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The Actions of Drugs on the Smooth Muscle of the Capsule and Blood Vessels of the Spleen

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I. Introduction

RECENT reviews of the spleen have considered either immunological aspects of its function (162) or its role in haemolysis (253, 255). These approaches will not be considered here. There have also been a number of general reviews on the pharmacology of vascular smooth muscle (222, 223). Somlyo and Somlyo (223) considered it "axiomatic that there can be marked differences among the effects of drugs on different regional circulatory beds or consecutive or nonconsecutive vascular segments and indeed among different smooth muscle fibres within the same vessel." This is the general theme of this review since we consider it now opportune to discuss drug action in one tissue, the spleen, in detail and to outline any differences in the responses from this tissue to the general trend both from a qualitative aspect and, where information exists, to compare quantitative aspects of the drug-smooth muscle interaction in the spleen. Furthermore, since two types of smooth muscle, vascular and non-vascular, are present in the spleen we have emphasised any differences in their responses to drugs.

The spleen has been used, generally, as a test organ or tool for research since the ease of isolation, well defined separate blood supply, and rich sympathetic innervation provide a preparation from which information can be obtained easily. Information on the responses of the smooth muscle of the spleen to drugs is scattered widely throughout the world literature and has not been reviewed comprehensively before. To facilitate the interpretation of drug action it is necessary that the majority of observations and references should be easily accessible in a framework of critical assessment.

II. General Considerations

A. Spleen Weight

There is considerable species variation in the size, structure, and physiological role of the spleen. In any species, the reported spleen weight differs amongst experimental groups. There are probably three main sources of variation. The condition of the animal from which the spleen is removed may influence the result considerably, since if surgical or traumatic shock is present, the spleen in those species in which it is capable of altering its volume may be contracted. On the other hand, if deep anaesthesia is present, it may be relaxed. The preparation for weighing is important since adequate removal of omentum and the presence or absence of stored blood within the organ may make a significant contribution to weight. Age of the animal is also an important factor since it has been established that the spleen of the young of many species does not function as a reservoir of blood but as a blood-forming organ and its comparatively large size after birth is explained by the presence of immature red cells, myeloid cells, megakaryocytes and actively developing lymphoid tissue (121, 171). The spleen weights and spleen weight/body weight ratio are listed for many species in table 1 and three groups are apparent. Species with high spleen weight/body weight ratios include the dog and cat; these species are known to possess a high density of smooth muscle in the capsule and trabeculae and experimental observations have confirmed that the spleen of these species actively contracts. A second group includes the species where the spleen contains little capsular and trabecular smooth muscle and where the available experimental evidence strongly suggests that active splenic contraction does not occur. The spleen weight/body weight ratio in these species, obtained by many groups of workers, is consistently low and may reflect the absence of a reservoir function. The rabbit forms a third group with a very low spleen weight/body weight ratio.

B. Histology

Distribution of the smooth muscle within the spleen. A discussion of the actions of drugs upon the spleen must include a consideration of the possible sites of action. The comparative histology has been reviewed exten-

TABLE 1

Relationship between splenic weight and body weight in different species

Spleen Wt., mean (range) ± S.E.	Spleen Wt./ Body Wt.	No.	Condition	Ref. No.
g	%			
Dog				
98 (29–185)	0.54	10	Anaesthetized	46
	0.25	23	No details	160
67	1.5	1	Estimate from x-ray outline. Post mortem check	12
31 (19–41)	0.19	4	Light anaesthesia, bled out	225
370 (345–394)	1.7	3	Anaesthetized, wt. after perfusion	188
300	2.0	16	Anaesthetized	96
51 ± 4.5	0.42	26	Anaesthetized, wt. after perfusion	124
104 (47–255)	0.86	23	Anaesthetized, trimmed	70
938 (430–1500)	3.0	5	No details	32
Cat				
5.1	0.22	10	Fresh post mortem after decapitation	254
21 (12–45)	0.7	19	Estimated from x-ray outline. Post mortem check	12
7.4 (3–11)	0.25	10	Post mortem—contracted by traumatic shock	184
27 (8–45)	0.8	2	Anaesthetized	202
8.9 ± 2.1	0.35	69	Anaesthetized	73
28 (12–56)	0.96	32	Anaesthetized, weighed *in situ*	112
24 ± 1.8	0.83	3	Anaesthetized, weighed *in situ*	224
12.3 (5–24)	0.43	10	Anaesthetized, trimmed	70
Mouse				
0.05	0.35	3	Fresh post mortem after decapitation	254
0.15 ± 0.005	0.69	180	Neck broken, trimmed	208
0.31 ± 0.05	1.03	100	Killed by stunning and bleeding	231
0.10 ± 0.01	0.39	24	Neck broken	49
Man				
142 (96–241)	0.25	12	Fresh post mortem after decapitation	254
151 (50–400)	0.24	3400	Post mortem after death by accident or disease	159
191 (60–350)	0.31	20	Fresh post mortem	259
212 (60–370)	0.30	8	Removed during surgery	6
194 (152–235)		2	No details	250
Monkey				
7.5	0.19	1	Fresh post mortem after decapitation	254
	0.13	7	No details	160
6.6 (5.7–7.5)	0.25	2	Estimate from x-ray outline. Post mortem check	12
Guinea pig				
0.54	0.13	1	Fresh post mortem after decapitation	254
0.90	0.18	1	Bled out	165
0.75 (0.35–1.2)	0.13	5	Neck broken, trimmed	70
0.97 ± 0.10	0.18	9	Anaesthetized	205

TABLE 1—*Continued*

Spleen Wt., mean (range) ± S.E.	Spleen Wt./ Body Wt.	No.	Condition	Ref. No.
g	%			
Rat				
0.66	0.27		Killed by ether, trimmed	150
0.53 ± 0.08	0.29	5	Killed by stunning and bleeding	231
0.64 ± 0.03	0.13	11	Anaesthetized, ether	105
0.59 ± 0.08	0.26	24	Decapitation	139
0.86 ± 0.07	0.23	58	Killed by ether or bleeding, trimmed	238
0.65 ± 0.09	0.27	26		
0.67 (0.5–0.9)	0.34	6	Killed by stunning, trimmed	70
Rabbit				
0.5	0.05		Fresh post mortem after decapitation	254
1.02 (0.1–5.0)	0.045	645	Killed by ether and bleeding, trimmed	39
1.4 (0.6–2.5)	0.058	26	Anaesthetized, trimmed	99

sively (121, 152, 240, 256) and the distribution of smooth muscle in the spleen of many species has been described (48, 235, 240). In most species the spleen has a framework of collagen containing a network of reticular fibres. The limiting capsule of the organ consists of an outer serosal layer with an inner layer of elastic, collagen, and smooth muscle fibres. The important and varying histological feature of this inner layer is the extent and distribution of the smooth muscle component. In many species (dog, cat) the capsular smooth muscle is abundant although in other species (man, rabbit) it is very sparse. The inner layer of the capsule, containing the smooth muscle, invaginates into the pulp of the spleen to form trabeculae dividing the organ into compartments or lobules. The presence and density of the smooth muscle in the capsule and the number of trabeculae is an indication of the reservoir function of the organ in any species. In some animals (cat, dog) the spleen acts as a store of erythrocytes for acute emergencies; contraction of the smooth muscle of the capsule and trabeculae reduces the capacity of the organ expelling red cells into the circulation. In this way the systemic haematocrit is raised and the oxygen-carrying capacity of the blood increased. In an important paper (171) McCance and Widdowson have shown that in contrast to the adult dog, the spleen of the newborn puppy has poorly developed trabeculae represented by delicate, rather inconspicuous networks of strands of spindle-shaped cells lacking the distinctive staining reactions of muscle. They are probably immature muscle cells which have not yet become contractile. The capsule of the spleen at birth also lacks mature muscle fibres. The trabeculae and capsule were a little better developed by the 17th day but still resembled those of the newborn rather than the adult.

Blood vessels and intermediate circulation. The blood supply and distribution of smooth muscle in the splenic vascular bed has been studied in the dog (164, 176) and other species (207, 240). The arterial blood is derived from a major branch of the coeliac axis, the gastroepiploic arcade system, and short gastric branches from the stomach. The enormous variation in the course of the human splenic artery has been well described (180). The main tortuous splenic arteries enter the spleen at the hilum, divide, and pass along the trabeculae dividing with them. Eventually when the arteries are reduced to about 250 μ in diameter they leave the trabeculae. In most species a periarterial

lymphatic sheath, the white pulp, accompanies the artery along its length; the artery is usually eccentric to the lymphopoietic centre. Upon attaining a particular size, the arteries lose their investment of lymphocytes and enter the red pulp. The pulp arteriole possesses a thin tunica of smooth muscle before narrowing and dividing into the sheathed arterioles with very thick walls of reticular tissue.

The nature of the splenic circulation between the arterioles and venules has been a field of controversy for many years. Bjorkman in 1947 (28), and more recently Grayson and Mendel (108) and Weiss (253) have reviewed the literature and critically discussed the various theories. A compromise between the "closed" circulation, where blood within the spleen always flowed within endothelially lined channels and the "open" pattern, where it was believed that the sheathed capillaries pour their blood into the pulp cords from which it passes through sinus wall stomata into the veins, is probably more correct. Subsequent investigation (220, 221) has resolved many of the original differences on the basis of species variation. In addition the difference in appearance of the red pulp in those species where the splenic capsule is contractile obviously depends upon the size of the spleen at fixation. The interpretation of drug action on the splenic vascular bed does not require the intermediate circulation of the spleen to be clearly defined. However, there is general agreement that at this stage of the microcirculation, the circulating blood cells come into intimate contact with the phagocytic cells of the reticuloendothelial system lining the pulp cords and venous sinuses. It is here that a further function of the spleen is revealed in the removal of effete erythrocytes, toxins, and cell debris. The venous sinuses consist of bands of reticulum with fibrillar elements; however, it is not suggested that these are contractile (98).

The venous sinuses empty into the pulp veins, lined by endothelium, which unite to form larger veins entering the trabeculae as trabecular veins. These latter channels appear to consist of only an endothelium supported by the smooth muscle and collagen of the trabeculae. They eventually drain into the splenic vein at the hilum. The influence of alterations in the tone of the trabecular smooth muscle on the venous drainage from the spleen and on the functions of the spleen as a filter and red cell store have not been examined sufficiently. It has been suggested that the spleen is segmental and that each segment is drained by its own vein (36, 37). In the rat additional drainage is afforded by collateral segmental veins and this is the channel by which substances pass from one splenic segment to another rather than diffusion through the pulp. Experimental advantage has been taken of this arrangement in the cat (30, 73) and two halves of the spleen have been perfused with separate circuits to give control and experimental preparations in the same organ.

Lymphatic supply. The lymphopoietic centres of the white pulp drain mainly into the venous system but also by afferent lymphatics in the larger trabeculae. Lymphatic channels draining the spleen are so inconspicuous that they are often not mentioned or their presence is denied in the histological literature. However, it has been observed that injection of a patent blue dye into the parenchyma of the spleen of rats, mice, and guinea pigs (104) was quickly and clearly visible in lymph capillaries and appeared later in lymph channels. Contractions of the lymphatic walls were seen to move the dyed lymph into the splenic lymph node. The actions of drugs on these contractions, presumably mediated through a sparse smooth muscle distribution, and on the lymphatic drainage from this important lymphopoietic centre is almost unknown.

Innervation. The innervation of the mammalian spleen has been investigated by classical staining techniques and there is general agreement that it is almost entirely sympathetic and postganglionic in origin (239, 240). A few nerve cells have been

found along the course of the splenic artery but none of these lie within the spleen. A parasympathetic innervation from the vagus nerve has been discounted in the cat (242) by a study involving counts of fibre types in the splenic nerves after chronic section of the vagus. This study also concluded that any sensory innervation to the spleen was small.

Recent studies in the cat spleen tracing the routes of nerve fibres have combined histochemical methods with either autoradiography (102) or electronmicroscopy (90). Only noradrenergic nerve fibres were present in the spleen and these were distributed sparsely suggesting to the author (90) that extensive diffusion of the transmitter must occur. The specific fluorescence of catecholamines which was confined to the nerve fibres among the smooth muscle of the capsule, trabeculae, arteries, and veins in the normal cat was not present after treating the cat with reserpine or after degeneration of the postganglionic splenic nerve fibres (102). Similar histochemical investigation in the dog (53) indicated that the normal spleen in this species has a very rich supply of adrenergic fibres situated around the vessels in the white and red pulp and in the trabecular network. Occasionally single terminal bundles were scattered in the red pulp without any obvious relation to blood vessels or trabeculae. No fluorescence characteristic of adrenergic neurons was observed if chronic denervation had been carried out.

C. Splenic Blood Flow

Splenic blood flow has been measured in many species and by many different methods and the reported values, even within the same species, show considerable variation. The resting splenic blood flows measured in several species appear in table 2. Possible sources of variation are different experimental approaches necessitated by different recording probes and limitations inherent in the methods, but the majority of measurements in all species give resting splenic blood flows of between 40 and 100 ml/min/100 g. No obvious species grouping is apparent as with spleen weights since the universal function of the spleen as a cellular sieve and its role in immunological processes necessitate a large blood flow. Therefore, the absence of a red cell reservoir function in the spleen of any particular species may not be apparent as a diminished splenic blood flow.

D. Appraisal of the Methods of Investigation

Many different preparations have been used for the study of the action of drugs on the spleen and it is apparent that many of the conflicting results in the literature have arisen from these variations in preparation. In the following brief, general discussion, the main advantages and disadvantages of the three basic techniques are considered.

In situ preparations. The majority of the earlier studies were performed on *in situ* preparations in which changes in splenic volume were measured with an oncometer or by plethysmography. Splenic blood flow was seldom recorded because of technical difficulties. If the spleen remained innervated and drugs were administered by intravenous injection, interpretation of the results was difficult because of the many sites outside the spleen at which a drug could act and thereby indirectly elicit changes in the smooth muscle of the spleen. It was considered that denervation of the spleen and bilateral adrenalectomy revealed a direct action on the spleen if subsequent administration of the drug evoked responses from the splenic capsule or blood vessels. Recent work has shown that substances released from the kidney or neurohypophysis can affect the smooth muscle of the spleen and this possibility should be considered in the analysis of the results of experiments in which drugs have been administered by intravenous injection.

Advances in instrumentation, particularly the use of non-cannulating electromagnetic flow probes, has made it possible to carry out *in situ* recording of splenic arterial

TABLE 2

Splenic blood flow in different species

Splenic Blood Flow, mean (range) ± S.E.	Splenic Blood Flow, mean (range) ± S.E.	No.	Method of Measurement	Ref. No.
ml/min	*ml/100 g/min*			
Man				
500–700	300 (approx.)		^{51}Cr labeled red cells	258
100–200	96 (60–140)	16	^{133}Xe clearance, wt. estimated from x-ray	257
150 (approx.)	83 (approx.)	4	^{51}Cr labeled red cells (assumed spleen wt.)	143
298		1	^{133}Xe clearance + electromagnetic flowmeter	32
313 ± 16	391 ± 21	7	Dye dilution	156
Dog				
30 (approx.)	10 (approx.)	12	Electromagnetic flowmeter	188
97			Thermostromuhr	116
57 (17–137)	58 (29–185)	10	Stromuhr in vein	46
30	10	16	Pressure/flow studies	96
20 ± 1.3	45 ± 4	26	Electromagnetic flowmeter	124
78 (60–100)		5	Nycotron electromagnetic flowmeter	3
47 (24–85)	52 (23–163)	18	Perfusion, rotameter	70
10–28			Venous outflow	199
	30–100		Exteriorized spleen, ^{133}Xe clearance	261
164 (71–356)	18 (6–36)		^{133}Xe clearance + electromagnetic flowmeter (very heavy spleens)	32
	128 ± 17	10	Dye dilution	156
Cat				
14 ± 2.4	52	21	Electromagnetic flowmeter	202
29 (12–88)	88 (41–163)	32	Electromagnetic flowmeter	112
7 ± 0.5		42	*In situ*, venous collection	31
41 ± 10.4	171	3	Electromagnetic flowmeter	224
6 (2–11)	42 (29–53)	4	*In situ*, venous collection	70
Rabbit				
1.7 (0.6–6.4)	108 (40–196)	15	Venous collection	99
Rat				
0.5	83		^{86}Rb dilution (assumed spleen wt.)	206
Guinea pig				
0.6 ± 0.09	66 ± 6.4	9	^{133}Xe clearance	205

blood flow. When drugs are administered intravenously, however, changes in systemic arterial pressure and consequently splenic perfusion pressure may occur. Any changes in splenic blood flow must then be related to the pressure-flow curves for the splenic vascular bed. These have not been studied extensively, and it is, therefore, essential to calculate changes in splenic vascular resistance. These difficulties have been overcome by either the administration of drugs by close arterial injection, or by the fine control of splenic perfusion pressure despite changes in the systemic blood pressure. This latter technique, used in conjunction with a recording of splenic weight to monitor changes in volume, is an excellent preparation for the study of reflex alterations in the activity of splenic vascular and capsular smooth muscle.

Isolated perfused spleens. This technique provides a controlled preparation, which is

probably the most satisfactory for a study of drug responses. The preparation may be perfused with either blood or an artificial perfusion medium at either constant flow or constant pressure. Perfusion of the spleen at constant flow eliminates the technical difficulties of continuously recording splenic arterial blood flow, but may result in very high perfusion pressures during vasoconstriction with consequent damage to small vessels and oedema, particularly with artificial perfusion media. Constant pressure perfusion is the more "physiological" method and there is an inverse relationship between the recorded changes in splenic arterial blood flow and the calculated changes in splenic vascular resistance. Volume changes can be measured by plethysmography with little risk of venous obstruction.

Drugs may be administered easily by close arterial injection, which limits their site of action to the spleen and facilitates the interpretation of experimental results. Distinct differences have been observed in the action of a drug on the spleen of the same species, when perfused with either blood or an artificial perfusion medium. This may be related to the immediate fate of a drug when added to blood (*e.g.*, enzymatic degradation or binding), or may be due to changes in the sensitivity of the smooth muscle caused by the artificial perfusion medium.

Isolated spleens or spleen strips. Preparations in organ baths limit observations to the responses of the capsular smooth muscle. Since the majority of species have a sparse distribution of smooth muscle in the splenic capsule, high concentrations of agonist drugs have been used, with a corresponding lack of specificity of response. The inevitable loss of tone in these preparations makes an analysis of relaxant drugs difficult unless tone is superimposed by drugs which cause contraction. Thus the qualitative nature of a response may be different from that obtained in the intact, *in situ* or perfused spleen preparation (*e.g.*, isoprenaline and histamine). The advantage of this type of preparation is its stability and the rapidity of its response, which facilitates the quantitative study of drug-receptor interaction.

III. Effects of Nerve Stimulation

The ability of the spleen to contract was predicted by the histologists, who had made detailed studies of structure. Rudolf Wagner in 1849 (248) was probably the first person actually to observe a contraction. He applied electrodes to the cross axis of the spleen of an anaesthetized dog and observed that the spleen instantly paled and the surface became wrinkled, almost like goose flesh. The substance of the spleen also felt much harder to the touch. Exactly the same phenomena were found with the cat spleen, whereas the rabbit spleen did not exhibit them. Wagner posed the question, "How far is the rich nerve supply to the spleen involved in these phenomena?" and added that he hoped to demonstrate this later. No report can be found, however, that he ever did. Three years later Henle (130) had access to the fresh dead body of a beheaded criminal and 35 min after death stimulated the nerves to the spleen and also attempted direct stimulation, but could not observe any effect. He then removed the spleen, which was previously pale and wrinkled, and observed that it became smoother and darker; evidence, he suggested, that the organ had contracted. During the following 10 years there were a number of reports of contraction of the spleen in various animals (89, 227, 241) but little further progress was made until Schiff in 1867 (211) reported contraction of the spleen in response to stimulation of the splanchnic nerves in cats and rabbits. The role of the splanchnic nerves was confirmed by Tarchanoff in 1874 (233) who observed that the contractions in response to stimulation of the medulla oblongata and various sensory nerves were dependent on an intact innervation. The possible involvement of the vagus nerve was suggested by Oehl in 1874 (186) when he obtained splenic contraction in response to stimulation of the peripheral end of the nerve in the neck of dogs, cats, and rabbits.

Bulgak (40) reviewed work on the subject up to 1877 and reported his own work, most of which had been published in Russian some 5 years previously. In contrast to previous reports (186), he could not observe any contraction of the spleen in response to stimulation of the peripheral end of the cut vagus nerve in either the neck or the thorax, but he reported contraction after stimulation of the central end. He suggested that this was an effect secondary to the depression of respiration. In addition he investigated the effects of stimulation of various regions of the spinal cord and found that only in the region of the nerve roots of the greater splanchnics did stimulation cause contraction of the spleen.

In 1881, Roy (203) published a paper, which was probably the first report of experiments in which changes in spleen volume were continuously recorded *in situ* by means of an oncometer. He demonstrated contraction of the spleen in response to electrical stimulation of the medulla oblongata, of the central end of sensory nerves (vagus and sciatic) and of the peripheral ends of the splanchnic and vagus nerves. He also showed that after section of the vagi and splanchnic nerves, stimulation of a sensory nerve still caused contraction and suggested that the "influence may pass from the cerebro-spinal centres to the spleen by some other route or routes than the nerves named." He also noted that a large fall in arterial blood pressure accompanied the splenic contraction produced by stimulation of the peripheral vagus but because of their different time-courses he felt that the two events were unrelated. Schäfer and Moore (209) carried out a detailed investigation of the contractility of the spleen of the dog and cat, which confirmed the motor innervation of the spleen through the splanchnic nerves. On the evidence of their results they concluded that there were no vagal fibres that directly influenced the contraction of the spleen thus supporting the views of Bulgak (40) rather than those of workers who had suggested a direct vagal innervation (186, 203). At the turn of the century, detailed anatomical studies of the "involuntary" nervous system were in progress and the sympathetic and parasympathetic subdivisions were defined.

A. Sympathetic Nerve Stimulation

Contraction of the spleen in response to stimulation of its pre- or postganglionic nerve supply had been demonstrated clearly in the dog and cat and this basic fact was subsequently confirmed by many groups with different techniques including *in situ* volume recording with a plethysmograph (14, 50, 229), volume recordings of isolated preparations perfused either with blood (33, 66, 110, 209) or an artificial saline medium (34, 92, 183, 236), at either constant flow (33, 34, 183, 236) or constant pressure (66, 92, 110). Contraction had also been recorded by measuring changes in length with either a Cushny myograph (7, 8) or with a transducer (161), changes in spleen surface area (47, 50, 226), changes in spleen weight (33, 110, 112, 183), or by changes in venous pressure in the isovolumetric spleen (251).

The most obvious response of the spleen to sympathetic nerve stimulation is contraction. A second response, vasoconstriction, was observed very early but received little attention because of technical difficulties. In 1900, Mall (176) reported that in experiments with the *in situ* dog spleen, stimulation of the nerves to the spleen or direct stimulation, resulted in the expulsion of blood as the spleen contracted and then a period followed when the venous outflow almost ceased. A more detailed investigation with the isolated dog spleen, perfused with Ringer-Locke solution gave similar results (212). The first continuous recording of splenic blood flow was probably that of Burton-Opitz (45, 46), who placed a "stromuhr" in the splenic vein of a dog and showed that on stimulation of either the preganglionic splanchnic or postganglionic splenic nerves, there was an "extraordinary increase" soon followed by an "equally

pronounced decrease" in flow. He pointed out that this primary increase was not due to vasodilatation, but to the squeezing out of blood by the contraction; only after the completion of this event did the vasoconstriction become apparent.

After these initial observations on the vascular responses of the spleen to sympathetic nerve stimulation, little additional information was obtained during the next 40 years, although Mertens (179) confirmed them with a thermostromuhr. The main progress during this period was the visual observation of vasoconstriction in the transilluminated spleens of small mammals, when the splenic pedicle was electrically stimulated (169). Improved techniques of blood flow recording made possible the simultaneous observations of changes in both volume and blood flow of the spleen. It was then observed that sympathetic nerve stimulation caused a profound vasoconstriction concomitant with the contraction of the splenic capsule and that the administration of an *alpha* adrenergic blocking agent abolished the contraction and converted the vasoconstriction to a small vasodilatation (65, 110, 112). However, a feature revealed by these simultaneous recordings of the vascular and capsular responses was the different time courses. Invariably the vascular response reached its maximum effect before that of the capsule and returned to the control state much more rapidly after the cessation of the stimulation. Indeed, the vascular response to short trains of stimuli returned to control before the capsule had reached its maximum response (33, 65, 236).

A single stimulation frequency had been investigated in most studies and when a wide range of sympathetic nerve stimulation frequencies was used (47) in the cat spleen it was found that a maximum contraction was obtained at about 4 Hz indicating that the tonic discharge was normally very low. This observation was confirmed in the isolated, blood perfused dog spleen (60, 61) where it was observed that at these low frequencies, which produced near maximal contractions, there was little or no response of the splenic vascular bed. Changes in vascular resistance only became apparent at higher frequencies with maximal vasoconstriction occurring at 7 to 10 Hz. There was thus a marked frequency separation of the vascular and capsular responses of the dog spleen, suggesting a capacitative function at lower frequencies of sympathetic activity and a resistance function operating under conditions of near maximum sympathetic activity. Investigations of a frequency separation in the cat spleen has produced conflicting results. In the intact blood perfused cat spleen (112) both capsular and vascular responses reach a maximum at stimulation frequencies of 2 to 5 Hz whilst in the saline perfused preparation (29) the response of the splenic capsule was almost maximal at 4 Hz but the response of the vascular bed was not maximal until 10 Hz. The majority of the studies on splenic responses to nerve stimulation have been made in either the dog or cat where contraction is obvious and easy to record and the storage function so definite. Information in other species is sparse.

Stukeley in 1723 (230) suggested that the human spleen might contract and act as a store of blood and Henle (130) provided some evidence but was unable to observe contraction after electrical stimulation of the nerve supply. Indirect evidence accumulated leading to the conclusion that the normal human spleen did not contract and function as a blood reservoir. Recently this general belief has been confirmed by a study of the normal human spleen removed at operation and subsequently perfused with McEwen's solution (5, 6). The results showed that when the splenic nerves are stimulated over a range of frequencies the vascular resistance increases up to a maximum at 10 Hz, similar to the vascular response in the dog spleen. The maximum reduction in volume was very small, about 5 ml, compared with the 50 to 70 ml reduc-

tion in volume observed in the slightly smaller spleen of the dog.

Stimulation of the splenic pedicle of the mouse, rat, rabbit, and guinea pig causes contraction observed visually whilst concomitant vasoconstriction was observed microscopically by transillumination (169). Splenic nerve stimulation in the isolated blood perfused rabbit spleen (99) causes a profound vasoconstriction and a reduction in spleen volume although how much of this is a passive effect has not been ascertained. Isolated rabbit spleen strips when subjected to electrical field stimulation in an organ bath show graded contractions with increasing frequency, reaching a maximum response at 8 Hz (111) and direct electrical stimulation of the rabbit spleen *in situ* causes contraction (178).

There is no doubt about the general nature of the smooth muscle responses of the spleen to stimulation of the sympathetic nerve supply. Vasoconstriction has been observed in all species studied and is accompanied by contraction of the capsule, the intensity of which depends considerably on the species. It has been suggested that these two smooth muscle components have anatomically distinct nerve supplies, both carried in the splenic nerve (50); this idea has received little or no support.

B. Parasympathetic Nerve Stimulation

The effect of stimulation of the vagus nerve on the smooth muscle of the spleen has been a subject of controversy for many years; conflicting reports have suggested that stimulation of the peripheral end of the vagus has either a direct effect on the spleen (186, 203) or no action (40, 209). However, it is clear that stimulation of the central end of the vagus does result in contraction of the spleen (40, 186, 203, 209, 229, 233). Magnus and Schäfer (174) clearly showed that although stimulation of the peripheral end of the vagus in the neck had no effect on the spleen, if insufficient care was taken to prevent the spread of current to surrounding tissues a reflex contraction could be elicited due to the accidental stimulation of the central end. Furthermore, the majority of evidence favoured the explanation that any contraction after stimulation of the peripheral vagus was a secondary response due to the fall in systemic blood pressure. Experiments in which atropine was administered in a dose sufficient to block the cardiac effects of vagal stimulation (174, 209) or in which the vagus was stimulated in the thorax or below the diaphragm (129, 177, 179) or in which any fall in blood pressure was prevented by the use of a compensator (177), revealed that stimulation of the peripheral vagus was not accompanied by contraction of the spleen. The passive nature of the reduction in spleen volume after peripheral vagus stimulation was demonstrated when it persisted after section of the preganglionic splanchnic nerves (229) or the postganglionic splenic nerves (137). In the latter case, the reduction in splenic volume was abolished by the administration of atropine to block the direct cardiac effects of vagal stimulation.

In contrast to the general consideration of whether stimulation of the peripheral vagus has a direct effect on the spleen causing contraction, are the results of a detailed study in which the peripheral vagus and the splanchnic nerves were stimulated at the same time or one after the other to observe mutual interaction (218, 219). The appearance of the spleen was observed and the time course of events noted. It was concluded that the peripheral vagal stimulation delayed the onset of the response to splanchnic stimulation and this was taken as a demonstration of the sympathetic and parasympathetic systems having antagonistic actions on the spleen.

In conclusion, on the basis of the physiological evidence, it is probable that stimulation of the peripheral end of the vagus nerve exerts no direct effect on the spleen, a view supported by histological evidence of the lack of any parasympathetic innervation to the organ (242).

IV. Catecholamines

A. Directly-acting Catecholamines

1. Adrenaline and noradrenaline. A. ACTIONS ON CAPSULAR SMOOTH MUSCLE. In 1895 Oliver and Schäfer (187) described the physiological effects of extracts of the suprarenal glands and reported the enormous contraction of the spleen of the dog produced by the injection of extracts of the calf suprarenal gland. These observations were confirmed rapidly in the classic paper by Schäfer and Moore (209) concerning the contractility and innervation of the spleen where injections were made into the splenic artery rather than a peripheral vein so emphasising the peripheral action of the active substance. In the succeeding 40 years little of significance was added to these initial observations but they were amply confirmed in many different species and preparations. Adrenaline has been shown to produce contraction of the spleen of the anaesthetized dog (7, 10, 92, 115, 122, 123, 131, 137, 141, 144, 155, 158, 191, 195, 226, 229), unanaesthetized dog (79, 120), anaesthetized cat (7, 54, 97, 137), and anaesthetized rabbit (75). Most of the early authors did not provide any volume calibrations with published records but all agreed that an increase in the volume of the spleen was never observed normally. It is probable that the few early reports (122, 123) of adrenaline causing an active dilatation of the spleen resulted from experimental artifacts due to venous occlusion and subsequent engorgement of the spleen and perhaps to the use of impure adrenal extracts. Two observations from the early period are of significant interest. It was shown (155) that very small doses of adrenaline which caused no change in systemic blood pressure in the dog were associated with marked splenic contraction. In a study on the onset of experimental asphyxia in the cat (52) it was observed that the spleen was almost maximally contracted before other sympathetically innervated structures began to respond. These were the first observations to suggest that, in these species, the capsule of the spleen had a very low threshold to stimulation either by nerves or circulating adrenaline. Furthermore, in the anaesthetized dog (115), where changes in spleen volume were recorded by plethysmography and thermostromuhrs were placed in both the splenic artery and vein, the temporal relationships between changes in splenic volume and splenic blood flow during an adrenaline infusion were clearly established. The observation by this group that contraction of the dog spleen was not always accompanied by a reduction in splenic blood flow is of particular interest in view of the subsequent studies in the same species (61).

After the demonstration of the presence of noradrenaline in the spleen (80) and its identification in the splenic venous effluent after sympathetic nerve stimulation (192) many authors have shown that injections of noradrenaline caused splenic contraction although the relative potency with respect to adrenaline varied with species and preparation. A large variation in the activity of (±)-noradrenaline relative to adrenaline of 0.5 to 5 times was found on the splenic capsule of the spinal cat (41). It was also found that the potency of noradrenaline declined during each experiment whilst that of adrenaline increased. Furthermore, it was observed that, in cats, a dose of noradrenaline which caused a greater pressor response than adrenaline induced a smaller contraction of the splenic capsule (138, 214). A careful study (47) of the cat spleen involving photography of the exteriorised flat surface showed that graded responses (contractions) were produced with intravenous infusions of (±)-adrenaline from a threshold dose of 0.2 μg/kg/min to a maximum contraction with 2.0 μg/kg/min. Noradrenaline was always less effective and generally the ratio of potency of adrenaline to noradrenaline was 2:1. The contractions of the splenic capsule caused by either catecholamine, whether administered by intravenous infusion or released from the adrenal glands by splanchnic nerve stimula-

tion were never as large as the maximum response to splenic nerve stimulation. These differences in response were related, by the author, to a limited access of blood-borne catecholamines to the receptor sites on the capsular smooth muscle due to the concomitant vasoconstriction. However, no simultaneous record of the vascular changes within the spleen was made. Changes in spleen weight have been used as an indication of volume variations in the cat spleen. The weight of the cat spleen decreases during the infusion of noradrenaline (114) and larger decreases in weight were obtained during infusions of the same doses of adrenaline. Although spleen volume changes were not recorded directly (202) the increased venous outflow accompanying the close arterial administration of noradrenaline to the cat spleen was observed to be less than that produced by the same dose of adrenaline in the same preparation.

The exteriorised spleen of the anaesthetized dog has been used to compare the actions of catecholamines (2). Adrenaline was found to be much more effective than noradrenaline in contracting the splenic capsule whether administered by close arterial or intravenous routes. Over a medium range it was 10 times more effective. In the same preparation the maximum contraction to adrenaline was always much greater than the maximum to noradrenaline. The reductions in weight of the dog spleen (110) were about the same for adrenaline as high sympathetic nerve stimulation (20 Hz) whilst noradrenaline was about half as active. There was no overshoot in the spleen weight after regaining control values. The blood flow in these experiments was slightly lower (30 ml/min for 18.5–24 kg dogs) than most comparable studies (see table 2) and the stagnant blood flow through the spleen may be a contributory factor to the long duration responses which were observed. Adrenaline produced a significantly greater decrease in weight of the dog spleen perfused with blood at constant flow (33) than noradrenaline although the increases in perfusion pressure were not different. In the dog spleen perfused with Krebs-Ringer at constant flow of 20 ml/min (183) a greater similarity between the effects of sympathetic nerve stimulation and injections of adrenaline was observed than most authors have reported. In these experiments the splenic contraction to adrenaline was 97% of the contractile response to nerve stimulation at 2 Hz whilst the response to injections of noradrenaline was very much less (31.4%). However, the authors did not state whether the splenic contraction to sympathetic nerve stimulation at 2 Hz was, in their experiments, maximal. Plateau capsular contractions to catecholamine infusions at 6 to 9 different arterial blood concentrations were investigated with the isolated dog spleen perfused with blood at constant pressure (59, 61). Concentration-dependent differences in the responses of the capsular and vascular smooth muscle systems were found. Low arterial blood concentrations of either adrenaline or noradrenaline (50 ng/ml) evoked the maximal contraction of the splenic capsule obtained with each amine but only small changes in splenic vascular resistance or conductance. In this series of experiments the maximum contractions of the splenic capsule to adrenaline and noradrenaline were not significantly different although at arterial blood concentrations producing submaximal responses adrenaline was always the more potent. Moreover, the threshold arterial concentration to produce a capsular response was lower for adrenaline than for noradrenaline. The maximum reduction in spleen volume produced by either adrenaline or noradrenaline was significantly less than the maximum contraction to sympathetic nerve stimulation. Since in these experiments on the dog spleen, the maximum contraction of the splenic capsule to either adrenaline or noradrenaline occurred at arterial doses below that at which there were significant reductions in splenic arterial blood flow, the differences between the maximum responses to either adrenaline or

noradrenaline infusion and nerve stimulation cannot, in this species, be explained on the basis of limited receptor access unless there are alterations in the microcirculation and distribution of blood within the spleen.

Various transducers have been sutured to the splenic capsule or placed around the splenic blood vessels of dogs and recovery allowed (117, 118). A continuous record of splenic diameter, splenic vein blood flow and splenic vein and systemic haematocrit has been obtained in the unanaesthetized animal with innervated spleen and intact cardiovascular reflexes. The intravenous administration of adrenaline produced a large decrease in spleen diameter which was estimated to be equivalent to a reduction in spleen volume of 85 ml; the recovery time was 13 min. Intravenous noradrenaline produced changes of the same magnitude but slightly more prolonged.

Experiments on the intact human spleen have been very limited. Results of experiments in which spleen size was indicated by palpation and counts of the circulating red blood cells were made, led to the suggestion that adrenaline, by subcutaneous injection, caused contraction of both the normal and the enlarged spleen (260). This was confirmed in the normal spleen of both child and adult by x-ray studies and red cell counts (19), and by measuring areas from x-rays after thorotrast (189, 247). Similar x-ray studies indicated contraction of the pathologically enlarged spleen to subcutaneous injection of adrenaline (181, 249, 252).

In one investigation (247), results of experiments on normal and enlarged spleens were compared and the small changes in the spleen outline in response to adrenaline were similar in both types. Direct observations were carried out in patients undergoing splenectomy (250). In nine cases the spleen was enlarged and in two it was normal; adrenaline was given close arterially and the spleen was observed and samples of splenic arterial and venous blood were collected. In six of the enlarged spleens, contraction was visible and the red cell count in the venous blood increased, but in the two normal spleens there was no evidence of contraction.

Experiments on the intact, isolated, normal human spleen perfused with McEwen's solution (6) reveal that very small reductions in volume accompanied the injections of either adrenaline or noradrenaline, despite large concomitant increases in splenic vascular resistance. The results suggested that within the limits of the accuracy of the experiments, which involved measurements of small changes in volume, imposed on a changing background, the two catecholamines were equipotent in their activity on the capsular smooth muscle of the human spleen.

B. ACTIONS ON SPLENIC VASCULAR SMOOTH MUSCLE. Despite the early observations (141) that the contraction of the dog spleen by adrenaline was accompanied by an initial increase in venous outflow, and that during the remainder of the capsular contraction the blood flow was reduced, the investigation of the actions of adrenaline on the splenic vascular bed was delayed mainly for technical reasons.

Mertens (1934) incorporated thermostromuhrs into the splenic artery and vein of dogs (179) and showed that intravenous adrenaline, at various doses, caused a large increase in splenic venous flow followed after approximately 2.5 min by a reduction in flow to about 50% of the control value. There was a slight initial increase in splenic arterial blood flow concomitant with the initial increase in systemic blood pressure; this was succeeded by a prolonged reduction in splenic arterial blood flow despite the raised systemic arterial blood pressure. These observations clearly indicated an increase in splenic vascular resistance concomitant with the phase of splenic capsular contraction. Extensive experimental observations on the actions of adrenaline on the splenic vascular bed of the dog appeared in 1939 (116) when thermostromuhrs were placed on the splenic artery and vein in

dogs and recovery allowed. Resting blood flows of 97 ml/min in the splenic artery and 91 ml/min in the splenic vein were recorded and adrenaline at a dose of 5 μg/kg body weight caused a transient but marked increase in flow to about twice the control levels; this was succeeded by a period of decreased flow in both the artery and vein. In a subsequent paper (115) changes in spleen volume were also recorded by plethysmography and the dissociation between the capsular and vascular events obtained, since it was observed that capsular contraction did not always result in reductions in splenic arterial blood flow. The capsular and vascular smooth muscle components were capable of independent activity. The periodic oscillations in splenic blood flow characteristic of adrenaline injections and previously described by Mertens (179) were analysed; they were large in amplitude with a 30 to 40 sec time-course. On the basis of the temporal relationship between the recording parameters the authors (115) interpreted these waves as arterial in origin. These periodic oscillations in splenic arterial blood flow have been reinvestigated recently (124). In the dog spleen where both the splenic artery and vein were cannulated to incorporate electromagnetic flowmeters (188) both adrenaline and noradrenaline caused an initial marked reduction in arterial inflow and after a brief delay an increase in venous outflow with a rise in splenic venous pressure. The recovery to control flow values was much longer after adrenaline. A prolonged secondary dilatation followed the administration of both amines. In the dog spleen perfused at constant flow with blood (33) the close arterial injection of noradrenaline (1 μg) produced an increase in splenic vascular resistance equivalent to splenic nerve stimulation at 5 Hz but a smaller increase in splenic venous pressure indicative of a smaller capsular contraction and emptying. When Krebs-Ringer solution has been used as the perfusion medium (183) distinct characteristics in the splenic vascular responses to adrenaline have been described. A triphasic response consisting of an initial rapid rise in pressure which was succeeded by a pronounced decrease and finally a secondary rise. No intermediate pressure decrease was observed with injection of noradrenaline. This triphasic pattern of vascular responses to adrenaline was not observed, however, in the experiments of another group (65) where the dog spleen was isolated and perfused with McEwen's solution at constant flow. The overall vasoconstrictor properties of adrenaline and noradrenaline have been confirmed in the isolated dog spleen perfused with blood at constant pressure where changes in splenic arterial blood flow have been measured with rotameters (57, 59, 61, 65). In one study (59, 61) plateau vascular responses were obtained from 6 to 9 different arterial concentrations of either adrenaline or noradrenaline. At low arterial concentrations of noradrenaline (0.5 μg/ml) when the contraction of the splenic capsule was almost maximal there were very small concomitant reductions of splenic arterial blood flow. Indeed in four of the nine experiments in this series arterial noradrenaline concentrations between 0.01 and 0.1 μg/ml were observed to induce splenic arterial vasodilatation. Higher blood concentrations of noradrenaline (0.1–1.0 μg/ml) evoked graded reductions in splenic arterial blood flow and at concentrations of 5.0 μg/ml almost complete cessation of splenic blood flow occurred. A similar dose response relationship was observed with adrenaline. In seven of the eight experiments low arterial concentrations (less than 0.1 μg/ml adrenaline) caused an increase in splenic blood flow indicating vasodilatation of the splenic bed. When the arterial concentration of adrenaline was increased above 0.05 μg/ml graded reductions in splenic arterial blood flow were observed to accompany the maximum contractions of the splenic capsule. Almost total cessation of splenic arterial blood flow occurred at concentrations of 1.0 μg/ml adrenaline. Furthermore, the maximum increases in splenic vascular resistance to

sympathetic nerve stimulation or to adrenaline or noradrenaline were not significantly different and in each case represented an almost complete cessation of blood flow through the organ. Lower blood concentrations of adrenaline and noradrenaline which elicited submaximal vascular responses indicated that the two catecholamines were equi-effective on the splenic vascular bed.

In the cat the actions of both adrenaline and noradrenaline on the splenic vascular bed have been investigated with non-cannulating electromagnetic flow probes on the splenic artery (114, 202) and also on the splenic vein (202) although the author states that stable recording of venous flows was only possible with constriction of the vessel with the recording probe. The mean splenic arterial blood flow was 14.3 ml/min and oscillations of splenic inflow and outflow were observed in all preparations including some that were acutely denervated (202). In 10 cats adrenaline was administered by the close arterial route (202) and small doses (0.03–0.3 μg) increased both arterial and venous splenic blood flows whilst larger doses (0.3–5.0 μg) increased splenic venous flow but reduced splenic arterial blood flow. These initial responses reached a peak within 30 to 40 sec and were followed by large oscillations in flow in both the splenic artery and vein. In another study (114) adrenaline was administered intravenously and the splenic perfusion pressure controlled by a micrometer clamp around the splenic artery. The vascular response to adrenaline was more variable and in 15 cats three flow patterns were observed of which the most frequent was a brief initial increase in arterial flow followed by a return towards control or even below. On three occasions a maintained vasodilatation was observed and on four a maintained vasoconstriction. Noradrenaline, when infused by the intravenous route (114), caused a reduction in splenic arterial flow which increased with dose and was well maintained for the duration of the infusion. However, when administered by the close arterial route noradrenaline caused a slight reduction in splenic arterial flow, of short duration, succeeded by a prolonged period of increased flow. No significant difference could be found in the vascular responses of the innervated or acutely denervated cat spleen to either adrenaline or noradrenaline (202).

Observations have been made on the vascular bed of the isolated spleen of man perfused with McEwen's solution at constant flow (6) or with Ringer-Locke solution at constant pressure (212). In the more recent investigation (6) increasing close arterial doses of either adrenaline or noradrenaline over the range 0.25 to 25 μg produced graded increases in perfusion pressure and therefore splenic vascular resistance. The ratio of potency of the two amines on the human splenic vascular bed varied with the dose. Adrenaline was the more potent vasoconstrictor at close arterial doses of less than 1 μg whilst at higher doses the two amines were equipotent.

A general conclusion from these intact, perfused spleen preparations is that both adrenaline and noradrenaline cause contraction of the splenic capsule and vasoconstriction of the splenic vascular bed. The magnitude of the capsular contraction varies considerably between species but in those species (dog and cat) where it is marked, adrenaline is more potent than noradrenaline although the maximum contraction to either amine is less than the maximum response evoked by sympathetic nerve stimulation. In these species the smooth muscle of the splenic capsule is more sensitive to circulating adrenaline and noradrenaline than the smooth muscle of the vascular bed. Furthermore, in the cat and dog low concentrations of adrenaline evoke splenic vasodilatation but over the greater range of blood concentrations both adrenaline and noradrenaline cause vasoconstriction and are equipotent.

C. *IN VITRO* PREPARATIONS. Adrenaline has been shown to contract isolated whole spleens or strips from many species including the dog (24, 26, 94, 95, 137, 204, 243), cat (23, 97, 101, 137, 146, 147, 151, 166–168,

204), kitten (34), man (204), rabbit (95, 136, 137, 148, 167, 168, 173, 204, 243), rat (167, 173), mouse (145), guinea pig (137, 173), sheep (167), pig (95, 173), ox (173), and kid (151). Strips of splenic artery and vein from sheep and cattle have also been shown to contract to adrenaline (167). Noradrenaline has been demonstrated to contract strips of spleen from the dog (232), cat (23, 38, 58, 97, 101, 146, 147, 163), rabbit (148, 170), and kid (232). On the isolated cat spleen strip the potency of adrenaline and noradrenaline has been found to be the same (101) or adrenaline greater than noradrenaline (23).

An extensive quantitative study (21) has evaluated the responses of isolated strips of cat spleen to sympathomimetic drugs and their antagonists. Concentrations of adrenaline (1×10^{-8} to 5×10^{-5} M) and of noradrenaline (3×10^{-7} to 3×10^{-5} M) produced concentration dependent contractions, each of which was initially rapid and then followed by a slower component that usually reached a peak within 3 to 5 min and was maintained. Adrenaline was more potent than noradrenaline. On these isolated strips of cat spleen the sensitivity of a second concentration activity series was consistently reduced below the sensitivity to the initial series. This reduction was usually more marked at concentrations below half maximal. In the second series the responses of the strips to a standard submaximal concentration of adrenaline and noradrenaline were reduced on the average by 77.6% and 45.4%, respectively. However, the maximal contracting effect of the catecholamines was not altered. The sensitivity of the strip to a subsequent series usually was further reduced. In a later publication (22) the actions of adrenaline and noradrenaline on the isolated spleen strip of the cat were analysed in terms of the mass action laws. In addition, strips from the kitten spleen have been shown to contract more rapidly with high doses of adrenaline although the maximum equilibrium contraction had been attained (197). The potency of the (+)- and (−)-isomers of noradrenaline on isolated strips of cats spleen differed very little (109) but whilst there was a 3-fold increase in the sensitivity to (−)-noradrenaline 7 days after denervation there was no change in the sensitivity to (+)-noradrenaline. The sensitivity of the capsular smooth muscle in different areas of the spleen to noradrenaline was examined and was not found to vary across the width of the spleen but the medial end of the spleen was significantly more sensitive to (−)-noradrenaline than the lateral end. However, the sensitivity to (−)-noradrenaline of both ends was increased by chronic denervation but the medial end remained the more sensitive.

The observations of Ignarro and Titus (145) on the whole isolated mouse spleen were that both (−)-noradrenaline and (−)-adrenaline caused contractions of equal rates and that both amines had equal intrinsic activity.

In the studies of Sheys and Green (216) the dissociation constants (K_A values) of several agonists were determined on rabbit spleen strips and aorta. (−)-Adrenaline was the only agonist studied that had the same EC50, K_A and efficacy in the two tissues. (−)-Noradrenaline had the same EC50 in the two tissues but different efficacies and K_A values. On the other hand (+)-noradrenaline had the same efficacy but significantly greater EC50 and K_A values in the spleen compared with the aorta suggesting that the receptors in the two tissues are different.

D. TRANSILLUMINATION STUDIES. The classical experiments of Knisely (153, 154) revealed that the local application of adrenaline (1:10,000) caused a decrease in the size of the rat spleen and induced many sinuses to empty. A period followed in which the arterial blood flowed in a pulsating stream through the capillaries, sinuses, and sinus system into the venules. A more extensive comparative study (169) evaluated the effects of intravenous and local administration of adrenaline on the splenic circulation of

mice, rats, rabbits, guinea pigs, and cats. Neither the route of administration nor the species altered the qualitative response which was uniform and transient although in the cat the responses were accentuated by the marked force of the trabecular contraction in this species. The first changes were a contraction of the spleen and constriction of the afferent pulp vessels as shown by the irregular, jerky flow in the arterioles and arterial capillaries and paling of the red pulp. As the effect passed off arterial vessels and trabeculae relaxed and after 3 to 5 min the patency to red and white cells of the smallest collateral pulp channels was restored. The vascular constriction produced by adrenaline was not localised to certain spots along the arterial vessels, but affected simultaneously their entire visible length. The venous tributaries of the pulp were not constricted by adrenaline although they were flattened by the concomitant splenic contraction but no decrease in diameter could be measured.

A detailed analysis of the effects of topically applied adrenaline and noradrenaline on the intermediate circulation of the mouse spleen (91) revealed that both substances cause vascular constriction; adrenaline was the more potent. Adrenaline caused constriction in the pulp arteries as well as terminal arterioles but the veins were never seen to react to adrenaline or indeed to any other substance studied. In 37 tests the time to induce constriction by adrenaline ranged from 10 to 50 sec (mean 31) with recovery in 5 to 120 sec (mean 32) after the re-establishment of the control perfusion fluid. Differences were observed in the sensitivity between two terminal arterioles branching from the same pulp artery. (−)-Noradrenaline was eight times less active than adrenaline in causing constriction but also desensitisation of the preparation was observed after the administration of (−)-noradrenaline; these observations are very different from those obtained in isolated whole spleens of the mouse (145). In this transilluminated preparation both adrenaline and noradrenaline evoke splenic contraction which was independent of the vasoconstriction since it occurred at a greater concentration of the drugs and the time-course was always different and usually shorter than the vascular reactions.

2. Isoprenaline. A. *IN VIVO* OBSERVATIONS. Small doses of isoprenaline (0.1–1.0 μg), administered by the close-arterial route, cause a profound vasodilatation in the dog spleen when arterial blood flow has been measured either with an electromagnetic flowmeter (124, 188) or a rotameter (70). These doses caused only small increases in splenic vein pressure and splenic volume (188). Although the increases in volume are small they are significantly greater than the increases in volume observed in the same preparations accompanying the vasodilatation evoked by either close-arterial bradykinin or histamine (70). It is, therefore, unlikely that the increase in volume to isoprenaline is purely passive in origin and an active relaxation of capsular smooth muscle is probably present. It is evident that the vasodilator response to isoprenaline depends on the basal vascular tone of the preparation and that if this is absent then the evoked responses are very different. Thus in the isolated dog spleen perfused with Krebs-Ringer solution at constant flow (183) isoprenaline (1–100 μg) given by close-arterial injection, produced a dose dependent increase in splenic vascular resistance and decrease in spleen weight. If the vascular smooth muscle tone was increased by sympathetic nerve stimulation at 2 Hz then the close arterial injection of isoprenaline (10 μg) produced a marked decrease in the splenic perfusion pressure at constant flow, indicating vasodilatation, and no change in weight. In the dog spleen perfused with blood (70) large doses of isoprenaline (100 μg) evoke vasoconstriction and contraction. These responses may be blocked by phenoxybenzamine unmasking the vasodilatation and capsular relaxation seen predominantly at low doses.

In the intact unanaesthetized dog where

the diameter of the innervated spleen was continuously monitored (118) 20 μg of isoprenaline given by intravenous injection produced a profound systemic hypotension with consequent reflex contraction of the spleen.

Two detailed studies by Ross (202) and Greenway and Stark (114) have elucidated the actions of isoprenaline in the cat spleen; both groups used non-cannulating flow probes. All effective doses of isoprenaline caused a rapid increase in splenic arterial and venous blood flow. Although the arterial and venous flow increases were similar in magnitude the time courses were different. The arterial flow increase developed more rapidly so that the arterial inflow reached a peak 8 to 10 sec before the maximum venous outflow. Both inflow and outflow returned to the control values over the next 5 to 10 min by means of a series of diminishing oscillations (202). The changes in spleen weight resulting from the intravenous administration of isoprenaline (114) were very small and biphasic; an increase in weight accompanied the initial vasodilatation but the succeeding systemic hypotension caused a decrease in weight which was not reflex since the splenic nerves had been divided. The changes in either direction were small not exceeding 15% of the control weight. The authors further analysed the initial increase in weight accompanying the vasodilatation by restricting the arterial inflow. If the increase in flow was prevented the small increase in spleen weight still occurred supporting their view that the active vasodilatation was accompanied by a small active relaxation of the splenic capsule rather than a passive increase in spleen size secondary to the vasodilatation.

B. *IN VITRO* OBSERVATIONS. In contrast to the blood perfused, intact spleen preparation, no effect is observed on isolated strips of spleen until high concentrations of isoprenaline are reached, when contraction of the strip is observed. Contractions to isoprenaline have been obtained from spleen strips of the cat (21, 58, 151, 163), kid (151, 232), rabbit (148, 151), and dog (232). These observations reflect the absence of basal tone in the isolated strip; if the preparations are slightly contracted with histamine, adrenaline, or noradrenaline, much lower doses of isoprenaline (4×10^{-8} to 4×10^{-5} M) cause a moderate relaxation in the cat (21), but not in the rabbit (216).

Low concentrations (5×10^{-9} to 5×10^{-7}) of (±)-isoprenaline produce relaxation of the whole mouse spleen (145), whereas at higher concentrations (5×10^{-6} to 5×10^{-3}) contraction prevails. Similar experiments with rat, guinea pig, rabbit, cat, and monkey strips or whole spleens did not reveal a similar pattern.

B. Indirectly-acting Catecholamines

1. Tyramine. Early observations (226) showed that tyramine, like adrenaline, evoked contraction of both the normal and acutely denervated spleen of the anaesthetized cat and that in the dog spleen the denervated portion did not appear to contract more rapidly or to a greater extent than the innervated portion. Recovery from the tyramine injection occurred within 12 to 15 min, about the same time as for a very large dose of adrenaline. Subsequently it was estimated (97) that tyramine required 250 times the dose of (−)-adrenaline to evoke the same response in the cat spleen. Isolated strips of cat spleen show a considerable tachyphylaxis to tyramine (146) in that the successive responses to 10^{-3} g/ml were reduced markedly. In the intact preparation, the spleen of the cat *in situ*, the question of a difference in the sensitivity of the smooth muscle of the capsule and blood vessels was indicated by the observations (72) that the splenic contraction to successive injections of tyramine declined much more rapidly than did the systemic pressor responses. The authors cited one experiment in which the splenic contractile response to tyramine declined by 90% after six injections whilst no change was observable in the pressor response. This tachyphylaxis could be temporarily arrested by a single large

injection of noradrenaline. In the isolated, blood perfused spleen of the dog (70) tyramine caused a graded vasoconstriction and reduction in spleen volume with a slower onset and longer duration of action than a dose of adrenaline which evoked a comparable maximum response. Both these responses were abolished by a dose of phenoxybenzamine which had abolished the responses to noradrenaline. The dose of tyramine required to be administered in this preparation to cause the same maximum effect was approximately 100 times that of noradrenaline. Studies on the isolated mouse spleen (145) revealed that tyramine evokes a smaller and slower contractile response than (−)-noradrenaline, (−)-adrenaline or (±)-isoprenaline. High concentrations of tyramine elicited a small relaxation which was antagonised by propranolol. Tachyphylaxis to tyramine was very obvious in this preparation and the dose response curve was very much steeper for tyramine than for adrenaline, noradrenaline or isoprenaline.

Analysis of the possible mode of action of tyramine showed that it produced a rise in blood pressure and splenic contraction in spinal cats (42), effects which were abolished by the prior treatment of the animals with reserpine for 2 days. The original responses were restored by an infusion of noradrenaline. These observations have been confirmed with the isolated strip of the spleen of the cat (146) and rabbit (216). However, in reserpine-treated rabbits restoration of the response of strips of spleen to tyramine could not be achieved by application of large doses of noradrenaline (170). Nevertheless, these observations formed the basis of the theory that tyramine exerts its action by releasing noradrenaline within the tissue from a neuronal store. Subsequent experiments have largely confirmed this view. Intravenous tyramine causes splenic vasoconstriction in the cat together with an increase in the perfusion pressure at constant flow and a concomitant increase in noradrenaline in the perfusate to three times the control level (228). In the isolated cat spleen perfused with Krebs-Ringer, tyramine (50 μg) induced pronounced contraction, slight vasoconstriction, and the release of tritium-labeled noradrenaline previously loaded into the organ. Chronic postganglionic sympathetic denervation of the spleen abolished all these responses (132). In the dog spleen, isolated and perfused with Tyrode at constant flow and loaded with tritium-labeled (±)-noradrenaline, tyramine in doses of 10 to 1,000 μg evoked a graded vasoconstriction and an overflow of labeled noradrenaline which remained constant with the dose of tyramine. Phenoxybenzamine prevented this overflow of noradrenaline (128).

2. Ephedrine. Early indirect evidence suggested that ephedrine caused contraction of the spleen since the intravenous administration to dogs (25) and guinea pigs (217) was accompanied by polycythaemia, leucocytosis, and increased platelet count. These effects did not occur in the dog after the removal of the spleen or injection of yohimbine. Direct observations on the exteriorised spleen of anaesthetized (10) and unanaesthetized dogs (71) confirmed that intravenous ephedrine leads to a long-lasting, slow, but marked contraction. In the conscious dog with implanted thermostromuhrs (116), intravenous ephedrine (1.2 mg/kg) produced a transitory rise in splenic arterial blood flow and a more marked initial increase in splenic venous flow. These transitory increases were followed by profound and lasting reductions in both splenic arterial and venous blood flows which returned to normal only after 15 min. A dose dependent contraction of the capsule and graded vasoconstriction were produced by close arterial ephedrine administered to the isolated, blood-perfused spleen of the dog (70). Both these responses were of slow onset and very long duration compared with the responses to noradrenaline or tyramine. To produce comparable maximum capsular and vascular responses, ephedrine required more than 250 times the dose of noradrenaline. In the cat spleen it had been calculated (97) that 10^4

times the dose of ephedrine was equivalent to noradrenaline.

The capsular and vascular responses to close arterial ephedrine in the dog spleen were considerably reduced by a dose of phenoxybenzamine which abolished the responses to noradrenaline and tyramine suggesting that ephedrine had a small but definite direct action of its own on the smooth muscle of the splenic vascular bed and capsule (70).

3. Amphetamine. Amphetamine given by intravenous injection to the intact unanaesthetized dog caused a significant contraction, slow in onset and long in duration, of the spleen located, by previous operation, in a subcutaneous pocket (190). In the anaesthetized dog with exposed spleen, a prolonged contraction and definite but transitory rise in blood pressure, were observed after the intravenous administration of 1 mg amphetamine (Benzedrine) (194). A second injection in the same animal was followed by a further splenic contraction but the blood pressure showed either no change or a slight fall. Graded vasoconstriction and capsular contraction were produced by close arterial amphetamine administered to the isolated, blood-perfused spleen of the dog (70). The responses were of slow onset and long duration. Amphetamine was approximately 150 times less effective on the splenic vascular and capsular smooth muscle than noradrenaline. The responses to amphetamine were significantly but not completely abolished by doses of phenoxybenzamine which abolished the responses to noradrenaline and tyramine, strongly suggesting that, like ephedrine, it has a direct action on the smooth muscle as well as acting through the release of stored noradrenaline.

Amphetamine causes contraction of the isolated strip of cat spleen (147), an effect antagonised by phenoxybenzamine. Protection to this blockade was given by addition of large doses of either adrenaline or 5-hydroxytryptamine suggesting that in this tissue all three compounds act through the same receptor.

V. Adrenoceptor Blocking Drugs

A. Alpha blocking Agents

Many of the earliest observations on the ability of adrenaline to contract the whole spleen or isolated strips also reported that this contraction was specifically antagonised by either yohimbine (24, 195) or ergotoxine (54, 135, 204). The phenomenon of adrenaline reversal, first described by Dale (54), was studied in the cat spleen (167) with various *alpha* blocking drugs. Ergotamine, yohimbine, or dibenamine abolished the capsule contraction after the administration of adrenaline but undulations in volume accompanied the injections. It was suggested that these transient increases in volume were not the result of an active capsular relaxation but passive changes accompanying vasodilatation of the splenic vascular bed. Relaxation of capsule strips with adrenaline after *alpha* blocking agents was never observed, but relaxation to adrenaline after *alpha* block was observed in strips of splenic vein or artery from sheep or cattle.

The actions of phenoxybenzamine have been described in the exteriorised dog spleen where both artery and vein were cannulated to incorporate flow probes (188). The drug itself had no significant effect on splenic arterial conductance or spleen volume in close-arterial doses up to 30 mg. However, the smallest close-arterial dose (0.1 and 0.3 mg) abolished the vasoconstrictor responses to adrenaline and noradrenaline and unmasked vasodilator responses to each, equal in magnitude to isoprenaline. The vasodilator response to adrenaline was further augmented by increasing the dose of phenoxybenzamine, but above 10 mg the response to noradrenaline diminished. In another series (110) spleen weight was continuously measured and recorded. The smallest dose of phenoxybenzamine markedly reduced the capsular contractions to adrenaline and noradrenaline whilst the largest dose caused both adrenaline and noradrenaline to increase spleen weight slightly al-

though nerve stimulation still caused a slight reduction in weight with concomitant vasodilatation. On the basis of their observations the authors suggested that, accepting Ahlquist's classification of adrenaline receptors (1), the splenic arterioles in the dog possess innervated *alpha* receptors and some *beta* receptors which cause vasodilatation but are not innervated. A much wider dose range of phenoxybenzamine was investigated in the isolated, blood-perfused spleen of the dog (65). The direct vascular effects were variable and short lasting although doses in excess of 0.5 mg usually induced transient vasoconstriction. The only effect of small close arterial doses of phenoxybenzamine (1–100 μg) was a partial block of the vascular and capsular smooth muscle responses to adrenaline, noradrenaline, and sympathetic nerve stimulation; there was no evidence of potentiated responses. The effects of low frequency stimulation (1 and 3 Hz) and noradrenaline were reduced to the same extent although the response to 10 Hz was significantly more resistant to blockade. The vascular component of the splenic response to sympathetic nerve stimulation was more sensitive to the blocking action of phenoxybenzamine than the capsular component. The vasoconstrictor responses to sympathetic nerve stimulation, noradrenaline, and adrenaline were all converted to vasodilatation by the prior administration of phenoxybenzamine whilst the contractions were abolished without any evidence of active relaxation. The predominant *alpha* receptor innervation to the capsule of the dog spleen and the presence of innervated *alpha* and *beta* receptors in the splenic vascular bed has been confirmed in the isolated dog spleen perfused with Krebs' solution (183). Moreover, there is now evidence that there are *beta* receptors in the capsule smooth muscle but these may not be innervated (70).

In the blood-perfused cat spleen (114) the pure vasoconstrictor role of noradrenaline was converted to vasodilatation by phenoxybenzamine whilst the biphasic response to adrenaline became purely vasodilator. There was now little change in weight to noradrenaline whilst adrenaline caused a slight increase in spleen weight similar to that evoked by isoprenaline. The smooth muscle of the blood vessels of the cat spleen therefore contain both *alpha* and *beta* receptors and the capsular smooth muscle a rich *alpha* distribution but sparse *beta* population. Which of these receptors in each tissue is innervated has not yet been established clearly.

Low concentrations of (±)-isoprenaline produce relaxation of the mouse spleen (145) whereas at high concentrations contraction prevails. Phenoxybenzamine and phentolamine antagonise the contractions at high concentrations leaving the relaxation component unaltered whilst a relaxation component to adrenaline was uncovered. The effects of the two *alpha* receptor antagonists on the contractile responses to (−)-noradrenaline, (−)-adrenaline and (±)-isoprenaline were examined and concentration curves characteristic of an irreversible or insurmountable antagonism of *alpha* receptors were obtained for phenoxybenzamine; with phentolamine, the maximal response was not depressed thus indicating reversible or surmountable antagonism for *alpha* receptors. In the spleen of this species the capsule contains both *alpha* and *beta* receptors although the functional significance of these observations is not yet known. Technical difficulties in the measurement of blood flow in the mouse spleen has so far prevented an investigation of the adrenaline receptors in the blood vessels.

In the human spleen, phenoxybenzamine was found to abolish the small contraction in response to sympathetic nerve stimulation, adrenaline and noradrenaline (6), and the vascular response to sympathetic nerve stimulation and injected noradrenaline, whilst a slight vasodilator component was uncovered with high doses of adrenaline. The spleen of man, therefore, contains predominantly *alpha* receptors in both the capsule and blood vessels.

Of course, more quantitative analysis has been possible on isolated strips of spleen and the effects of adrenergic antagonists have been elucidated in the cat spleen (21, 163). Tolazoline (21) in concentrations from 2×10^{-5} to 2×10^{-4} M produced concentration dependent surmountable inhibition of adrenaline, noradrenaline, and isoprenaline and as the concentration of tolazoline was increased the concentration-activity curves for the agonists were shifted progressively to the right. Tolazoline 2×10^{-4} M depressed the maximal contracting effect of the catecholamines. Dibenamine, in concentrations of 1.7×10^{-7} to 3.4×10^{-6} M produced concentration dependent insurmountable depression of the maximal contracting effect of adrenaline, noradrenaline, and isoprenaline; some shift of the curves to the right was also observed. The relative affinities of the adrenergic blocking drugs for catecholamine receptors was determined and it was seen that tolazoline antagonised all three catecholamines equally effectively as did dibenamine. Tolazoline, however, possessed a lower receptor affinity than dibenamine. Isolated strips from the spleens of kids and dogs (232) revealed that the contractions to noradrenaline and isoprenaline were inhibited by dibenamine (10^{-7}) in the dog and reduced to 8% and 12% of control respectively in the kid.

An interesting approach has been that of Sheys and Green (216) who found that the dissociation constants (K_B) of phentolamine, azopetine, and yohimbine were significantly greater in splenic strips than in aortic strips from the same rabbit. They pointed out that the difference, although significant for phentolamine, was not as large as the 100 times difference in the pA_2 in the cat spleen (109) compared with the cat aorta (234). However, two other antagonists, ethomoxane and dihydroergotamine were approximately equipotent.

B. Beta blocking Agents

In the cat spleen *in vivo* (202) doses of adrenaline or noradrenaline which increased splenic arterial blood flow, caused a reduction in flow in the same animal after the administration of propranolol. Also doses of adrenaline or noradrenaline which reduced splenic arterial blood flow before blockade now provoked a greater reduction. The increased venous outflow caused by adrenaline and noradrenaline and partly due to splenic capsular contraction, was reduced but not abolished after *beta* blockade. The effects of isoprenaline on either splenic inflow or outflow were abolished by propranolol. All these effects were seen whether the catecholamines were administered by either close arterial or intravenous routes. In a similar study (114) the splenic conductance and weight responses to noradrenaline were unchanged by propranolol whilst the biphasic conductance changes to adrenaline altered to one of pure vasoconstriction. Moreover, the vasodilatation evoked by noradrenaline and adrenaline after phenoxybenzamine was either much reduced or abolished by *beta* receptor block as was the slight weight increase accompanying the administration of adrenaline.

In the dog spleen the vasoconstrictor responses to adrenaline and noradrenaline became very similar in magnitude after propranolol (183) whilst the vasodilator response to sympathetic nerve stimulation seen after phenoxybenzamine was abolished (65, 183) and the vasodilator responses to adrenaline and noradrenaline after phenoxybenzamine (65) converted to a slight vasoconstriction by the administration of propranolol. The vascular response to isoprenaline was abolished (70).

The experiments of Ignarro and Titus on the mouse spleen (145) indicated that the relaxation produced by low concentrations of (±)-isoprenaline were completely antagonised by either MJ1999 or propranolol and that the relaxation caused by adrenaline in the presence of phenoxybenzamine was also blocked by MJ1999.

Dichloroisoproterenol (DCI) generally augmented the contractile effects of adrenaline and noradrenaline in isolated strips of

cat spleen (21). Concentrations of 7×10^{-5} M or greater usually depressed the responses to lower concentrations of adrenaline and noradrenaline whilst augmenting the responses to higher concentrations. The maximal contracting effect of the agonists was not increased; thus DCI usually increased the slope of the concentration activity curves. In a few spleen strips DCI augmented all concentrations of noradrenaline thus producing a parallel shift of the curve to the left. In contrast DCI inhibited the contractile effects of isoprenaline and concentrations of 1.8×10^{-5} to 1.4×10^{-4} M DCI produced progressively increasing surmountable inhibition of isoprenaline contractions. Concentrations greater than 1.4×10^{-4} M usually depressed the maximal contracting effect of isoprenaline. Augmentation of the responses to isoprenaline by DCI was never observed. The affinity of DCI for isoprenaline contractile receptors was of a low order of magnitude.

A species variation has been observed in the effects of *beta* receptor blocking agents on the responses of splenic strips to catecholamines (151). Adrenaline and isoprenaline were administered to produce submaximal contractions which were augmented by pronethalol (10^{-8} to 10^{-6}) in the kid but not in the cat or rabbit. These observations suggest the absence of *beta* receptors in the capsule of these two species. Similarly propranolol (3×10^{-8} M) has no effect on the dose response curve to adrenaline in the rabbit splenic strip from which it was also concluded that the rabbit splenic capsule contains no *beta* receptors (216). However, higher doses of the *beta* receptor antagonist reduced the responses in all three species and 10^{-4} caused complete blockade. The authors (151) suggested that the inhibitory effects of high concentrations of pronethalol on the contraction of the splenic strips to adrenaline, acetylcholine, and isoprenaline was not due to a specific action of the drug on the *beta* receptors. In strips of rabbit spleen both pronethalol (1×10^{-5}) and DCI (1.5×10^{-5}) inhibited the contractions to isoprenaline, adrenaline, and noradrenaline (148). Isolated strips of dog spleen contract to isoprenaline (10^{-5} to 10^{-6}); these responses were augmented by propranolol 10^{-7} whilst higher concentrations (10^{-4}) greatly reduced the contractions to isoprenaline as well as those to adrenaline and barium chloride. The results with the lower concentrations of blocking drug would suggest the presence of some *beta* receptors but it was suggested by the author (232) that at the high dose level isoprenaline acts by stimulation of *alpha* receptors and that *beta* receptors do not exist in the splenic capsule of the dog.

VI. Cholinergic Drugs

The first observation of the action of acetylcholine on the spleen was in 1918 by Hunt (144) who recorded changes in cat spleen volume with a plethysmograph and studied the *in situ* responses to intravenous injections of acetylcholine. His results were variable but most commonly a decrease in volume followed by an increase was observed. This was interpreted as an active vasodilatation, with the initial decrease in volume being due to the fall in systemic blood pressure. This interpretation was supported by the results of another series of experiments in which the isolated spleen was perfused with Ringer's solution and an increase in the venous outflow was observed after injection of acetylcholine. Subsequent work in which changes in volume were recorded in the cat or dog spleen produced varied results, but it was generally considered that acetylcholine, given intravenously, caused a reduction in spleen volume (84, 106). Experiments on the exteriorised dog spleen suggested either a shortening of the spleen in response to acetylcholine (71) or an increase in area (135). This latter work must be treated with caution as almost every drug tested, even those which in other workers' hands have consistently produced capsular contraction, resulted in an increase in area. The possibility that venous occlusion oc-

curred with their technique must be considered.

The effect of intravenous acetylcholine on splenic blood flow of the conscious dog with implanted thermostromuhrs on the splenic artery (116) was a brief, slight increase in splenic arterial blood flow.

In an attempt to avoid the complications of secondary effects on the spleen, caused by the action of acetylcholine on the whole animal, the isolated, intact spleen or a strip preparation has been used extensively. Results with this preparation have shown almost universally that acetylcholine causes contraction (23, 38, 84, 94, 100, 137, 146, 173, 204, 243), although the dose required varied considerably. There has been a report, however, that acetylcholine induced relaxation of the human spleen strip and also of dog and rabbit strips which have been already contracted by adrenaline (95, 204, 243). The splenic contractions produced by acetylcholine were small compared with that of adrenaline (23, 173) and if a strip was contracted maximally to acetylcholine the subsequent addition of adrenaline provoked further contraction (94, 243).

The effects of acetylcholine on the intact spleen can only be studied satisfactorily when it is administered by the close arterial route and systemic effects therefore avoided. This procedure has produced variable results in the isolated spleen perfused with Ringer's solution (135, 144). A detailed analysis on the actions of acetylcholine on the isolated blood perfused dog spleen by Farber (82) showed that a wide range of close arterial doses of acetylcholine produced a contraction which was not influenced by denervation. However, in a further series of experiments he showed that if the *in situ* spleen of one dog with the splenic nerve connections maintained, was perfused by the circulation of a second, then the intravenous injection of acetylcholine into the first dog caused contraction of the spleen despite the fact that no acetylcholine entered the spleen through the circulation. It was concluded that acetylcholine exerts some action on the spleen *via* the extrinsic nerve supply. Even by close arterial injection, varying effects of acetylcholine on the spleen have been reported including contraction (38, 93) and vasodilatation with an increase in volume (110).

The cause of these conflicting results was demonstrated by Daly and Scott (57) with the isolated blood perfused spleen of the dog. Acetylcholine was administered by close arterial injection over the dose range 0.01 to 100 μg and it was found that at the lower doses there was a reduction in vascular resistance together with a small increase in volume attributable to the passive effect of vasodilatation. At the higher dose level, vasodilatation still occurred together with a contraction of the spleen. These results have been confirmed in the dog spleen both for high (33) and low doses (66, 124). However, in the dog spleen perfused with McEwen's solution, close arterial acetylcholine caused contraction only, with no evidence of a vascular action (161) presumably due to a lack of basal vascular tone.

The effects of various drugs have been studied to analyse the nature of the action of acetylcholine on the spleen. Eserine has been shown to potentiate it (93, 137, 204), while another anticholinesterase, tetraethylpyrophosphate (TEPP) given intravenously to the dog caused splenic contraction (215). This latter observation, however, was shown to be a result of increased sympathetic discharge to the spleen and the secretion of catecholamines by the adrenal medulla, inasmuch as the contraction was abolished by splenic denervation and adrenalectomy. There have been many reports that atropine blocks the action of acetylcholine on the spleen (84, 93, 110, 144, 204), but also some that describe the action of acetylcholine persisting after atropine (82, 106). In one study (57), a wide range of doses of acetylcholine was administered by close arterial injection and the conclusion was reached that the splenic vasodilatation and increase in volume produced in response to low doses of acetylcholine was blocked by atropine.

In these atropinised preparations, however, doses of acetylcholine 1.5 to 10 times that required to produce the muscarinic effects, now caused contraction of the spleen. This latter response was abolished by hexamethonium. These results clearly suggest that in addition to its muscarinic action, acetylcholine also had a nicotinic action on the spleen. Support for this idea was provided by experiments in which nicotine and 1:1-dimethyl-4-phenylpiperazinium iodide (DMPP) were shown to cause contractions of the spleen, which were abolished or reduced by an *alpha* adrenoceptor blocking agent (57).

The nature of this nicotinic action of acetylcholine on the spleen requires some explanation and Daly and Scott (57) listed four possible sites of action. They considered an action on sympathetic ganglion cells, excitation of postganglionic nerve fibres or endings, stimulation of sensory endings with the initiation of an axon reflex, and an effect causing the release of stores of adrenaline and noradrenaline beyond the nerve endings. This last possibility was taken by Burn and Rand as evidence in support of their cholinergic link theory of sympathetic action (38, 43, 44). The arguments for and against this theory have been comprehensively reviewed (88), and will not be considered here. An action of acetylcholine on ganglion cells in the splenic nerve was discounted since histological examination revealed very few ganglion cells and also because the nicotinic contraction could not be evoked after section and degeneration of the splenic nerves (57). The possibility that acetylcholine was directly exciting the postganglionic fibres or endings was studied extensively by Ferry (87) who recorded the discharge in the splenic nerve fibres after the close arterial injection of acetylcholine. This discharge was not affected by atropine or an *alpha* adrenoceptor blocking agent, substances which would abolish the direct effects of acetylcholine or any released catecholamines on the smooth muscle and so rule out the excitation of any mechanoreceptors. The discharge could still be elicted after the sensory innervation of the spleen had degenerated, but it was abolished by hexamethonium, a specific action described by workers investigating the excitation of nerve fibres by acetylcholine (76, 107). The conclusion was, therefore, that acetylcholine "excites the sympathetic postganglionic nerves of the spleen somewhere near their endings" and this explains the numerous reports of sympathomimetic effects being produced by substances with nicotinic-like actions.

Some discrepancies remained, however, since when the nicotinic action of acetylcholine was blocked by hexamethonium, large doses of acetylcholine still caused some contraction of the spleen, which was blocked by atropine (57) thereby suggesting a direct muscarinic action on the capsular smooth muscle described by others on strips of spleen (84, 93, 204). Furthermore, if the nicotinic action of acetylcholine is on the postganglionic nerve endings (87), then a close arterial injection of acetylcholine (5 to 100 μg) which caused contraction might be expected to evoke vasoconstriction rather than the vasodilation which has been described as accompanying the contraction (57). This discrepancy might be explained by postulating that the direct muscarinic action causing vasodilatation overrides the indirect nicotinic action evoking vasoconstriction.

Other parasympathomimetic drugs have been shown to behave in a similar manner to acetylcholine in respect of their actions on the spleen. Methacholine (110, 188) produced vasodilatation and a small increase in spleen volume after close arterial injection in a perfused preparation whilst both pilocarpine and arecoline contract splenic strips (100, 204) and excised spleen (35).

Little work has been reported to compare the effectiveness of acetylcholine on the spleens from different species. Information from two studies investigating splenic strip contractions (173, 204) suggest that the order of magnitude of capsular response was

dog > rabbit > pig > rat and guinea pig > ox. Human splenic strips failed to contract and with high concentrations of acetylcholine showed some relaxation.

In contrast to all other reports, acetylcholine applied topically to the mouse spleen has been shown, by direct observation of a transilluminated portion, to cause vasoconstriction (91). This appeared to be a muscarinic action since it was blocked by atropine and not affected by hexamethonium.

In conclusion, it must be said that the action of acetylcholine on the spleen is extremely complex. In the intact animal preparation only the effects elicited by close arterial injection can be considered without some degree of uncertainty, since intravenous injections cause systemic effects which influence the spleen by indirect and reflex pathways. The direct effects of acetylcholine appear to be 3-fold; at all doses, a muscarinic effect on the vascular smooth muscle causing vasodilatation except possibly in the mouse spleen, a nicotinic effect on the postganglionic nerve terminals causing capsular contraction, and at high doses a muscarinic effect on the capsular smooth muscle evoking contraction. The effect of acetylcholine on the spleen, therefore, depends on the dose level since this will determine which effect predominates. The muscarinic vasodilatation has the lowest threshold since this effect can be elicited with only a small, accompanying, passive increase in volume. Contraction of the capsular smooth muscle only becomes apparent at higher dose levels.

VII. Polypeptides

There are three groups of polypeptides which are active on smooth muscle, particularly vascular smooth muscle. These are the neurohypophyseal peptides, angiotensin, and the plasma kinins and related hypotensive polypeptides.

A. Neurohypophyseal Peptides

The actions of these peptides on the spleen were studied originally by injection of the crude extracts of the pituitary gland. Magnus and Schäfer (1901) (175) were probably the first to demonstrate that in the dog in which spleen volume was being recorded by plethysmography, an intravenous injection of the extract of the pituitary (probably posterior lobe only), resulted in a rise in systemic blood pressure and a reduction in spleen volume. Similar observations were made in 1909 in the pithed cat by Dale (55) while testing extracts of posterior lobe. Experiments on conscious dogs in which a spleen plethysmograph had been positioned at a previous operation (120) or in which the spleen had been exteriorised (71) revealed that injections of posterior pituitary extract (Pituitrin) resulted in a reduction in volume or shortening of the spleen.

The nature of the reduction in spleen volume was investigated by perfusing the spleen at constant flow with Ringer's solution (35); addition of posterior pituitary extract to the perfusing fluid resulted in splenic vasoconstriction, but only a small reduction in volume which was thought to be a passive effect due to the vasoconstriction. This view was supported by the results of experiments with excised spleens (35) or splenic strips (204) in organ baths, when the addition of posterior pituitary extract had no effect. Indeed it was shown that strips of rabbit and dog spleen already contracted with adrenaline were relaxed by the subsequent addition of posterior pituitary extract (204); the effect was more marked in the rabbit than dog.

In a study of splenic blood flow in the anaesthetized dog, the intravenous injection of Hypophysin caused a reduction in splenic arterial blood flow and an initial increase in venous flow followed by a sustained reduction (179). In conscious dogs, in which thermostromuhrs had been placed on either the splenic artery or splenic vein (116), the intravenous injection of pituitary extract (Pitressin) caused a marked and long-lasting reduction in the flow of both splenic artery and vein.

Although the pressor activity of pituitary extracts was shown to be due to the posterior lobe as early as 1898 (142, 210) controversy continued well into the 1920's about whether the gland synthesised a single hormone with several different actions, or a number of hormones. This uncertainty was resolved gradually by the separation of progressively purer antidiuretic-pressor and oxytocic preparations (78, 149). It is, however, difficult to evaluate the results of these early experiments with pituitary extracts without precise knowledge of the preparation used. When the formulae of the two active principles of the posterior pituitary were determined, and the synthesis became possible (245, 246), detailed investigations of the actions of vasopressin and oxytocin commenced.

1. Vasopressin. Comparatively little work has been carried out on the action of vasopressin on the spleen and the results that have been reported are somewhat conflicting. It was shown that with strips of cat spleen. Pitressin caused contraction (101), but in the isolated cat spleen perfused with Krebs-Ringer solution, vasopressin had virtually no effect on the volume of the spleen, as recorded by plethysmography, despite a profound vasoconstriction in the organ (132). An extensive investigation of the responses of the cat spleen to vasopressin has been carried out in which splenic arterial blood flow was recorded by an electromagnetic flowmeter and changes in spleen volume registered by changes in spleen weight. Again, vasopressin induced a profound vasoconstriction but there were negligible or no changes in spleen volume (51, 113, 114).

The responses of the dog spleen are rather different. In the isolated preparation perfused with blood at constant pressure, close arterial injection of vasopressin caused intense vasoconstriction and a marked decrease in spleen volume, both responses being dose dependent (69). Although the intense vasoconstriction must contribute to the reduction in volume, its magnitude and time-course relative to the vasoconstrictor response suggest that, in the dog spleen, vasopressin causes contraction of the smooth muscle of both the capsule and blood vessels.

The only other species in which the action of vasopressin on the spleen has been established is man where it is known that, because of the sparse distribution of smooth muscle, the capsule is generally unresponsive to drugs. Excised normal human spleens, perfused at constant flow, show negligible changes in volume but intense vasoconstriction when vasopressin is administered (6).

2. Oxytocin. Very little work has been carried out on the action of oxytocin on splenic smooth muscle. Experiments with strips of cat spleen demonstrated that, unlike vasopressin, addition of oxytocin (Orasthin) had no effect (101).

In the dog spleen, oxytocin was found to be similar to vasopressin in that it caused both vasoconstriction and a reduction in volume (69). However, both responses were smaller than those evoked by the same dose of vasopressin.

In contrast to these observations oxytocin appeared to cause a slight vasodilatation in the perfused human spleen (5), but this response was subsequently shown to be due to the preservative chlorobutanol (6). Since no reduction in volume was detected in these experiments oxytocin, even in very high doses, is apparently without effect on either the capsular or vascular smooth muscle of the human spleen.

In conclusion, the actions of the neurohypophyseal peptides on the spleen must be considered with regard to their possible physiological significance. Experiments in the cat and human spleen indicate that oxytocin has little or no action, while the doses required to produce comparatively small responses in the dog spleen suggest that the action is of no importance. However, direct evidence suggests that the release of vasopressin by the posterior pituitary contributes to the vascular response to haemorrhage in

the cat, and that the spleen has a role in this response, but only as a site of vasoconstriction (224). This is supported by the finding that as a result of haemorrhage, the blood concentration of vasopressin may rise as high as 1 mU/ml in the cat (16). Blood concentrations of this magnitude, achieved by intravenous infusions, have been shown to cause splenic vasoconstriction with little concomitant reduction in spleen volume (51, 113, 114). In the dog, close arterial injections of 10 mU of vasopressin with a splenic arterial blood flow of 40 ml/min would produce an arterial concentration of the order of 1 mU/ml, which is the concentration occurring in the intact animal as a result of haemorrhage (27, 201, 213). At this dose level, vasopressin causes both vasoconstriction and reduction in volume (69), suggesting that in haemorrhage, in the dog, the spleen may contribute to the maintenance of peripheral resistance by vasoconstriction and to the increase in circulating blood volume by a reduction in splenic capacity. However, concomitant increase in sympathetic activity and increases in blood levels of the adrenal catecholamines and angiotensin would affect splenic smooth muscle and make the role of vasopressin difficult to assess.

B. Hypertensive peptides

Angiotensin. Active angiotensin is formed by a series of events initiated by the release of the enzyme renin from the kidney. The inactive decapeptide angiotensin I is subsequently split from the plasma substrate angiotensinogen and rapidly converted to the highly active octapeptide angiotensin II. Although the converting enzyme is present in the plasma it has been shown that the rate of conversion is too slow to account for the *in vivo* conversion (185), and the lung has been suggested as the major site of conversion in the body (9, 244).

The pressor action of angiotensin II is well established, but the exact nature of its action is uncertain and in the intact animal may consist of three components, a central action, the release of catecholamines from stores in the adrenal medullae and sympathetic nerve endings, and a direct action on the smooth muscle cells. To study the actions on the spleen, effects elicited through central stimulation and the release of adrenal catecholamines must be avoided. This usually has been achieved with the isolated, perfused spleen when angiotensin II has been administered by close arterial injection.

In all reports, angiotensin II causes constriction of the splenic vascular bed but not always is this response accompanied by contraction of the splenic capsule. In the dog spleen, perfused with blood at constant flow, there was no evidence of capsular contraction (33). A similar result was obtained with the cat spleen perfused with a modified Krebs' solution (237). However, in contrast, with basically the same technique of perfusion of the cat spleen, the vasoconstriction was shown to be accompanied by longitudinal contractions of the spleen (17), or by a reduction in volume when recorded by a plethysmograph (132). In the isolated dog spleen, perfused with blood at constant pressure, close arterial injections of angiotensin II produced very marked contractions of the splenic capsule as well as vasoconstriction (59, 65, 69). The variability of these results is probably due to the very different perfusion techniques but the evidence, on the whole, favours the view that there is some active capsular contraction of the dog and cat spleen in response to injection of angiotensin II.

Experiments on the spleen *in vivo* also support the view that there is both splenic contraction and vasoconstriction in response to angiotensin. In the cat, in which splenic arterial blood flow was recorded with an electromagnetic flowmeter and changes in volume were recorded as changes in weight, it was found that with intravenous infusions of angiotensin II at low rates (0.06–0.25 μg/min), there was vasoconstriction but little or no change in volume. At high infusion rates (0.5–1.0 μg/min), however, the degree of

vasoconstriction increased and a marked reduction in spleen volume occurred (113, 114). These responses were not modified by adrenalectomy (114) so that an increase in circulating catecholamines released from the adrenal medullae by angiotensin did not contribute to the response.

There is no doubt that the main splenic response to angiotensin II is vasoconstriction since in both the dog (59) and cat (113, 114) low doses induce vasoconstriction without any concomitant change in volume. In contrast the predominant splenic response to either catecholamines or splenic nerve stimulation is contraction of the capsular smooth muscle. Direct comparison of the splenic smooth muscle responses to angiotensin and nerve stimulation has been made in the cat and for the same vasoconstriction, the volume reduction to nerve stimulation is much greater than that to angiotensin (114). In the dog a direct comparison of the splenic vascular and capsular smooth muscle responses to angiotensin and catecholamines (69) indicates that doses of these drugs which elicit the same reduction in spleen volume produce different increases in splenic vascular resistance and that angiotensin is considerably the most potent.

A different question is whether the action of angiotensin II on the smooth muscle of the perfused spleen is a direct one on the muscle cells or indirect through the release of noradrenaline stored in the sympathetic nerve endings. The latter view was favoured by experiments (17) in which the splenic responses to angiotensin displayed tachyphylaxis and in which angiotensin potentiated the splenic responses to sympathetic nerve stimulation. However, the splenic responses to angiotensin are unaltered by either chronic denervation or reserpine pretreatment (132). In addition, phenoxybenzamine, an *alpha* receptor blocking agent, does not reduce or abolish the splenic responses to angiotensin (6, 65); on the contrary, it appears to potentiate them (69). In general, the evidence suggests that the major part of the splenic response to angiotensin II is a direct action on the capsular and vascular smooth muscle.

The actions of angiotensin II have been investigated in the isolated perfused human spleen and close arterial injections caused intense vasoconstriction but negligible changes in spleen volume (5, 6).

The responses of the spleen to angiotensin would seem to be of importance in the cardiovascular response of the cat to haemorrhage (51, 224). The response would appear to be limited to constriction of the splenic vascular bed with little or no capsular contraction. In the dog there is no direct evidence of a similar nature but it has been shown that after haemorrhage, the rate of formation of angiotensin is increased by 0.25 to 1.5 μg/min resulting in an increase in the blood angiotensin concentration of 0.1 to 0.33 ng/ml (134, 196). Possibly in prolonged haemorrhage the blood concentration might reach 1.0 ng/ml the threshold required for splenic vasoconstriction (62), but it is unlikely that the threshold concentration of 50 ng/ml for capsular contraction would be achieved (59).

In conclusion, it appears likely that in the dog and the cat, sufficient quantities of angiotensin may be released as a result of haemorrhage to contribute to splenic vasoconstriction, but not to contraction of the capsule.

C. Hypotensive Peptides

Kinins. The plasma kinins are a group of hypotensive polypeptides, the most important being the nonapeptide bradykinin and the decapeptide kallidin. The related peptides eledoisin and physalaemin are not of mammalian origin but their pharmacological properties are similar to those of the plasma kinins.

Bradykinin, like angiotensin, releases catecholamines from the adrenal medulla of both the cat (83) and the dog (200). The possibility that these polypeptides have some action on the stores of noradrenaline in the sympathetic nerve endings must be considered when studying the actions of the plasma kinins on the smooth muscle of the spleen.

An early observation demonstrated that the introduction of bradykinin by close arterial injection to the spleen of either the dog or cat resulted in hypertension, hyperpnoea, and vocalisation. These responses are almost identical to those obtained when bradykinin was given into the femoral artery and which were shown to be reflex by denervation experiments. Consequently, the responses to intrasplenic injections of bradykinin were thought to be reflex responses to visceral pain (119).

These observations led to a study of the actions of bradykinin on the spleen of the cat (74); two preparations were used. An *in vitro* preparation, in which the venous outflow and longitudinal contractions of the spleen were recorded, showed that close arterial injections of bradykinin (2 to 50 μg) had no effect. However, in the *in vivo*, innervated, spleen preparation, perfused at constant pressure with Tyrode's solution, close-arterial injections of bradykinin (1 to 5 μg) caused vasoconstriction and contraction of the splenic capsule. These two responses were abolished or reduced by transection of the spinal cord, or administration of either ganglion blocking agents or phenoxybenzamine; it was concluded, therefore, that bradykinin had no direct action on the spleen of the cat but elicited reflex adrenergic responses.

In the isolated spleen of the dog perfused with Krebs-Ringer solution at constant flow (182), bradykinin (5 to 1000 ng) injected into the splenic artery had small and variable effects, which were shown to be due to the solvent. When some vascular tone was induced in the preparation by sympathetic nerve stimulation, close arterial injections of bradykinin now consistently caused splenic vasodilatation unaccompanied by any change in the weight of the spleen and indicating no change in the tone of the capsular smooth muscle. Furthermore, since the vasodilatation was not blocked by phenoxybenzamine the release of catecholamines from the nerve terminals could play no part in the response to bradykinin. In the same investigation kallidin, physalaemin, and eledoisin were also shown to cause splenic vasodilatation and the order of potency was physalaemin > kallidin > eledoisin > bradykinin. In the blood perfused spleen of the dog (70) close arterial injections of bradykinin (0.5–5 μg) consistently caused vasodilatation, a response which showed little dependence on dose and which was not accompanied by any appreciable change in volume. The splenic vasodilatation was not affected by either *alpha* or *beta* adrenoceptor blocking drugs and it was suggested that the effect was a direct action or the vascular smooth muscle.

In conclusion, it appears that the release of catecholamines from stores in the postganglionic sympathetic nerve endings plays no part in the response of splenic smooth muscle to bradykinin. The direct action of bradykinin on the dog spleen causes dilatation of the vascular bed with no effect on the smooth muscle of the capsule. This effect has not been demonstrated in the cat. However, in the intact animal, this direct vasodilatation would probably be overridden by adrenergic vasoconstrictor responses elicited by reflexes activated by the action of bradykinin on sensory receptors within the spleen.

VIII. Vasoactive Amines

A. Histamine

The action of histamine on smooth muscle is very complex since there is considerable variation both between species and also between different types of smooth muscle within the same animal. In addition, it has been shown that histamine has a dual action in that it releases catecholamines from stores in sympathetic nerve endings as well as having a direct action on the smooth muscle (81, 172).

Dale and Laidlaw in 1910 (56) recorded blood pressure and spleen volume by plethysmograph in the decerebrate cat and found that intravenous histamine (β-iminazolylethylamine) resulted in a fall in blood pressure and a reduction in spleen volume. They thought that the reduction in volume was independent of the fall in blood pressure,

since the volume recovered before there was any restoration of normal blood pressure. This early suggestion that histamine contracted the splenic capsule has been confirmed in many species and different preparations. In a similar preparation to that used by Dale and Laidlaw it was shown that the splenic volume reduction to histamine also occurred in the excised spleen perfused with blood at constant pressure (13). In both the dog and cat, with the spleen displayed on a graticule, intravenous histamine caused a reduction in area and a reduction in calculated volume (226). Histamine has been shown to cause contraction of either splenic strips or complete spleens of the cat (72, 146, 204), dog (84, 204), rabbit (173, 204), guinea pig, rat, pig, and ox (173) when these preparations are suspended in suitable solutions in an organ bath. However, the view that histamine causes contraction of the splenic capsule by a direct action has not been unanimous. The reduction in volume of the *in situ* dog spleen to intravenous histamine was considered to be an effect secondary to the fall in blood pressure (84) and with a preparation of the cat spleen, histamine was described as having a dilator action which presumably referred to an action on the capsule although very little detail of the observation is given (97). Histamine has been described as having no action on the rabbit spleen (100).

There have been few reports of the effect of histamine on the vascular smooth muscle of the spleen. In the conscious dog with thermostromuhrs placed on either the splenic artery or the splenic vein, intravenous histamine caused a reduction in arterial blood flow and a transient increase followed by a prolonged decrease in venous flow (116). These results suggest that histamine causes both vasoconstriction and capsular contraction of the spleen, although these effects may not have been direct actions of histamine on the splenic smooth muscle. Visual observation of the transilluminated mouse spleen revealed both vasoconstriction and contraction in response to the topical application of histamine (91); little detail is given about the capsular effect.

Thus the generally accepted view of the direct action of histamine on the spleen is that it causes vasoconstriction and contraction of the capsule although the possibility of the effects arising from the release of catecholamines stored in the nerve endings has not been eliminated. Recently, the action of histamine on both the capsular and vascular smooth muscle has been studied in the isolated, blood perfused dog spleen (70) by close arterial injection. Doses (1–100 μg) consistently caused a reduction in volume and usually vasodilatation, although with the higher doses, there was occasionally a transient vasoconstriction followed by vasodilatation.

In the human spleen, area has been calculated from the outline obtained by x-ray in both the normal subject (189) and in patients with splenomegaly (181). Intravenously administered histamine caused a small increase in area in the normal spleen and a decrease in the enlarged spleen. In the patients with splenomegaly, a fall in blood pressure was observed, so that the action of histamine on the spleen may have been brought about reflexly.

Finally the possibility of histamine acting directly on the spleen and causing contraction as a part of the body response in anaphylaxis should be considered. There have been a few conflicting references on this subject and in none of the work have reflex effects mediated either through the sympathetic innervation or by adrenal medullary secretions been eliminated. The spleen has been shown to be contracted in the guinea pig in histamine shock (165) and also in cats killed by histamine (12) although in this latter case it was noted that the degree of contraction was less than in cats killed by other methods. However, the problem was specifically investigated in cats sensitized with human serum and it was concluded that the spleen was passive during anaphylaxis (157).

B. 5-Hydroxytryptamine

5-Hydroxytryptamine (5HT) (5–20 μg) administered by close arterial injection into the *in situ* dog spleen, innervated but cross perfused (198), caused a very small reduction in spleen volume of 3.5 to 7.5 ml lasting for 60 to 90 sec. In the totally isolated dog spleen perfused with Tyrode, 5HT (1–20 μg) given by close-arterial injection caused a 6-ml contraction for 1 to 3 min. This contraction of the splenic capsule provoked an initial increase in splenic venous flow which was succeeded by a vasoconstriction which remained unaltered whilst the spleen volume remained the same or returned to normal (199). In the *in situ* dog spleen perfused with blood, in which the splenic arterial blood flow was measured with non-cannulating flow probes, 5HT caused vasoconstriction which was blocked by methysergide (124). A slight reduction in volume and slight vasoconstriction in the isolated, blood perfused, dog spleen was evoked by close arterial 5HT (5 μg); increasing the dose administered to 25 μg caused a profound vasoconstriction which completely arrested the blood flow through the spleen (70).

Isolated strips of cat spleen responded to 5HT by a contraction which was blocked by phenoxybenzamine, bromolysergic acid diethylamide, and dihydroergotamine (147, 148). Considerable protection to the phenoxybenzamine blockade was afforded by adrenaline thereby suggesting that both adrenaline and 5HT react with the same receptor site on splenic smooth muscle. Moreover, the contraction to 5HT was not persistent; it declined despite the continued presence of 5HT in the bath fluid. Adrenaline was also ineffective if added at this stage. 5HT had little effect on the spleen strips from reserpine treated cats (1.0 mg/kg, 24 hr before) but the responses were greatly increased after high doses of noradrenaline. These observations suggested to the author that 5HT acted, in this tissue, by the release of noradrenaline.

IX. Prostaglandins

The prostaglandins are a group of acidic lipids widely distributed in animal tissues; their general pharmacology and modes of action have been reviewed recently (140). Two prostaglandins E_2 and $F_{2\alpha}$, have been detected in the splenic venous effluent after splenic nerve stimulation in the isolated, blood-perfused spleen of the dog (63, 64), and also after splenic nerve stimulation and injections of adrenaline, noradrenaline, and histamine in the dog spleen perfused with Krebs-dextran solution (86, 103).

The actions of prostaglandins E_1 and E_2 have been studied by close-arterial infusions into the spleen to examine their effects on the two smooth muscle components. E_2 caused a transient reduction in splenic vascular resistance, which did not persist throughout the infusion period (66). E_1, however, was a very potent vasodilator of the splenic vascular bed; the response was prolonged and lasted throughout the infusion period. A slight increase in volume accompanied the vasodilatation produced by each prostaglandin. These vascular responses were in no way altered by the prior administration of either *alpha* or *beta* adrenoceptor blocking drugs to the spleen. In these experiments on the blood-perfused dog spleen, the splenic smooth muscle responses of vasoconstriction and capsular contraction produced by sympathetic nerve stimulation or injections of either adrenaline, noradrenaline, or angiotensin were not altered by the concomitant infusion of prostaglandin E_1 in a dose (10^{-8}M), which itself caused a marked reduction in splenic vascular resistance. Similarly, the infusion of E_2, one of the prostaglandins released from the spleen, did not alter the splenic smooth muscle responses to either adrenaline or noradrenaline administered by close arterial injection. The interaction of higher concentrations of E_1 (127) and E_2 (126) with adrenergic stimuli has been examined in the cat spleen, perfused with a modified Krebs-Henseleit solution and prostaglandin E_1 in concentrations up to 10^{-6} M produced a marked antagonism of the

vascular responses to high rates of sympathetic nerve stimulation (10 Hz) and noradrenaline). The antagonism was of the same time-course and extent to each stimulus. In this preparation low concentrations of prostaglandin E_2 were found to reduce the pressor, as well as the splenic contractile responses to sympathetic nerve stimulation (10 Hz). Higher concentrations of E_2 further depressed the contractile response while the vascular response tended to increase again, although it usually remained below that obtained during the preceeding control stimulation. The effect of prostaglandin E_2 on the splenic capsular response to noradrenaline was weak and inconsistent but the effect on the vascular response to noradrenaline was highly dose dependent. In these experiments, therefore, low doses of prostaglandin E_2 reduced the pressor response while high doses produced a distinct potentiation. The discrepancy between these observations and those in the dog may reflect a real species variation, but the possibility cannot be ruled out that it may be related to the different experimental techniques and different concentrations of prostaglandins at the effector sites. The doses of E_1 which produced a profound reduction in splenic vascular resistance in the dog (66) were apparently without effect on the blood vessels of the cat spleen, perfused with Krebs-Henseleit solution (127). This suggests that the progressive loss of basal tone observed in the cat spleen preparation (126) prevented the appearance of any vasodilator properties of the molecule being manifest. It raises the question of the validity of responses in preparations where an artificial perfusion medium is used. However, a contributory factor to these differences may be the binding of the prostaglandins in the blood stream so that administration by this route does not necessarily reflect the potential activity of the prostaglandins when they are released within the organ of origin. On the basis of his observation Hedqvist (126) has proposed that the prostaglandins may play a modulatory role on the sympathetic innervation within the spleen by antagonising the effects of nerve stimulation and circulating catecholamines. Indeed, the observation that indomethacin, which inhibits the synthesis of prostaglandin, potentiates the splenic smooth muscle responses to injected adrenaline (85) adds strong support to this view.

The effects of prostaglandin $F_{2\alpha}$ on the smooth muscle responses of the dog spleen have been elucidated (68) and whilst low blood concentrations (less than 10 μg/ml blood) caused a marked reduction in splenic vascular resistance, which was reversed by phenoxybenzamine, higher arterial concentrations caused splenic vasoconstriction. On the other hand, $F_{2\alpha}$ had little action at any arterial concentration on the splenic capsular smooth muscle. Furthermore, the splenic smooth muscle responses to sympathetic nerve stimulation (1 and 3 Hz), adrenaline and noradrenaline were not altered significantly by the concomitant infusion of a dose of $F_{2\alpha}$ which itself produced a slight vasodilatation.

Preliminary observations (67) have revealed the splenic smooth muscle responses to prostaglandins $F_{1\alpha}$, A_1 and A_2 to be, predominantly, mild vasodilatation with concomitant slight increases in spleen volume in the dog. A slight interaction of A_1 and A_2 with nerve stimulation and injections of adrenaline was observed. However, A_1 increased the response while A_2 caused a reduction. A_1 was not affected by prior administration of phenoxybenzamine while A_2, like $F_{2\alpha}$, was reversed.

X. Conclusions

The question should be considered as to what extent the changing levels of circulating substances are involved in the splenic responses under physiological conditions. Involvement of the spleen in the cardiovascular responses has been demonstrated in the intact cat and dog to a number of physiological stimuli such as haemorrhage (12, 15, 53, 113, 193, 262), asphyxia (47, 120, 209), hypoxia (4, 158), anaesthesia (71, 120, 125), exercise (11, 15), emotion (10, 120), carotid

sinus baroreceptor stimulation (77, 133, 137), and nasal mucosa stimulation (3). In the majority of the cases only the splenic capsular contraction has been investigated and this was abolished by denervation of the spleen (15, 53, 77, 137, 193, 209, 262). This observation has often led to the conclusion that only the direct innervation of the spleen is involved in the response. There are a number of contradictory reports which suggest the participation of blood borne influences, particularly catecholamines from the adrenal medullae (10, 47, 133).

In the dog, splenic responses have been demonstrated as a result of stimulation of the nasal mucous membrane (3). A small increase in splenic vascular resistance and a small decrease in spleen volume were observed and it was suggested that both effects were entirely due to the direct sympathetic innervation. Indirect evidence, already discussed, suggests that as a result of physiological stimuli, the arterial blood concentrations of catecholamines, angiotensin, and vasopressin could all reach levels which may have effects on the spleen. It is probable that, at these low blood concentrations, angiotensin and vasopressin would cause vasoconstriction only, with little or no effect on the capsular smooth muscle.

The responses of the human spleen have not been investigated extensively. Contraction of the human spleen to stimuli such as emotion, exercise, and changes in body temperature (18, 20, 189) have been reported. The methods of detecting the contractions were indirect and subject to error. The general conclusion from direct observation (6) is that the normal human spleen is not capable of active contraction. In contrast there was direct evidence of marked contraction of the smooth muscle of the splenic vascular bed. Further investigation is required to ascertain whether any of the vasoactive compounds, catecholamines, angiotensin or vasopressin, are released in sufficient quantities to cause changes in splenic vascular resistance.

The most complete analysis of the role of the spleen in the response to a physiological stimulus has been that of Greenway and Stark (113, 114, 224); both capsular and vascular responses of the cat spleen to haemorrhage were studied. The capsular contraction was reduced by splenic denervation and abolished by combined denervation and adrenalectomy. The splenic vasoconstriction, however, was still evident after these combined procedures and persisted after nephrectomy. Only after the additional procedure of hypophysectomy was the splenic vasoconstriction in response to haemorrhage abolished. It was concluded, therefore, that, in contrast to the contraction of the splenic capsule which was caused by the direct sympathetic innervation with a small adrenal medullary component, the splenic vasoconstriction was caused by the overlapping mechanisms of direct sympathetic innervation, increased secretion of catecholamines from the adrenal medullae, and raised circulating blood levels of both angiotensin and vasopressin.

A number of points emerge in this review which need to be considered in the interpretation of the actions of drugs on splenic smooth muscle. Different experimental techniques impose limitations on the nature and validity of the information derived from them. The action of some drugs (*e.g.*, isoprenaline, histamine) on isolated strips of spleen is qualitatively different from the response obtained from the intact spleen by close arterial injection. Information derived from one type of experimental preparation should be related to data derived from other preparations with caution. The large variations in structure and function of the spleen in different species is now well documented. In the dog and cat there is ample smooth muscle in the capsule and splenic contraction is capable of significantly increasing the systemic haematocrit and blood volume. In normal man splenic contraction is very small and the expelled blood volume insignificant. The actions of drugs on the splenic capsule reflects these differences in the density of the effector cells. The splenic vascular responses

to drugs have been established in only a few species and in general are qualitatively similar although some species variation does occur, for example, to oxytocin. It has become apparent that there is a difference in the sensitivity of the capsular and vascular smooth muscle to most drugs. Catecholamines are very potent on the splenic capsule and slightly less active on the vascular bed whilst the polypeptide hormones are very active on the splenic vascular bed but have little action on the capsule even in those species where a reservoir role of the spleen is well established. Therefore, two systems of smooth muscle are present in the spleen and may show qualitative and quantitative differences in their responses to the same drug.

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REFERENCES

1. Ahlquist, R. P.: A study of the adrenotropic receptors. Amer. J. Physiol. **153**: 586-600, 1948.
2. Ahlquist, R. P., Taylor, J. P., Sydow, V. and Rawson, C. W.: Comparative aspects of epinephrine and levarterenol in the intact, anaesthetized dog. J. Pharmacol. Exp. Ther. **110**: 352-360, 1954.
3. Angell James, J. E. and Daly, M. deB.: Reflex respiratory and cardiovascular effects of stimulation of receptors in the nose of the dog. J. Physiol. (London) **220**: 673-696, 1972.
4. Antal, J. and Schleinzer, R.: Die Speicherfunktion der Milz in ihrer Abhängigkeit von der Beatmung des Blutes. Pflügers Arch. Gesamte Physiol. Menchen Tiere **245**: 680-696, 1942.
5. Ayers, A. B., Davies, B. N. and Withrington, P. G.: The responses of the isolated, perfused spleen of man to sympathetic nerve stimulation, catecholamines and polypeptides. Brit. J. Pharmacol. **40**: 575P, 1970.
6. Ayers, A. B., Davies, B. N. and Withrington, P. G.: Responses of the isolated, perfused human spleen to sympathetic nerve stimulation, catecholamines and polypeptides. Brit. J. Pharmacol. **44**: 17-30, 1972.
7. Bacq, Z. M. and Fredericq, H.: Sur la nature adrénergique de l'inervation motrice de la rate. Action de la cocaine et du 933F. C.R. Séances Soc. Biol. **118**: 1611-1612, 1935.
8. Bacq, Z. M. and Fredericq, H.: Sur la nature de l'innervation de la rate. Action de divers agents pharmacodynamiques. Arch. Int. Physiol. **41**: 322-333, 1935.
9. Bakhle, Y. S., Reynard, A. M. and Vane, J. R.: Metabolism of angiotensins in isolated perfused tissues. Nature (London) **222**: 956-959, 1969.
10. Barcroft, J.: Some effects of emotion on the volume of the spleen. J. Physiol. (London) **68**: 375-382, 1929.
11. Barcroft, J. and Florey, H.: The effect of exercise on the vascular conditions in the spleen and colon. J. Physiol. (London) **68**: 181-189, 1929.
12. Barcroft, J., Harris, H. A., Orahovats, D. and Weiss, R.: A contribution to the physiology of the spleen. J. Physiol. (London) **60**: 443-456, 1925.
13. Barcroft, J. and Nisimaru, Y.: Cause of rhythmical contraction of the spleen. J. Physiol. (London) **74**: 299-310, 1932.
14. Barcroft, J., Nisimaru, Y. and Puri, S. R.: The action of the splanchnic nerves on the spleen. J. Physiol. (London) **74**: 321-326, 1932.
15. Barcroft, J. and Stephens, J. G.: Observations upon the size of the spleen. J. Physiol. (London) **64**: 1-22, 1927.
16. Beleslin, D., Bisset, G. W., Haldah, J. and Polak, R. L.: The release of vasopressin without oxytocin in response to haemorrhage. Proc. Roy. Soc. Ser. B Biol. Sci. **166**: 443-458, 1967.
17. Benelli, G., Della Bella, D. and Gandini, A.: Angiotensin and peripheral sympathetic nerve activity. Brit. J. Pharmacol. Chemother. **22**: 211-219, 1964.
18. Benhamou, Jude and Marchioni: La Splénocontraction à l'effort chez l'homme normal. C. R. Séances Soc. Biol. **100**: 456-458, 1929.
19. Benhamou, Jude and Marchioni: La splénocontraction à l'adrenaline chez l'homme normal. C. R. Séances Soc. Biol. **100**: 458-460, 1929.
20. Benhamou, Marchioni and Nauchy: La splénocontraction à l'émotion chez l'homme normal. C. R. Séances Soc. Biol. **100**: 461-463, 1929.
21. Bickerton, R. K.: The response of isolated strips of cat spleen to sympathomimetic drugs and their antagonists. J. Pharmacol. Exp. Ther. **142**: 99-110, 1963.
22. Bickerton, R. K., O'Bleness, G. V. and Rockhold, W. T.: Threshold and the effects of catecholamines on isolated strips of cat spleen. Arch. Int. Pharmacodyn. Thér. **161**: 431-441, 1966.
23. Bickerton, R. K., Rockhold, W. T. and Micalizzi, E. R.: Use of isolated strips of cat spleen for the assay of α-adrenergic blocking compounds. J. Pharm. Sci. **51**: 837-840, 1962.
24. Binet, L.: Action de l'adrénaline sur la rate. La Méd. **8**: 919-924, 1927.
25. Binet, L., Arnaudet, A., Fournier, B. and Kaplan, M.: Mobilisation par l'éphédrine des éléments figures du sang en réserve dans la rate. C. R. Séances Soc. Biol. **98**: 1282-1284, 1928.
26. Binet, L., Cardot, H. and Fournier, B.: Splénocontraction et polyglobulie par l'adrénaline et l'extrait de Genêt. C. R. Séances Soc. Biol. **96**: 521-524, 1927.
27. Biro, G. P., Forsling, M. L. and Wilmott, R. W.: Personal communication, 1972.
28. Bjorkman, S.: The splenic circulation. Acta Med. Scand. **128**: suppl. **191**: 1-89, 1947.
29. Blakeley, A. G. H.: The responses of the spleen to nerve stimulation in relation to the frequency of splenic nerve discharge. Proc. Roy. Soc. Ser. B Biol. Sci. **171**: 201-211, 1968.
30. Blakeley, A. G. H., Brown, G. L., Dearneley, D. P. and Geffen, L. B.: Simultaneous perfusion of two separate circulations in the spleen. J. Physiol. (London) **181**: 22P, 1965.
31. Blakeley, A. G. H., Brown, G. L., Dearnaley, D. P. and Woods, R. I.: Perfusion of the spleen with blood containing prostaglandin E_1 : transmitter liberation and uptake. Proc. Roy. Soc. Ser. B Biol. Sci. **174**: 281-292, 1969.
32. Blendis, L. M., Roberts, V. C., Spiro, M. and Williams, R.: The comparative measurement of splenic blood flow using 133 Xenon and an electromagnetic flowmeter. Cardiovasc. Res. **4**: 44-49, 1970.
33. Boatman, D. L. and Brody, M. J.: Analysis of vascular responses in the spleen. Amer. J. Physiol. **207**: 155-161, 1964.
34. Boer, S. de and Carroll, D. C.: The mechanism of the splenic reaction to general CO poisoning. J. Physiol. (London) **59**: 312-332, 1924.
35. Boer, S. de and Carroll, D. C.: The significance of the

action of pituitrin on the splenic volume. J. Physiol. (London) 59: 381–386, 1924.

36. Braithwaite, J. L. and Adams, D. J.: Vascular components in the rat spleen. Nature (London) 178: 1178–1179, 1956.
37. Braithwaite, J. L. and Adams, D. J.: The venous drainage of the rat spleen. J. Anat. 91: 352–357, 1957.
38. Brandon, K. W. and Rand, M. J.: Acetylcholine and the sympathetic innervation of the spleen. J. Physiol. (London) 157: 18–32, 1961.
39. Brown, W. H., Pearce, L. and van Allen, M.: Organ weights of normal rabbits. J. Exp. Med. 43: 733–741, 1926.
40. Bulgak, J.: Ueber die Contractionen und die Innervation der Milz. Virchows Arch. Pathol. Anat. Physiol. Klin. Med. 69: 181–213, 1877.
41. Burn, J. H. and Hutcheon, D. E.: The action of noradrenaline. Brit. J. Pharmacol. Chemother. 4: 373–380, 1949.
42. Burn, J. H. and Rand, M. J.: The action of sympathomimetic amines in animals treated with reserpine. J. Physiol. (London) 144: 314–336, 1958.
43. Burn, J. H. and Rand, M. J.: Sympathetic postganglionic mechanism. Nature (London) 184: 163–165, 1959.
44. Burn, J. H. and Rand, M. J.: Sympathetic postganglionic cholinergic fibres. Brit. J. Pharmacol. Chemother. 15: 56–66, 1960.
45. Burton-Opitz, R.: Some data regarding the portal circulation. Proc. Soc. Exp. Biol. Med. 5: 45–46, 1907.
46. Burton-Opitz, R.: Über die Strömung des Blutes in dem Gebiete der Pfortader. II. Das Stromvolum der Vena lienalis. Pflügers Arch. Gesamte Physiol. Menschen Tiere 129: 189–216, 1909.
47. Celander, O.: The range of control exercised by the sympathicoadrenal system. Acta Physiol. Scand., suppl. 116: 1954.
48. Chatterjee, B. N. and Cruickshank, E. W. H.: Comparative histological study of spleens of various vertebrates with reference to bone marrow and blood. Indian J. Med. Res. 16: 870–886, 1929.
49. Clegg, E. J.: Weight changes in different organs of the mouse at two levels of reduced atmospheric pressure. J. Appl. Physiol. 30: 764–767, 1971.
50. Cleland, J. G. P. and Tait, J.: Nervous connections of the mammalian spleen, including an account of certain visceromotor and other abdominal reflexes. Quart. J. Exp. Physiol. Cog. Med. 17: 179–204, 1927.
51. Cohen, M. M., Sitar, D. S., McNeil, J. R. and Greenway, C. V.: Vasopressin and angiotensin on resistance vessels of spleen, intestine and liver. Amer. J. Physiol. 218: 1704–1706, 1970.
52. Cook, S. F. and Olmstead, J. M. D.: The effect of asphyxia on the spleen of curarised and uncurarised decapitate cats. Amer. J. Physiol. 92: 249–252, 1930.
53. Dahlström, A. B. and Zetterström, B. E. M.: Noradrenaline stores in nerve terminals of the spleen; changes during haemorrhagic shock. Science 147: 1583–1585, 1965.
54. Dale, H. H.: On some physiological actions of ergot. J. Physiol. (London) 34: 163–206, 1906.
55. Dale, H. H.: The action of extracts of the pituitary body. Biochem. J. 4: 427–447, 1909.
56. Dale, H. H. and Laidlaw, P. P.: The physiological action of β-iminazolylethylamine. J. Physiol. (London) 41: 318–344, 1910.
57. Daly, M. de B. and Scott, M. J.: The effects of acetylcholine on the volume and vascular resistance of the dog's spleen. J. Physiol. (London) 156: 246–259, 1961.
58. Davidson, W. J. and Innes, I. R.: Dissociation of potentiation of isoprenaline by cocaine from inhibition of uptake in cat spleen. Brit. J. Pharmacol. 39: 175–181, 1970.
59. Davies, B. N., Gamble, J. and Withrington, P. G.: Effects of noradrenaline, adrenaline and angiotensin on vascular and capsular smooth muscle of the spleen of the dog. Brit. J. Pharmacol. Chemother. 32: 424P, 1968.
60. Davies, B. N., Gamble, J. and Withrington, P. G.: The separation of the vascular and capsular smooth muscle responses to sympathetic nerve stimulation in the dog's spleen. J. Physiol. (London) 196: 42–43P, 1968.
61. Davies, B. N., Gamble, J. and Withrington, P. G.: Frequency-dependent differences in the responses of the capsular and vascular smooth muscle of the spleen of the dog to sympathetic nerve stimulation. J. Physiol. (London) 228: 13–25, 1973.
62. Davies, B. N., Gamble, J. and Withrington, P. G.: Unpublished observation.
63. Davies, B. N., Horton, E. W. and Withrington, P. G.: The occurrence of prostaglandin E_2 in the splenic venous blood of the dog following splenic nerve stimulation. J. Physiol. (London) 188: 38–39P, 1967.
64. Davies, B. N., Horton, E. W. and Withrington, P. G.: The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. Brit. J. Pharmacol. Chemother. 32: 127–135, 1968.
65. Davies, B. N., Robinson, B. H. and Withrington, P. G.: The effects of graded doses of phenoxybenzamine on the vascular and capsular responses of the isolated blood perfused dogs spleen to sympathetic nerve stimulation and catecholamines. Arch. Int. Pharmacodyn. Thér. 180: 143–154, 1969.
66. Davies, B. N. and Withrington, P. G.: The effects of prostaglandin E_1 and E_2 on the smooth muscle of the dog spleen and on its responses to catecholamines, angiotensin and nerve stimulation. Brit. J. Pharmacol. Chemother. 32: 136–144, 1967.
67. Davies, B. N. and Withrington, P. G.: Actions of prostaglandins A_1, A_2, E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$, on splenic vascular and capsular smooth muscle and their interactions with sympathetic nerve stimulation, catecholamines and angiotensin. *In* Prostaglandins, Peptides and Amines, ed. by P. Mantegazza and E. W. Horton, pp. 53–56, Academic Press, London and New York, 1969.
68. Davies, B. N. and Withrington, P. G.: Actions of prostaglandin $F_{1\alpha}$ on the splenic vascular and capsular smooth muscle in the dog. Brit. J. Pharmacol. 41: 1–7, 1971.
69. Davies, B. N. and Withrington, P. G.: The responses of the isolated, blood perfused spleen of the dog to angiotensin, oxytocin and vasopressin. Brit. J. Pharmacol., submitted for publication, 1973.
70. Davies, B. N. and Withrington, P. G.: Unpublished observations.
71. Davis, J. E.: Response of the exteriorised spleen to ephedrine, acetylcholine, pilocarpine and pituitrin. Proc. Soc. Exp. Biol. Med. 36: 71–72, 1937.
72. Day, M. D. and Rand, M. J.: Tachyphylaxis to some sympathomimetic amines in relation to amine oxidase. Brit. J. Pharmacol. Chemother. 21: 84–96, 1963.
73. Dearnaley, D. P. and Geffen, L. B.: Effect of nerve stimulation on the noradrenaline content of the spleen. Proc. Roy. Soc. Ser. B Biol. Sci. 166: 303–315, 1966.
74. Della Bella, D. and Benelli, G.: Bradykinin and the spleen: Different reaction 'in vitro' and 'in vivo'. *In* Prostaglandins, Peptides and Amines, ed. by P. Mantegazza and E. W. Horton, pp. 99–107, Academic Press, London and New York, 1969.
75. Donatelli, L. and Martinetti, R.: Studio comparativo dell'azione degli adrenalinici sulla milza di coniglio. Boll. Soc. Ital. Biol. Sper. 15: 921–924, 1941.
76. Douglas, W. W. and Ritchie, J. M.: The excitatory action of acetylcholine on cutaneous non-myelinated fibres. J. Physiol. (London) 150: 501–514, 1960.
77. Driver, R. L. and Vogt, M.: Carotid sinus reflex and contraction of the spleen. Brit. J. Pharmacol. Chemother. 5: 505–509, 1950.
78. Dudley, H. W.: On the active principles of the pituitary gland. J. Pharmacol. Exp. Ther. 21: 103–122, 1923.
79. Durant, R. R. and McNinch, J. H.: Vasomotor responses to perfused adrenaline in the unanaesthetised dog. Amer. J. Physiol. 85: 364, 1928.

80. EULER, U. S. VON.: Presence of substance with sympathin E properties in spleen extracts. Acta Physiol. Scand. **11**: 168-186, 1946.
81. EVERETT, S. D. AND MANN, S. P.: Catecholamine release by histamine from the isolated intestine of the chick. Eur. J. Pharmacol. **1**: 310-320, 1967.
82. FARBER, S.: The action of acetylcholine on the volume of the spleen of the dog. Arch. Int. Pharmacodyn. Thér. **53**: 367-376, 1936.
83. FELDBERG, W. AND LEWIS, G. P.: The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. J. Physiol. (London) **171**: 98-108, 1964.
84. FERGUSON, J., IVY, A. C. AND GREENGARD, H.: Observations on the response of the spleen to intravenous injection of certain secretin preparations, acetylcholine and histamine. Amer. J. Physiol. **117**: 701-707, 1936.
85. FERRIERA, S. H., MONCADA, S., AND VANE, J. R.: Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature, New Biol. **231**: 237-239, 1971.
86. FERRIERA, S. H. AND VANE, J. R.: Prostaglandins: Their disappearance from and release into the circulation. Nature (London) **216**: 868-873, 1967.
87. FERRY, C. B.: The sympathomimetic effect of acetylcholine on the spleen of the cat. J. Physiol. (London) **167**: 487-504, 1963.
88. FERRY, C. B.: Cholinergic link hypothesis in adrenergic neuroeffector transmission. Physiol. Rev. **46**: 420-456, 1966.
89. FICK, L.: Zur Mechanik der Blutwegung. Reichert du Bois Arch. **1**: 8-13, 1859. Cited by J. Bulgak, 1877 (40).
90. FILLENZ, M.: Innervation of the cat spleen. Proc. Roy. Soc. Ser. B Biol. Sci. **174**: 459-468, 1970.
91. FLEMING, W. W. AND PARPART, A. K.: Effects of topically applied epinephrine, norepinephrine and histamine on the intermediate circulation of the mouse spleen. Angiology **9**: 294-302, 1958.
92. FORTINO, S. AND STOICULESCU, P.: Studii experimentale asupra inervatiei splenice. Stud. Cercet. Fiziol. **1**: 119-143, 1956.
93. FORTINO, S. AND STOICULESCU, P.: Action des médiateurs nerveux sur la rate. Possibilité d'existence de substances receptrices bivalentes au niveau des cellules effectrices spléniques. J. Physiol. (Paris) **48**: 1011-1034, 1956.
94. FREDERICQ, H.: Le nature parasympathique probable de l'innervation motrice de la rate du chien. C. R. Séances Soc. Biol. **101**: 1164-1165, 1929.
95. FREY, W. AND TONIETTI, F.: Der Einfluss der vegetativen Nerven auf die Milz und die Lymphocyten des Bluts. Z. Gesamte Exp. Med. **44**: 597-608, 1925.
96. FROHLICH, E. D. AND GILLENWATER, J. Y.: Pressure-flow relationships in the perfused dog spleen. Amer. J. Physiol. **204**: 645-648, 1963.
97. GADDUM, J. H., PEART, W. S. AND VOGT, M.: The estimation of adrenaline and allied substances in the blood. J. Physiol. (London) **108**: 467-481, 1949.
98. GALINDO, B. AND FREEMAN, J. A.: Fine structure of splenic pulp. Anat. Rec. **147**: 25-42, 1963.
99. GAMBLE, J.: Personal communication.
100. GENAZZANI, E. AND NITO, E.DE.: Su di un nuovo methodo biologico per il dosaggio dell' adrenalina. Boll. Soc. Ital. Biol. Sper. **28**: 1283-1286, 1952.
101. GEUS, J. P. DE, BERNARDS, J. A. AND VERDUYN, W. H.: On the rhythmic activity of the cat spleen. Arch. Int. Pharmacodyn. Thér. **136**: 353-384, 1962.
102. GILLESPIE, J. S. AND KIRPEKAR, S. M.: The histological localisation of noradrenaline in the cat spleen. J. Physiol. (London) **187**: 69-79, 1966.
103. GILMORE, N., VANE, J. R. AND WYLLIE, J. H.: Prostaglandins released by the spleen. Nature (London) **218**: 1135-1140, 1968.
104. GODART, S. J. AND HAMILTON, W. F.: Lymphatic drainage of the spleen. Amer. J. Physiol. **204**: 1107-1114. 1963.
105. GOLLNICK, P. D.: Chronic effect of exercise on liver cholesterol of normal and hypercholesteremic rats. Amer. J. Physiol. **205**: 453-456, 1963.
106. GOTSEV, T.: Über die Wirkung des Acetylcholins auf Blutgefässe, Blutdruck und Vasomotorenzentren. Arch. Exp. Pathol. Pharmakol. (Naunyn-Schmiedebergs) **181**: 207-214, 1936.
107. GRAY, J. A. B. AND DIAMOND, J.: Pharmacological properties of sensory receptors and their relation to those of the autonomic nervous system. Brit. Med. Bull. **13**: 185-188, 1957.
108. GRAYSON, J. AND MENDEL, D.: Physiology of the Splanchnic Circulation, Edward Arnold, London, 1965.
109. GREEN, R. D. AND FLEMING, W. W.: Analysis of supersensitivity in the isolated spleen of the cat. J. Pharmacol. Exp. Ther. **162**: 254-262, 1968.
110. GREEN, H. D., OTTIS, K. AND KITCHEN, T.: Autonomic stimulation and blockade on canine splenic inflow, outflow and weight. Amer. J. Physiol. **198**: 424-428, 1960.
111. GREENBERG, R.: The effects of phentolamine and phenoxybenzamine on the responses of the isolated spleen strip to electrical stimulation and noradrenaline. Arch. Int. Pharmacodyn. Thér. **184**: 227-232, 1970.
112. GREENWAY, C. V., LAWSON, A. E. AND STARK, R. D.: Vascular responses of the spleen to nerve stimulation during normal and reduced blood flow. J. Physiol. (London) **194**: 421-433, 1968.
113. GREENWAY, C. V. AND STARK, R. D.: Vascular responses of the spleen to rapid haemorrhage in the anaesthetized cat. J. Physiol. (London) **204**: 169-179, 1969.
114. GREENWAY, C. V. AND STARK, R. D.: The vascular response of the spleen to intravenous infusions of catecholamines, angiotensin and vasopressin in the anaesthetized cat. Brit. J. Pharmacol. **38**: 583-592, 1970.
115. GRINDLAY, J. H., HERRICK, J. F. AND BALDES, E. J.: Rhythmicity of the spleen in relation to blood flow. Amer. J. Physiol. **127**: 119-126, 1939.
116. GRINDLAY, J. H., HERRICK, J. F. AND MANN, F. C.: Measurement of the blood flow of the spleen. Amer. J. Physiol. **127**: 106-118, 1939.
117. GUNTHEROTH, W. G., MCGOUGH, G. A. AND MULLINS, G. L.: Continuous recording of splenic diameter, vein flow and haematocrit in the intact dog. Amer. J. Physiol. **213**: 690-694, 1967.
118. GUNTHEROTH, W. G., AND MULLINS, G. L.: Liver and spleen as venous reservoirs. Amer. J. Physiol. **204**: 35-41, 1963.
119. GUZMAN, F., BRAUN, C. AND LIM, R. K. S.: Visceral pain and the pseudoaffective response to intra-arterial injections of bradykinin and other algesic agents. Arch. Int. Pharmacodyn. Thér. **136**: 353-384, 1962.
120. HARGIS, E. H. AND MANN, F. C.: A plethysmographic study of the changes in the volume of the spleen in the intact animal. Amer. J. Physiol. **75**: 180-200, 1925.
121. HARTMANN, A.: Handbuch der Mikroskopischen Anatomie des Menschen, Part 1: Die Milz, vol. 6 pp. 397-563, Verlag von Julius Springer, Berlin, 1930.
122. HARTMANN, F. A. AND LANG, R. S.: Actions of adrenaline on the spleen. J. Pharmacol. Exp. Ther. **13**: 417-427, 1919.
123. HARTMANN, F. A. AND MCPHEDRAN, L.: Further observations on the differential action of adrenalin. Amer. J. Physiol. **43**: 311-327, 1917.
124. HASHIMOTO, K. AND SATOH, S.: Enhancement of the post-occlusive oscillation in the splenic circulation by adenosine. J. Physiol. (London) **218**: 295-304, 1971.
125. HAUSNER, E., ESSEX, H. E. AND MANN, F. C.: Roentogenologic observations of the spleen of the dog under ether, sodium amytal, pentobarbital sodium, and pentothal sodium anaesthesia. Amer. J. Physiol. **121**: 387-391, 1938.
126. HEDQVIST, P.: Control by prostaglandin E_2 of sympathetic neurotransmission in the spleen. Life Sci. **9**: 269-278, 1970.
127. HEDQVIST, P. AND BRUNDIN, J.: Inhibition by prostaglandin E_1 of noradrenaline release and of effector response

to nerve stimulation in the cats spleen. Life Sci. 8: 389-395, 1969.

128. Hedqvist, P., Oliverio, A. and Stjarne, L.: Inhibition by phenoxybenzamine of the noradrenaline releasing effect of tyramine. Acta Physiol. Scand. 72: 385-391, 1968.
129. Heirman, P.: A propos de l'action du vague sur les contractions de la rate et sur l'adrénaline-sécrétion. Arch. Int. Physiol. 42: 318-322, 1936.
130. Henle, J.: Versuche und Beobachtungen an einen Enthaupteten. Z. Rat. Med. 2: 299-312, 1852.
131. Henschen, C. and Reissinger, H.: Beitrage zur Klinischen Physiologie der Milz: Experimentelle Untersuchungen über die Volumenschwankungen und die Kontraktilität der Milz, über ihre Durchblutung und über die Sperrmechanismen der Milzarterie. Deut. Z. Chir. 210: 1-35, 1928.
132. Hertting, G. and Suko, J.: Influence of angiotensin, vasopressin or changes in flow rate on vasoconstriction, changes in volume and ^{3}H-noradrenaline release following postganglionic sympathetic nerve stimulation in the isolated cat spleen. Brit. J. Pharmacol. Chemother. 26: 686-696, 1966.
133. Heymans, C.: Le sinus carotidien isolé et perfusé, zone réflexogène régulatrice de l'adrénalinosecretion. C. R. Séances Soc. Biol. 100: 199-201, 1929.
134. Hodge, R. L., Lowe, R. D. and Vane, J. R.: The effect of alteration of blood volume on the concentration of circulating angiotensin in anaesthetised dogs. J. Physiol. (London) 185: 613-626, 1966.
135. Hodgson, P. and Olmsted, J. M. D.: Dilatation of the spleen. Stimulation of nerves; effects of autonomic drugs. Proc. Soc. Exp. Biol. Med. 30: 478-480, 1933.
136. Holler, H. and Kiehtreiber, A.: Die Bestimmung von sympathikolytischen Substanzen an der isolierten Milz. Wien. Med. Wochenschr. 104: 559-560, 1954.
137. Holtz, P., Bachmann, F., Engelhardt, A. and Greef, K.: Die Milzwirkung des Adrenalins und Arterenols. Pflügers Arch. Gesamte Physiol. Menschen Tiere 255: 232-250, 1952.
138. Holtz, P. and Schümann, H. J.: Karotissinusentlastung und Nebennieren. Arch. Exp. Pathol. Pharmakol. (Naunyn-Schmiedebergs) 206: 49-64, 1949.
139. Horne, T.: Changes in whole body and spleen weight in the rat during exposure to high oxygen pressure. J. Physiol. (London) 187: 35-36 P, 1966.
140. Horton, E. W.: Hypotheses on physiological roles of prostaglandins. Physiol. Rev. 49: 122-161, 1969.
141. Hoskings, R. G. and Lee Gunning, R. E.: The effects of adrenin on the distribution of the blood. II. Volume changes and venous discharge in the spleen. Amer. J. Physiol. 43: 298-303, 1917.
142. Howell, W. H.: The physiological effects of extracts of the hypophysis cerebri and infundibular body. J. Exp. Med. 3: 245-258, 1898.
143. Hughes Jones, N. C., Mollison, P. L. and Veall, N.: Removal of incompatible red cells by the spleen. Brit. J. Haematol. 3: 125-133, 1957.
144. Hunt, R.: Vasodilator reactions. 1. Amer. J. Physiol. 45: 197-230, 1918.
145. Ignarro, L. J. and Titus, E.: The presence of antagonisitically acting alpha and beta adrenergic receptors in the mouse spleen. J. Pharmacol. Exp. Ther. 160: 72-80, 1968.
146. Innes, I. R.: An action of 5-hydroxytryptamine on adrenaline receptors. Brit. J. Pharmacol. Chemother. 19: 427-441, 1962.
147. Innes, I. R.: Smooth muscle receptors for amphetamine and 5-hydroxytryptamine. Abstr. XXII Int. Physiol. Congr., p. 591, 1962.
148. Jacobi, H. and Fontaine, R.: Untersuchung über die beta-rezeptorenblockende Wirkung an der Kaninchen-Milz. Arztliche Forschung 20: 308-309, 1966.
149. Kamm, O., Aldrich, T. B., Grote, I. W., Rowe, L. W. and Bugbee, E. P.: The active principles of the posterior lobe of the pituitary gland. J. Amer. Chem. Soc. 50: 573-601, 1928.
150. Kimledorf, D. J. and Baum, S. J.: Alteration in organ and body growth of rats following daily exhaustive exercise, X irradiation and post-irradiation exercise. Growth 18: 79-96, 1954.
151. Kizaki, S. and Abiko, Y.: The blocking action of pronethalol on the contraction of isolated spleen strips produced by adrenaline, acetylcholine or isoproterenol. Jap. J. Pharmacol. 16: 306-311, 1966.
152. Klemperer, P.: The Spleen. *In* Handbook of Haematology, ed. by H. Downey, vol. 3, pp. 1587-1754, Paul B. Hoeber, Inc., New York, 1938.
153. Knisely, M. H.: Spleen studies. I. Microscopic observations of the circulatory system of living unstimulated mammalian spleens. Anat. Rec. 65: 23-50, 1936.
154. Knisely, M. H.: Spleen studies. II. Microscopic observations on the circulatory system of living traumatized spleens and of dying spleens. Anat. Rec. 65: 131-148, 1936.
155. Koppanyi, T. and Dooley, M. S.: Responses of the kidney and spleen to subcutaneously administered epinephrin. Proc. Soc. Exp. Biol. Med. 26: 443-445, 1929.
156. Koyama, K.: Haemodynamics of the spleen in Banti's syndrome. Tohoku J. Exp. Med. 93: 199-217, 1967.
157. Krafka, J., McCrea, F. D. and Vogt, E.: The activity of the spleen during anaphylactic shock. J. Physiol. (London) 68: 292-294, 1929.
158. Kramer, K. and Luft, U. C.: Mobilisation of red cells and oxygen from the spleen in severe hypoxia. Amer. J. Physiol. 165: 215-228, 1951.
159. Krumbhaar, E. B. and Lippincott, S. W.: The postmortem weight of the normal human spleen at different ages. Amer. J. Med. Sci. 197: 344-358, 1939.
160. Krumbhaar, E. B. and Musser, J. H.: The effect of splenectomy on the hemopoietic system of Macacus Rhesus. Arch. Intern. Med. 31: 686-700, 1923.
161. Leaders, F. E. and Dayrit, C.: The cholinergic component in the sympathetic innervation to the spleen. J. Pharmacol. Exp. Ther. 147: 145-152, 1965.
162. Lennert, K. and Harms, D.: (Die Milz: The Spleen.) Structure, Function, Pathology, Clinical Aspects, Therapy. ed. by K. Lennert and D. Harms, Springer-Verlag, Berlin, 1970.
163. Leszkovszky, G. P. and Tardos, L.: Potentiation by cocaine and 3,3-di(p-aminophenyl)-propylamine (TK_{174}) of the effect of isoprenaline and noradrenaline on isolated strips of cats spleen. J. Pharm. Pharmacol. 20: 377-380, 1968.
164. Li, P. L., Garven, H. S. D. and Mole, R. H.: Microscopic anatomy of the vascular system of the dog's spleen. J. Roy. Microsc. Soc. 49: 109-119, 1929.
165. Lindgren, A. G. H.: Kapillarstudien bei Schockzuständen. Arch. Exp. Pathol. Pharmakol. (Naunyn-Schmiedebergs) 176: 96-110, 1934.
166. Lippay, F., Mitchell, G. G. and Irving, E.: Rhythmic activity of the isolated mammalian spleen. Aust. J. Exp. Biol. Med. Sci. 31: 267-272, 1953.
167. Lippay, F., Mitchell, G. G. and Potter, B. J.: The role of the spleen in the adrenaline reversal and the theory of this phenomenon. Aust. J. Exp. Biol. Med. Sci. 28: 517-542, 1950.
168. Loewe, S. and Faure, W.: Unmittelbarer Nachweis der Muskeltätigkeit der Milzkapsel. Klin. Wochenschr. 4: 1358-1359, 1925.
169. MacKenzie, D. W., Whipple, A. O. and Wintersteiner, M. P.: Studies on the microscopic anatomy and physiology of living transilluminated mammalian spleens. Amer. J. Anat. 68: 397-456, 1941.
170. MacMillan, W. H. and Rand, M. J.: The effects in rabbits of thyroidectomy and treatment with triiodothyronine on the sensitivity to noradrenaline and the content of noradrenaline in aorta and spleen. J. Pharm. Pharmacol. 14: 257-267, 1962.

171. McCance, R. A. and Widdowson, E. M.: The size and function of the spleen in young puppies. J. Physiol. (London) **129**: 636–638, 1955.
172. Maengwyn-Davis, G. D.: The dual mode of action of histamine in the cat isolated tracheal chain. J. Pharm. Pharmacol. **20**: 572–573, 1968.
173. Magee, D. F.: The action of some drugs on the excised spleen. Quart. J. Pharm. Pharmacol. **19**: 44–48, 1946.
174. Magnus, R. and Schäfer, E. A.: Does the vagus contain motor fibres for the spleen? J. Physiol. (London) **27**: iii, 1901.
175. Magnus, R. and Schäfer, E. A.: The action of pituitary extracts on the kidney. J. Physiol. (London) **27**: ix, 1901.
176. Mall, F. P.: The architecture and blood vessels of the dog's spleen. Z. Morphol. Anthropol. **2**: 1–42, 1900.
177. Masuda, T.: The action of the vagus on the spleen. J. Physiol. (London) **62**: 289–300, 1927.
178. Menkin, V.: The effect of splenic contraction induced by faradic stimulation on the leucocyte level of the splenic vein. Amer. J. Physiol. **88**: 737–746, 1929.
179. Mertens, O.: Die Milz als Kreislauforgan. Nachrichtenbl. Gesamte Wiss. Gottingen Biol. **1**: 261–283, 1935.
180. Michels, N. A.: Variational anatomy of spleen and splenic artery. Amer. J. Anat. **70**: 21–72, 1942.
181. Miller, D. K. and Rhoads, C. P.: The effect of splenic contraction on the formed elements of the blood in one case of anemia and splenomegaly. J. Clin. Invest. **12**: 1009–1020, 1933.
182. Moerman, E. J., Scapagnini, U. and de Schaepdryver, A. F.: Effects of bradykinin, kallidin, physalaemin and eledoisin on the perfused, isolated, dog spleen. *In* Prostaglandins, Peptides and Amines, ed. by P. Mantegazza and E. W. Horton, pp. 109–117, Academic Press, London and New York, 1969.
183. Moerman, E. J., Scapagnini, U. and de Schaepdryver, A. F.: Adrenergic receptors in the isolated perfused dog spleen. Eur. J. Pharmacol. **5**: 279–285, 1969.
184. Moore, R. M.: The volume of the spleen in traumatic shock. Amer. J. Physiol. **89**: 508–514, 1929.
185. Ng, K. K. F. and Vane, J. R.: Fate of angiotensin I in the circulation. Nature (London) **218**: 144–150, 1968.
186. Oehl, E.: Zur Physiologie der Milz. Gazeta Lombarda (Schmidts Jahrb) **141**: 275, 1869. Cited by J. Bulgak, 1877 (40) and E. A. Schäfer and B. Moore, 1896 (209).
187. Oliver, G. and Schäfer, E. A.: The physiological effects of extracts of the suprarenal capsules. J. Physiol. (London) **18**: 230–276, 1895.
188. Ottis, K., Davis, J. E. and Green, H. D.: Effects of adrenergic and cholinergic drugs on splenic inflow and outflow before and during adrenergic blockade. Amer. J. Physiol. **189**: 599–604, 1957.
189. Paffenholz, W. and Schürmeyer, A.: Änderungen der Milz- und Lebergrösse in Röntgenbild unter verschiedenen Kreislaufbedingungen. Klin. Wochenschr. **10**: 2076–2078, 1931.
190. Palitz, L. L.: A simple method for recording splenic volume changes in the intact, unanaesthetised dog. Effects of epinephrine, pilocarpine, atropine and benezedrine on splenic volume. J. Lab. Clin. Med. **24**: 1296–1301, 1939.
191. Parin, W. W.: Mechanism of contraction following stimulation of sensory nerves. Pflügers Arch. Gesamte Physiol. Menschen Tiere **228**: 329–343, 1931.
192. Peart, W. S.: Nature of splenic sympathin. J. Physiol. (London) **108**: 491–501, 1949.
193. Pinardi, T. G.: Effects of denervation on the haemodynamics of the spleen during irreversible shock. Acta Physiol. Latinoamer. **18**: 248–252, 1968.
194. Pinkston, J. L. and Pinkston, J. O.: Changes in spleen size, blood pressure and erythrocyte count after administration of benzedrine sulphate in dogs. J. Lab. Clin. Med. **24**: 1038–1045, 1939.
195. Raymond-Hamet, M.: Sur les vasomoteurs spléniques. C. R. Hebd. Séances Acad. Sci. Paris **184**: 1672–1674, 1927.
196. Regoli, D. and Vane, J. R.: The continuous estimation of angiotensin formed in the circulation of the dog. J. Physiol. (London) **183**: 513–531, 1966.
197. Reiffenstein, R. J.: Effects of cocaine on the rate of contraction to noradrenaline in the cats spleen strip: mode of action of cocaine. Brit. J. Pharmacol. Chemother. **32**: 591–597, 1968.
198. Riva Sanseverino, E. and Urbano, A.: Effetti della 5-HT sul volume della milza di cane artificialmente perfusa. Boll. Soc. Ital. Biol. Sper. **37**: 1554–1556, 1961.
199. Riva Sanseverino, E. and Urbano, A.: Variazioni circulatorie della Milza di cane per effetto della 5-HT. Boll. Soc. Ital. Biol. Sper. **38**: 518–521, 1962.
200. Robinson, R. L.: Stimulation of the catecholamine output of the isolated, perfused adrenal gland of the dog by angiotensin and bradykinin. J. Pharmacol. Exp. Ther. **156**: 252–257, 1967.
201. Rocha e Silva, M. and Rosenberg, M.: The release of vasopressin in response to haemorrhage and its role in the mechanism of blood pressure regulation. J. Physiol. (London) **202**: 535–557, 1969.
202. Ross, G.: Effects of catecholamines on splenic blood flow in the cat. Amer. J. Physiol. **213**: 1079–1083, 1967.
203. Roy, C. S.: The physiology and pathology of the spleen. J. Physiol. (London) **3**: 203–228, 1881.
204. Saad, K.: The effects of drugs on the isolated splenic capsule of man and other animals. Quart. J. Pharm. Pharmacol. **8**: 31–38, 1935.
205. Sandberg, G.: Splenic blood flow in the guinea-pig measured with xenon 133, and calculation of the venous output of lymphocytes from the spleen. Acta Physiol. Scand. **84**: 208–216, 1972.
206. Sapirstein, L. A., Sapirstein, E. H. and Bredemeyer, A.: Effect of haemorrhage on the cardiac output and its distribution in the rat. Circ. Res. **8**: 135–148, 1960.
207. Schabadash, A.: Beiträge zur vergleichenden Anatomie der Milzarterien. Z. Anat. Entwicklungsgesch. **104**: 502, 1935.
208. Schaepdryver, A. F. de and Preziosi, P.: Pharmacological depletion of adrenaline and noradrenaline in various organs of mice. Arch. Int. Pharmacodyn. Thér. **121**: 177–221, 1959.
209. Schäfer, E. A. and Moore, B.: On the contractility and innervation of the spleen. J. Physiol. (London) **20**: 1–50, 1896.
210. Schäfer, E. A. and Vincent, S.: The physiological effects of extracts of the pituitary body. J. Physiol. (London) **25**: 87–97, 1899.
211. Schiff, M.: Über die Funktion der Milz. Leçons sur la physiologie de la digestion tII, xxxv leçon, 416–452, 1867. Cited by J. Bulgak, 1877 (40) and E. A. Schäfer and B. Moore, 1896 (209).
212. Schkawera, G. L.: Über die Wirkung verschiedener Gifte und anderer Agenzien auf die glatte Muskulatur und die Gefässe der isolierten Milz bei Tieren und Menschen. Z. Gesamte Exp. Med. **33**: 339–349, 1923.
213. Schrier, R. W., Verroust, P. J., Jones, J. J., Fabian, M., Lee, J. and de Wardener, H. E.: Effect of isotonic saline infusion and acute haemorrhage on plasma oxytocin and vasopressin concentrations in dogs. Clin. Sci. (London) **35**: 433–443, 1968.
214. Schumann, H. J.: Vergleichende Untersuchungen über die Wirkung von Adrenalin, Arterenol und Epinin auf Blutdruck, Milzvolumen, Darm und Blutzucker. Arch. Exp. Pathol. Pharmakol. (Naunyn-Schmiedebergs) **206**: 164–170, 1949.
215. Scott, M. J.: The effects of anticholinesterases upon the spleen of the dog. J. Physiol. (London) **139**: 489–496, 1957.
216. Sheys, E. M. and Green, R. D.: A quantitative study of alpha adrenergic receptors in the spleen and aorta of the rabbit. J. Pharmacol. Exp. Ther. **180**: 317–325, 1972.
217. Simpson, S., Levy and Cadness, B. H. E.: The effects of ephedrine on erythrocytes, leucocytes and platelets in the

normal and splenectomised guinea pig. J. Pharmacol. Exp. Ther. **56**: 389–395, 1936.

218. SKRAMLIK, E. VON.: Die Milz. Mit besonderer Berücksichtigung des vergleichenden Standpunktes. Ergeb. Biol. **2**: 505–553, 1927.
219. SKRAMLIK, E. VON. AND DURAN-CAO, M.: Über die Beziehungen des Vagus zum Sympathicus bei der Milz. Z. Gesamte Exp. Med. **45**: 460–474, 1925.
220. SNOOK, T.: A comparative study of the vascular arrangements in mammalian spleens. Amer. J. Anat. **87**: 31–61, 1950.
221. SNOOK, T.: The histology of the vascular terminations in the rabbits spleen. Anat. Rec. **130**: 711–729, 1958.
222. SOMLYO, A. P. AND SOMLYO, A. V.: Vascular smooth muscle. I. Normal structure, pathology, biochemistry and biophysics. Pharmacol. Rev. **20**: 197–272, 1968.
223. SOMLYO, A. P. AND SOMLYO, A. V.: Vascular smooth muscle. II. Pharmacology of Normal and Hypertensive vessels. Pharmacol. Rev. **22**: 249–353, 1970.
224. STARK, R. D., MCNEILL, J. R. AND GREENWAY, C. V.: Sympathetic and hypophyseal roles in the splenic response to haemorrhage. Amer. J. Physiol. **220**: 837–840, 1971.
225. STEINHAUS, A. H., HOYT, L. A. AND RICE, H. A.: Studies in the physiology of exercise. X. The effect of running and swimming on the organ weights of growing dogs. Amer. J. Physiol. **99**: 512–520, 1932.
226. STEPHENS, J. G.: Effects of vaso-dilator and vaso-constrictor substances on normal and denervated spleens. J. Physiol. (London) **99**: 127–134, 1940.
227. STINSTRA, G.: Physiologie de functione lienis, Groningen, 1854. Cited by J. Bulgak, 1877 (40).
228. STJÄRNE, L.: Tyramine effects on catecholamine release from spleen and adrenals in the cat. Acta Physiol. Scand. **51**: 224–229, 1961.
229. STRASSER, A. AND WOLF, H.: Über die Blutversorgung der Milz. Pflügers Arch. Gesamte Physiol. Menschen Tiere **108**: 590–626, 1905.
230. STUKELEY, W.: On the Spleen, Its Description and History, Uses and Diseases, Particularly the Vapours, with Their Remedy. London, 1723.
231. SULLIVAN, T. J.: Effect of antibiotics on the 5-hydroxytryptamine content of the small intestine and other organs in rats and mice. Brit. J. Pharmacol. Chemother. **16**: 90–98, 1961.
232. TAKANO, S.: A study of the contraction of splenic strips in kids and dogs: with special reference to the cholinergic and adrenergic receptors. Jap. J. Pharmacol. **19**: 563–568, 1969.
233. TARCHANOFF, F. VON.: Über die Innervation der Milz und deren Beziehung zur Leucocythämie. Pflügers Arch· Gesamte Physiol. Menschen Tiere **8**: 97–100, 1874.
234. TAYLOR, J. AND GREEN, R. D.: Analysis of reserpine induced supersensitivity in aortic strips of rabbits. J. Pharmacol. Exp. Ther. **177**: 127–135, 1971.
235. TEHRER, J. AND GRAHAME, T.: Capsule and trabeculae of spleens of domestic mammals. J. Anat. **65**: 473–481, 1931.
236. THOENEN, H., HÜRLIMANN, A. AND HAEFELY, W.: The effect of postganglionic stimulation on the isolated, perfused spleen of the cat. Helv. Physiol. Pharmacol. Acta **21**: 17–26, 1963.
237. THOENEN, H., HÜRLIMANN, A. AND HAEFELY, W.: The effect of angiotensin on the response to postganglionic sympathetic stimulation of the cat spleen; lack of facilitation of norepinephrine liberation. Med. Pharmacol. Exp. **13**: 379–387, 1965.
238. TIPTON, C. M., THARP, G. D. AND SCHILD, R. J.: Exercise' hypophysectomy and spleen weight. J. Appl. Physiol. **21**: 1163–1167, 1966.
239. TISCHENDORF, F.: Beobachtungen über die feinere Innervation der Milz. Köln Universitäts-Verlag, 1948.
240. TISCHENDORF, F.: Blutgefäss und Lymphgefässapparat innersekretorische Drüsen. *In* Handbuch der Mikroskopischen Anatomie des Menchen, ed. by W. v. Möllendorf and W. Bargmann, vol. 6, Springer-Verlag, Berlin, 1969.
241. TOMSA: Die Lymphwege der Milz. Sitzungsberichte der Wiener Akademie, **6**: 652–666, 1863. Cited by J. Bulgak, 1877 (40).
242. UTTERBACK, R. A.: The innervation of the spleen. J. Comp. Neurol. **81**: 55–66, 1944.
243. VAIREL, J.: Action de l'adrénaline et de l'acétylcholine sur la rate. J. Physiol. Pathol. Gén. **31**: 42–52, 1933.
244. VANE, J. R.: The release and fate of vaso-active hormones in the circulation. Brit. J. Pharmacol. **35**: 209–242, 1969.
245. VIGNEAUD, V. DU, LAWLER, H. C. AND POPENOE, E. A.: Enzymatic cleavage of glycinamide from vasopressin and a proposed structure for this pressor-antidiuretic hormone of the posterior pituitary. J. Amer. Chem. Soc. **75**: 4880–4881, 1953.
246. VIGNEAUD, V. DU, RESSLER, C., SWAN, J. M., ROBERTS, C. W. KATSOYANNIS, P. G. AND GORDON, S.: The synthesis of an octapeptide amide with the hormonal activity of oxytocin. J. Amer. Chem. Soc. **75**: 4879–4880, 1953.
247. VOLICER, L. AND VESIN, S.: Die Veränderungen der Milz nach Adrenalin im Rötgenbilde und die gleichzeitigen Veränderungen des Blutbildes. Z. Klin. Med. **122**: 57–82, 1932.
248. WAGNER, R.: Nachrichtenbl. Gesamte Wiss (Göttingen), no. 8, p. 89–96, 1849.
249. WATSON, C. J.: Haemolytic jaundice and macrocytic haemolytic anaemia: certain observations in a series of 35 cases. Ann. Intern. Med. **12**: 1782–1796, 1939.
250. WATSON, C. J. AND PAINE, J. R.: A study of the splenic venous blood with particular reference to the haematocrit percentage and the haemoglobin concentration of the erythrocytes before and after splenic arterial injection of adrenaline. Amer. J. Med. Sci. **205**: 493–501, 1943.
251. WEBB-PEPLOE, M. W.: The isovolumetric spleen: index of reflex changes in splanchnic vascular capacity. Amer. J. Physiol. **216**: 407–413, 1969.
252. WEIL, P. E. AND WALL, P. I.: De la splénocontraction adrénalique dans les splénomégalies chroniques. Press. Med. **37**: 1357–1359, 1929.
253. WEISS, L.: The structure of the normal spleen. Seminars Haematol. **2**: 205–228, 1965.
254. WELCKER, H. AND BRANDT. A.: Gewichtswerthe der Körperorgane bei dem Menschen und den Thieren. Arch. Anthropol. **28**: 1–89, 1902.
255. WENNBERG, E. AND WEISS, L.: Structure of the spleen and haemolysis. Annu. Rev. Med. **20**: 29–40, 1969.
256. WHITING, A. J.: On the comparative histology and physiology of the spleen. Trans. Roy. Soc. Edinburgh **38**: 253–316, 1897.
257. WILLIAMS, R., CONDON, R. E., WILLIAMS, H. S., BLENDIS, L. M. AND KREEL, L.: Splenic blood flow in cirrhosis and portal hypertension. Clin. Sci. **34**: 441–452, 1968.
258. WOLF, R. AND FISCHER, J.: Das Milzminutenvolumen. *In* Die Milz: The Spleen, ed. by K. Lennert and D. Harms, pp. 113–117, Springer-Verlag, Berlin, 1970.
259. WU, P. P. T.: The maximal volume of the human spleen. Surg. Gynecol. Obstet. Int. Abstr. Surg. **77**: 74–78, 1943.
260. YANG, C. S.: Influence of adrenalin on human spleen and blood picture. Chin. J. Physiol. **2**: 163–171, 1928.
261. ZETTERSTRÖM, B.: Cited by Sandberg (205).
262. ZETTERSTRÖM, B. E. M., PALMERIO, C. AND FINE, J.: Protection of function and vascular integrity of the spleen in traumatic shock by denervation. Proc. Soc. Exp. Biol. Med. **117**: 373–376, 1964.