at *A* for 1½ min, at *B* for 2 min 20 sec, at *C*, *D* and *F* for 1 min each time, and at *E* for 7 min. ⌊, duration of injections. Time in 10 sec.

the injection of a similar dose into the femoral vein. The actual height of pressure reached on intravenous injection of the noradrenaline, however, is as high and sometimes even higher than the rise attained on injection of the same dose into the splenic artery. Finally, the pressor response on intravenous and also on intraportal injection is followed by a fall of pressure below the pre-injection level. This does not occur when the noradrenaline is injected into the splenic artery: the level of arterial blood pressure remains for many minutes 10–20 mm higher than the pre-injection level. Associated with the pressor responses there is bradycardia, slowing and inhibition of respiration. The degree of these changes in heart rate and respiration depends on the intensity of the pressor responses.

There is this difference between the results obtained with noradrenaline and adrenaline. The pressor effect of noradrenaline on injection into the splenic vein is relatively high in comparison with the pressor response of adrenaline in corresponding experiments, because the destruction of adrenaline on its passage through the liver is greater than that of noradrenaline. This is illustrated in Fig. 3, in which the pressor responses to noradrenaline and adrenaline in cats on intraportal and intravenous injections are compared. Similar results have been obtained in dogs. These results confirm similar observations by West (1948).

Splenic extracts. The effects of splenic extracts, prepared according to von Euler, could not be explained by the presence of small amounts of Na_2SO_4 which the extracts contained, because injections of Na_2SO_4 in amounts equivalent to those present in the extracts were ineffective.

Splenic extracts injected into the splenic artery contracted the spleen and produced strong pressor effects which could not be accounted for by discharge of blood from the contracting spleen.

A comparison of Fig. 2 *D–F* shows that, like noradrenaline, the pressure response is more pronounced on injection into the splenic artery than into the splenic vein, and that the blood pressure rises more steeply and to a higher level, but for a shorter time, on intravenous injection.

The effects of splenic extracts resembled more those of noradrenaline than of adrenaline, but there was the following difference: whereas the pressor response of an intravenous or intraportal injection of noradrenaline was followed by a secondary fall in blood pressure below the pre-injection level, this did not occur after the injection of splenic extracts.

Sympathol. The hypertensive effect of sympathol, which is not a naturally occurring sympathomimetic substance, causes effects like adrenaline when injected into the splenic artery, splenic vein and femoral vein. In some experiments the pressor response on injection into the splenic artery lasted so long that the blood pressure did not return to its pre-injection level within 20 min.

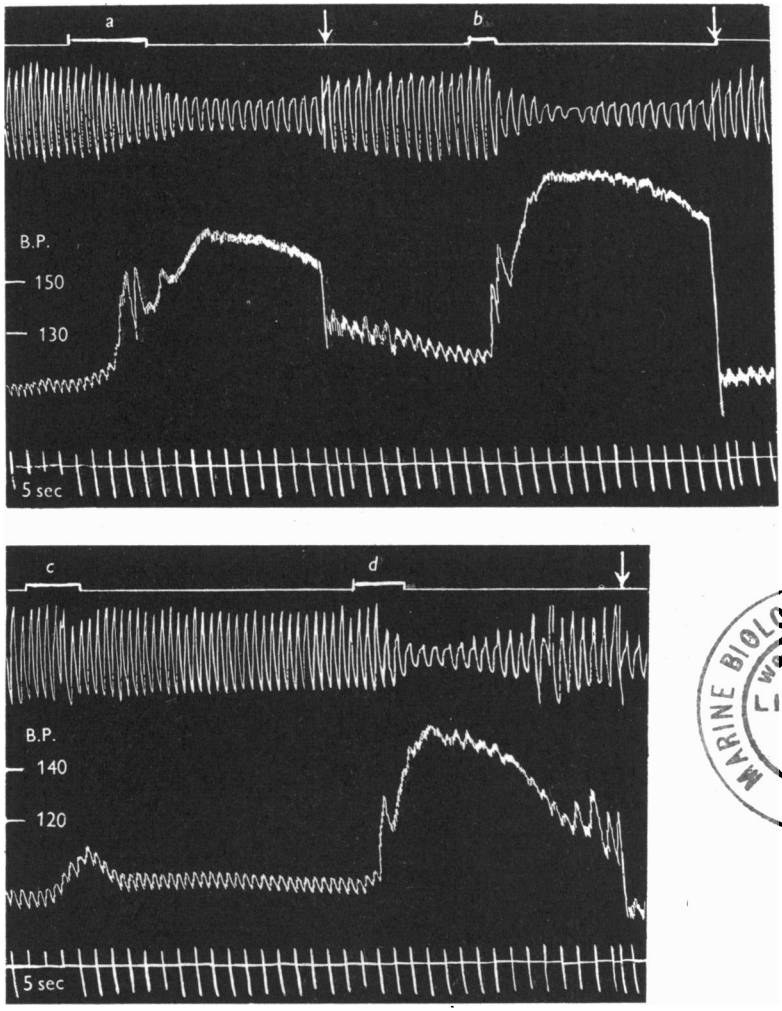

Fig. 3. Respiration (upper tracing) and arterial blood pressure (lower tracing) from 6 kg cat. Comparison of intraportal and intravenous injections of noradrenaline and adrenaline. At *a* 8 μg/kg noradrenaline injected into the splenic, and at *b* into the jugular vein. At *c* 16 μg/kg adrenaline injected into the splenic, and at *d* into the jugular vein. At the arrows drum stopped for 1 min. Time in 5 sec.

DISCUSSION

The finding that an injection of a given dose of either adrenaline, noradrenaline, sympathol or splenic extract into the splenic artery has a more pronounced and longer-lasting pressor effect on the arterial blood pressure than its injection into the portal vein via the splenic vein cannot be explained by

the fact that the injection of these substances into the splenic artery causes a discharge of splenic blood into the portal circulation by the contracting spleen, and thereby increasing the circulating blood volume, because the amounts of blood discharged are small and it could be shown that such amounts of blood injected into the splenic vein had no effect, or scarcely any, on the arterial blood pressure. Further, the difference in the pressor responses of these substances when injected into the splenic artery and vein occurred also when the experiments were made in conditions in which the spleen was contracted either by previous stimulation of the splenic nerves or by a previous injection of these substances into the splenic artery.

The old observation of Roy (1881) that the rhythmic contractions of the spleen are associated with rises in arterial blood pressure is therefore perhaps not wholly explained by the mechanical effect of a discharge of blood from the spleen, as was suggested by Barcroft, but by the release into the portal blood of vaso-active substances. Peart (1949) had found that the release of relatively large amounts of noradrenaline on stimulation of the splenic nerves does not occur when stimulation is repeated at short intervals. In the present experiments, with injections of sympathomimetic substances into the splenic artery, it is shown, however, that the pressor principle seemed to be released from the spleen into the circulation even when the injections were repeated at relatively short intervals.

Since splenic extracts contain relatively large amounts of noradrenaline, it is natural to assume that the pressor principle released from the spleen is noradrenaline itself, or that noradrenaline is at least responsible to a great extent for the effects observed. However, there are a few findings which suggest that the active pressor principle discharged from the spleen is not only noradrenaline nor noradrenaline plus adrenaline, but that, in addition, an unknown pressor principle is discharged. The pressor effect of noradrenaline and adrenaline when injected intravenously or intraportally is followed by a fall of blood pressure below the pre-injection level. This secondary depressor effect is absent after intravenous and intraportal injections of splenic extracts, as well as after the injection of the sympathomimetic substances into the splenic artery.

Domenjoz & Fleisch (1940) found no real difference between the effect of adrenaline when injected into the splenic artery or intravenously. In the present experiments the differences in the effect of adrenaline when injected into these vessels was also not as pronounced as when the comparison was made between an injection into the splenic artery and the splenic vein. The main difference between an injection of adrenaline into the splenic artery and into the femoral vein consisted in the more prolonged pressor effect of the arterial injection. If Domenjoz & Fleisch had also compared the response of adrenaline when injected into the splenic artery with that of an injection

into the splenic vein, they would probably also have come to the conclusion that there must be a fundamental difference in the mechanism by which the apparently comparable pressor responses are produced on intravenous injections and on injections into the splenic artery.

The possibility must therefore be considered that when the spleen is made to contract, either by sympathomimetic substances or by stimulation of the splenic nerves (Granaat, 1952), the resulting pressor response is due to a release of noradrenaline, perhaps of some adrenaline, and, in addition, of an unknown active principle, which would account for the finding that injections of sympathomimetic substances into the splenic artery have a prolonged 'tonic' effect on the circulation, particularly in dogs with a low blood pressure. The possibility that the spleen may secrete a hypertensive or tonic principle which is not identical with noradrenaline and adrenaline has also been suggested by Rein, Mertens & Bücherl (1949) and Rein (1951), and attributes a new function to this organ: i.e. that of releasing vaso-active substances into the circulation when contracting. Since rhythmic contractions of the spleen occur at regular intervals, the secretion of such substances would be a contributory factor in the maintenance of an efficient circulation. The results here described do not give any evidence of whether these substances have first to pass through the liver in order to become active, as suggested by Rein.

The idea that the spleen is a storage organ for vaso-active substances which are released on its contraction raises the question whether these substances are formed in the spleen, or whether the spleen is able to absorb from the circulation the sympathomimetic substances released elsewhere and to store and modify them for future use. This possibility finds some support in the observations of Euler & Uddén (1951).

SUMMARY

1. A comparison is made of injections of sympathomimetic substances into the splenic artery, splenic and femoral vein, in order to find out if the pressor response associated with contraction of the spleen is accounted for by an increased circulatory blood volume, or by the release into the circulation of vaso-active substances. Most of the experiments were performed in dogs, some in cats.

2. The injections of 10–80 ml. of blood into the portal circulation of dogs, or of a few ml. into that of cats, produces scarcely any pressor effect.

3. The injection of adrenaline, noradrenaline and sympathol into the splenic artery of dogs produces a pressor response which is greater and longer lasting than that produced by an injection of equal amounts of these substances into the splenic vein.

4. The pressor effect of an injection of adrenaline, noradrenaline or sympathol into the splenic artery is approximately as great as that produced

by an injection of the same amounts of these substances into the femoral vein, but the duration of the effect is more prolonged with the arterial injections.

5. The pressor responses to splenic extracts on injection into the splenic artery, portal or femoral vein resemble those of noradrenaline.

6. An injection of a small dose of adrenaline into the splenic artery of a dog does not produce a fall of blood pressure as observed by West in corresponding experiments on cats.

7. Comparison of the effects of intraportal and intravenous injections of noradrenaline and adrenaline confirms the results of West that the liver has a greater ability to inactivate adrenaline than noradrenaline.

8. It is concluded that the pressor response obtained by injections of sympathomimetic substances into the spleen causes the release from the spleen of noradrenaline and an unknown vaso-active substance into the circulation, and that these substances are also released from the spleen when made to contract by stimulation of the splenic nerves.

9. It is concluded that the rise of blood pressure associated with splenic contraction is not wholly accounted for by the mechanical effect of increasing the circulating blood volume due to the discharge of the blood stored in the dilated spleen, but by the release of these vaso-active substances.

10. The possibility is discussed that by the release of these vaso-active substances the spleen may exert a 'tonic' effect on the blood pressure in normal and pathological conditions.

REFERENCES

Bacq, Z. M. (1947). La substance sympathicomiméthique des extraits de rate. *C.R. Soc. Biol., Paris*, **141**, 537–538.

Barcroft, J. & Nisimaru, Y. (1932). Undulatory changes of blood-pressure. *J. Physiol.* **74**, 299–301.

Cicardo, V. H. (1952). Liberation of noradrenaline in the neurogene arterial hypertension. *Arch. int. Pharmacodyn.* **90**, 285–291.

Dawes, G. S. (1946). Amidines, guanidines and adrenaline inactivation in liver. *Brit. J. Pharmacol.* **1**, 21–37.

Domenjoz, R. & Fleisch, A. (1940). Die Wirkung kreislaufaktiver Pharmaka auf die Speicherfunktion der Milz. *Arch. exp. Path. Pharmak.* **195**, 609–1616.

Euler, U. S. v. (1946). The presence of a substance with sympathin E properties in spleen extracts. *Acta physiol. scand.* **11**, 168–186.

Euler, U. S. v. (1949). Distribution of sympathin N and sympathin A in spleen and splenic nerves of cattle. *Acta physiol. scand.* **19**, 207–214.

Euler, U. S. v. & Purkhold, A. (1951). Effect of sympathetic denervation on the noradrenaline and adrenaline content of the spleen kidney and salivary glands in the sheep. *Acta physiol. scand.* **24**, 212–217.

Euler, U. S. v. & Uddén, P. (1951). Increase in noradrenaline content of organs after administration of DOPA in the cat. *Experientia*, **7**, 465–466.

Feldberg, W. & Lewin, H. (1928). Versuche über Milz und Blutvolumen. *Pflüg. Arch. ges. Physiol.* **219**, 246–259.

Granaat, D. (1952). *De Milt in de Regulatie van de Arteriële Bloeddruk.* Amsterdam: Jacob van Campen.

GUYTON, A. C., BATSON, H. M. & SMITH, CH. M. (1951). Adjustments of the circulatory system following very rapid transfusion or hemorrhage. *Amer. J. Physiol.* **164**, 351–359.

GUYTON, A. C., BATSON, H. M., SMITH, CH. M. & ARMSTRONG, G. G. (1951). Method for studying competence of the body's blood-pressure, regulatory mechanisms and effect of pressoreceptor denervation. *Amer. J. Physiol.* **164**, 360–369.

KENDRICK, D. B. & UIHLEIN, A. (1942). Shock in control and splenectomized animals under ether and pentobarbital sodium anaesthesia. *Surgery*, **12**, 76–80.

LEWIS, R. N., WERLE, J. M. & WIGGERS, C. J. (1942). The behavior of the spleen in hemorrhagic hypotension and shock. *Amer. J. Physiol.* **138**, 205–212.

MANN, M. & WEST, G. B. (1950). The nature of hepatic and splenic sympathin. *Brit. J. Pharmacol.* **5**, 173–177.

PEART, W. S. (1949). The nature of splenic sympathin. *J. Physiol.* **108**, 491–501.

REIN, H. (1951). Die Beeinflussung von Coronar- oder Hypoxie-bedingten Myokard-Insuffizienzen durch Milz und Leber. *Pflüg. Arch. ges. Physiol.* **253**, 435–458.

REIN, H., MERTENS, O. & BÜCHERL, E. (1949). Über ein Regulationssystem 'Milz-Leber' für den oxydativen Stoffwechsel der Körpergewebe und besonders des Herzens. *Naturwissenschaften*, **36**, 233–268.

ROY, C. S. (1881). The physiology and pathology of the spleen. *J. Physiol.* **3**, 203–229.

SCHÄFER, E. A. & MOORE, B. (1896). On the contractility and innervation of the spleen. *J. Physiol.* **20**, 1–50.

SCHÜMANN, H. J. (1949). Vergleichende Untersuchungen über die Wirkung von Adrenalin, Arterenol und Epinin auf Blutdruck, Milzvolumen, Darm und Blutzucker. *Arch. exp. Path. Pharmak.* **206**, 164–170.

WEST, G. B. (1948). Injections of adrenaline and noradrenaline and further studies on liver sympathin. *Brit. J. Pharmacol.* **3**, 189–197.

WEST, G. B. (1950). Further studies on sympathin. *Brit. J. Pharmacol.* **5**, 165–172.