OBSERVATIONS ON FIBRIN THROMBI PRODUCED BY ENDOTOXINS: AN EXPERIMENTAL STUDY

G. B. D. SCOTT

From the Department of Morbid Anatomy, The Royal Free Hospital School of Medicine London, W.C.1

Received for publication August 18, 1961

STAMMLER (1959) demonstrated that the deposition of material, resembling fibrin, in the renal glomeruli of pregnant rats fed on a diet deficient in tocopherol was associated with the production of bilateral renal cortical necrosis, while Selzer and Parker (1951) speculated whether the occlusive lesions of the hepatic veins seen in Seneciosis resulted from the organisation of thrombi.

The possibility is thus raised that diverse morbid anatomical changes may result from the deposition of thrombi in small vessels, due to a wide variety of apparently unrelated causes.

In the generalized Shwartzman reaction, produced by 2 intravenous injections of endotoxin, given 24 hr. apart, eosinophilic hyaline material, often referred to as fibrinoid, is formed in the small vessels of many organs and differing views exist as to its origin. Booth, Muirhead and Montgomery (1956) believed it to be derived from the vessel walls, while Gerber (1936) considered it to be fibrin formed from the circulating blood.

The electron-microscopic studies of Pappas, Ross and Thomas (1958) have confirmed the latter view, as have the demonstration of temporary increases in the coagulability of the blood accompanying the deposition of this material (McKay and Shapiro, 1958) and the inhibition of the latter by either anticoagulants (Good and Thomas, 1953; Shapiro and McKay, 1958) or an increase in the fibrinolytic activity of the blood (Kliman and McKay, 1958).

Because of its accompanying thrombotic lesions the generalized Shwartzman reaction seemed to present a suitable phenomenon for the study of the natural history of fibrin thrombi in small vessels and for the investigation of the concept that the deposition of such thrombi might be the basis for many disease processes.

It was thus decided to investigate the generalized and localized Shwartzman reactions with particular reference to the formation, distribution and fate of the accompanying thrombi.

METHOD

One hundred and nineteen male Dutch rabbits, weighing 1–2 kg., were fed on the S.G.1 diet and allowed unrestricted drinking water containing a drachm of nitrofurazone per 5 l. to inhibit coccidiosis infestation.

Two lipopolysaccharides of *Escherichia coli*, manufactured by Difco Laboratories, Detroit (ref. Nos.: 0127 : B8 and 0111 : B4), henceforth referred to as endotoxins A and B respectively were dissolved in sterile normal saline to give a concentration of 0·1 mg. of endotoxin per ml. of saline.

The generalized Shwartzman reaction was produced in 71 animals, which received 2 injections of 0·2 mg. of endotoxin into the marginal ear veins 24 hr. apart. In 36 of these the

reaction was produced using each and the same antigen as preparer and provoker as well as by using each antigen in turn as preparer followed by the other as provoker, these procedures being henceforth referred to as the " uncrossed " and " crossed " reactions respectively. All animals were killed 24 hr. later.

The same reaction was produced, using endotoxin A, in 35 rabbits which were allowed to survive for periods ranging from 10 min. to 18 days after the second intravenous injection. A group of 6 rabbits received one intravenous injection of endotoxin A only, being killed 24 hr. later, while 6 animals received normal saline only.

The localized Shwartzman reaction was produced in 24 animals, which received 0·01 mg. of endotoxin intradermally into one ear and 0·2 mg. into the marginal vein of the other ear 24 hr. later. Both endotoxins were used either singly or in combination as in the generalized reactions. The site of the intradermal injection was inspected after a further 24 hr., and if a haemorrhagic reaction was present, the animal was killed. If no reaction was evident, a further inspection was made 4 hr. later and the animal was killed whether or not a reaction had occurred. The skin reactions were graded as faint, moderate or severe. Six animals received an intradermal injection only, being killed 24 hr. later, and 6 animals received an intradermal followed by an intravenous injection of saline.

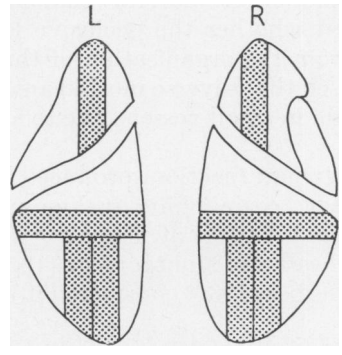


FIG. 1.—Diagram of posterior view of lungs showing positions of blocks taken for microscopy.

All animals were killed with intravenous Nembutal, any which died being autopsied. The liver, kidneys, spleen, suprarenals, lungs and heart were removed and, after 24 hr. fixation in 10 per cent formol saline, numerous blocks were taken from all areas of the liver. Blocks were also taken from both kidneys, spleen and adrenals and a horizontal block was taken through both ventricles of the heart, while standard blocks were taken from the upper and lower lobes of both lungs (Fig. 1).

After paraffin embedding, sections were stained with haematoxylin and eosin, Weigert's fibrin stain, picro-Mallory, phosphotungstic acid haematoxylin and for reticulin.

The numbers of thrombi seen in each organ were graded by the same method as that used by McKay and Shapiro (1958) and ranged from + where only an occasional thrombus was present, to ++++ where widespread thrombosis had occurred.

RESULTS

Although 13 animals died shortly after the first or second intravenous injections and 3 died 2, 3 and 7 days later respectively, the majority survived for the requisite periods.

Considerable numbers of thrombi were found in both the generalized and localized reactions but not in animals receiving saline only, while very scanty thrombi were seen in animals receiving a single intravenous injection of endotoxin A.

Occasional cysts of coccidiosis were encountered in some livers. Of 6 rabbits receiving an intradermal injection of endotoxin A, one showed thrombi in the

liver 24 hr. later. This animal was the only example of acute hepatic coccidiosis encountered, with numerous coccidia in the biliary epithelium and intense inflammation of the portal tracts (Smetana, 1933).

Six animals showed heavy infestation of the liver with coccidiosis and although the severity of the infestation and the accompanying necrosis did not appear to make any appreciable difference to the total number of thrombi found in the liver and elsewhere, these animals were not included in any comparative studies. Scanty thrombi were found in the liver of one such animal dying after one intravenous injection of endotoxin A.

The natural history of fibrin thrombi

Although very scanty ill-formed fibrin masses were seen as early as 10 min. after the second intravenous injection, by 2 hr. many compact rounded fibrin thrombi, in which nucleated white blood cells had become incorporated, were either occluding small vessels or adhering to their walls, the latter variety being covered by polymorphonuclear leucocytes and mononuclear cells. Some of these cells became elongated (Fig. 2) so that by 4 hr. many mural thrombi were covered by flat cells resembling endothelium.

The maximum number of thrombi was found 1–24 hr. after the second injection, after which the number identifiable decreased rapidly, only occasional thrombi being seen up to 10 days, and none after this.

Apart from occasional examples in the lungs, which will be described later, no organization of occlusive or mural thrombi was seen and no vascular lesions resembling those described following the organization of arterial emboli (Harrison, 1948 ; Heard, 1952) or venous thrombi (Scott, 1956 ; Filshie and Scott, 1958) were identified.

Liver.—In both the generalized and localized Shwartzman reactions, thrombi were present in the central and hepatic veins, being usually occlusive in the former and mural in the latter. Small numbers of thrombi were also identified in the liver sinusoids and one animal showed massive sinusoidal involvement only.

Thrombi were never seen in the hepatic arteries but were present in portal veins in the only 3 animals in which coccidiosis infestation had apparently resulted in severe scarring of the portal areas, the central and hepatic veins also being involved.

Small scanty foci of necrosis, infiltrated with inflammatory cells, were sometimes seen in livers containing thrombi. The presence of these lesions, which were by no means a constant accompaniment of hepatic thrombosis, was not related to the number of thrombi present in the liver. The lesions themselves were transient and did not result in hepatic fibrosis.

Lungs.—Thrombi were seen in pulmonary vessels of all sizes in both the generalized and localized reactions, but difficulty was experienced in distinguishing many pulmonary arterioles and arteries from pulmonary veins. Although certain criteria were used for identifying the first two varieties, namely the presence of an internal elastic lamina, a relatively thick muscular tunica media, obvious continuity with a recognizable pulmonary artery and the proximity of a bronchus or bronchiole, many small vessels remained unidentified.

Thrombi were more frequent in the dependent parts of the lower lobes than in the upper lobes and were present in recognizable pulmonary arteries, veins and capillaries as well as in numbers of small unidentifiable vessels. The thrombi in

pulmonary arteries were often composed of irregular masses of fibrin, the spaces between them and the vessel wall being filled with red and white corpuscles (Fig. 3). They occurred sometimes at arterial bifurcations and extended into the smaller branches. These appearances, which were suggestive of impacted emboli, were not seen in recognizable pulmonary veins in which the thrombi were often mural and multiple (Fig. 4).

With the passage of time neither organization of venous thrombi nor lesions resembling the end-results of their organization (Scott, 1956 ; Filshie and Scott, 1958) could be found after prolonged search. Partial organization of thrombotic masses lying in pulmonary arteries by cells resembling fibroblasts, without cellular infiltration of the arterial wall or periarterial tissues, was occasionally seen between the second and tenth days (Fig. 5). During this period some thrombi appeared to be disintegrating (Fig. 6) and very scanty cellular fibrotic intimal lesions were found with great difficulty (Fig. 7). No microscopical changes were seen in the lung parenchyma.

Kidneys.—Renal involvement occurred in both the generalized and localized reactions, usually appearing macroscopically as bilateral renal cortical necrosis 12–24 hr. after the second injection (Fig. 8), although microscopical changes were occasionally seen as early as $1\frac{1}{2}$ hr.

The glomerular capillaries were the first vessels to contain fibrin, which was often seen as a layer on the endothelium, leaving a patent though reduced lumen (Fig. 9). Only a few capillaries of individual glomeruli were initially involved but ultimately the whole tuft became filled with fibrin, the very infrequent arterial and venous involvement only occurring after the glomeruli were filled.

Although, in 2 animals, affected glomeruli were seen in the presence of normal renal parenchyma, they were usually surrounded by necrotic tubules, being either confined to segments of the cortex or distributed over the whole of the latter, producing necrosis of approximately its outer two-thirds.

Glomeruli, free from fibrin, varied greatly in size, cellularity and vascularity, but similar variations were seen in those receiving saline only. Hyaline droplet change was sometimes seen in the tubular epithelium of animals showing moderate glomerular involvement but not in those cases in which the glomeruli did not contain fibrin.

The kidneys of one rabbit, killed after 3 days, showed haemorrhagic spots on their surfaces and, microscopically, extensive necrosis of tubular epithelium, the tubules being filled with necrotic material. The surviving tubular epithelial cells showed great pleomorphism and in some areas they were flattened and

---

EXPLANATION OF PLATES.

FIG. 2.—Thrombus covered by mononuclear cells, two of which are elongated. H. and E. ×. 320

FIG. 3.—Pulmonary artery partially filled with fibrin mass ; the remainder of the lumen contains red and white corpuscles. H. and E. ×80.

FIG. 4.—Pulmonary vein containing three separate thrombi. H. and E. ×80.

FIG. 5.—Pulmonary artery containing an organising thrombus. H. and E. ×80.

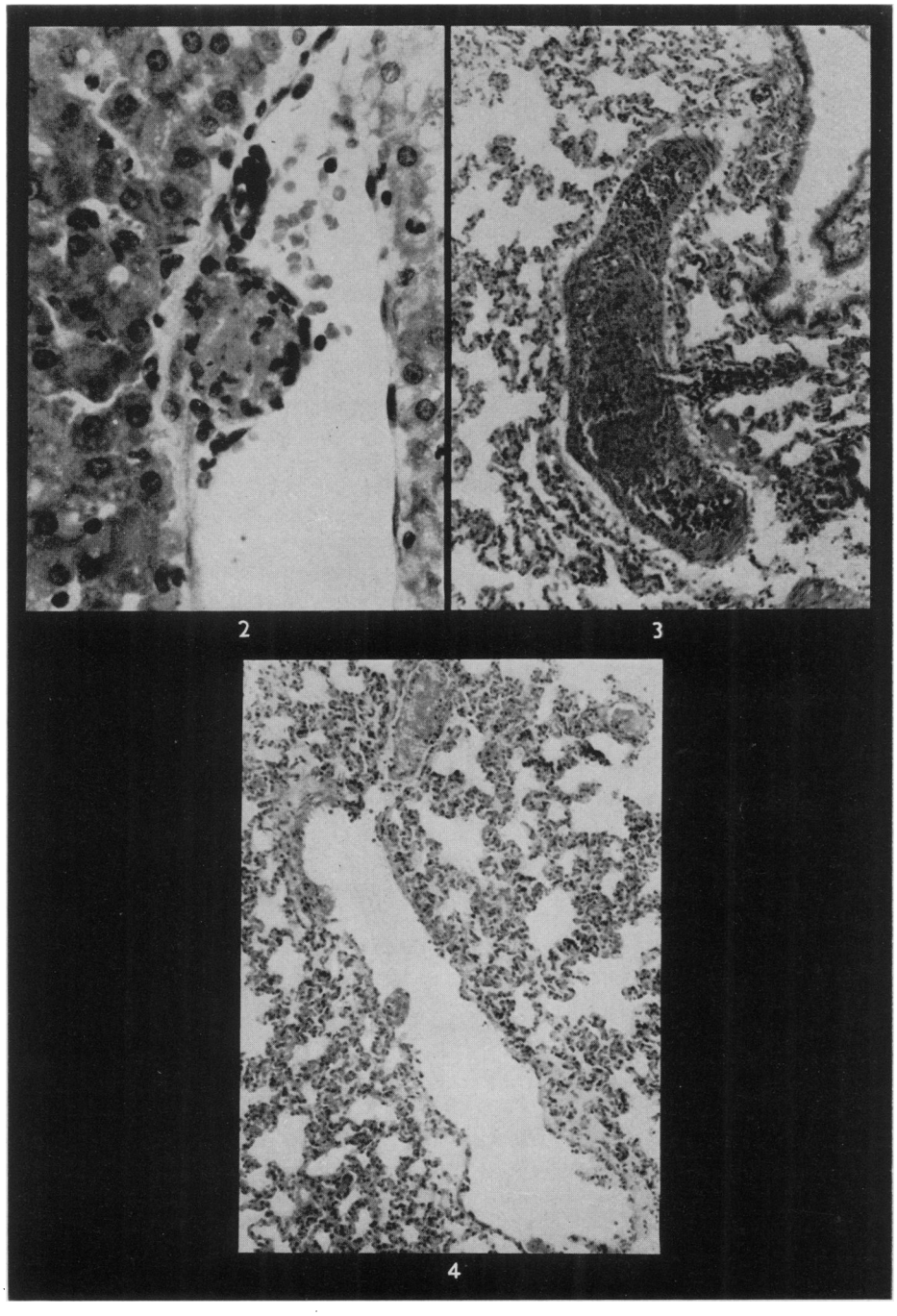
FIG. 6.—Pulmonary artery containing a disintegrating thrombus. H. and E. ×80.

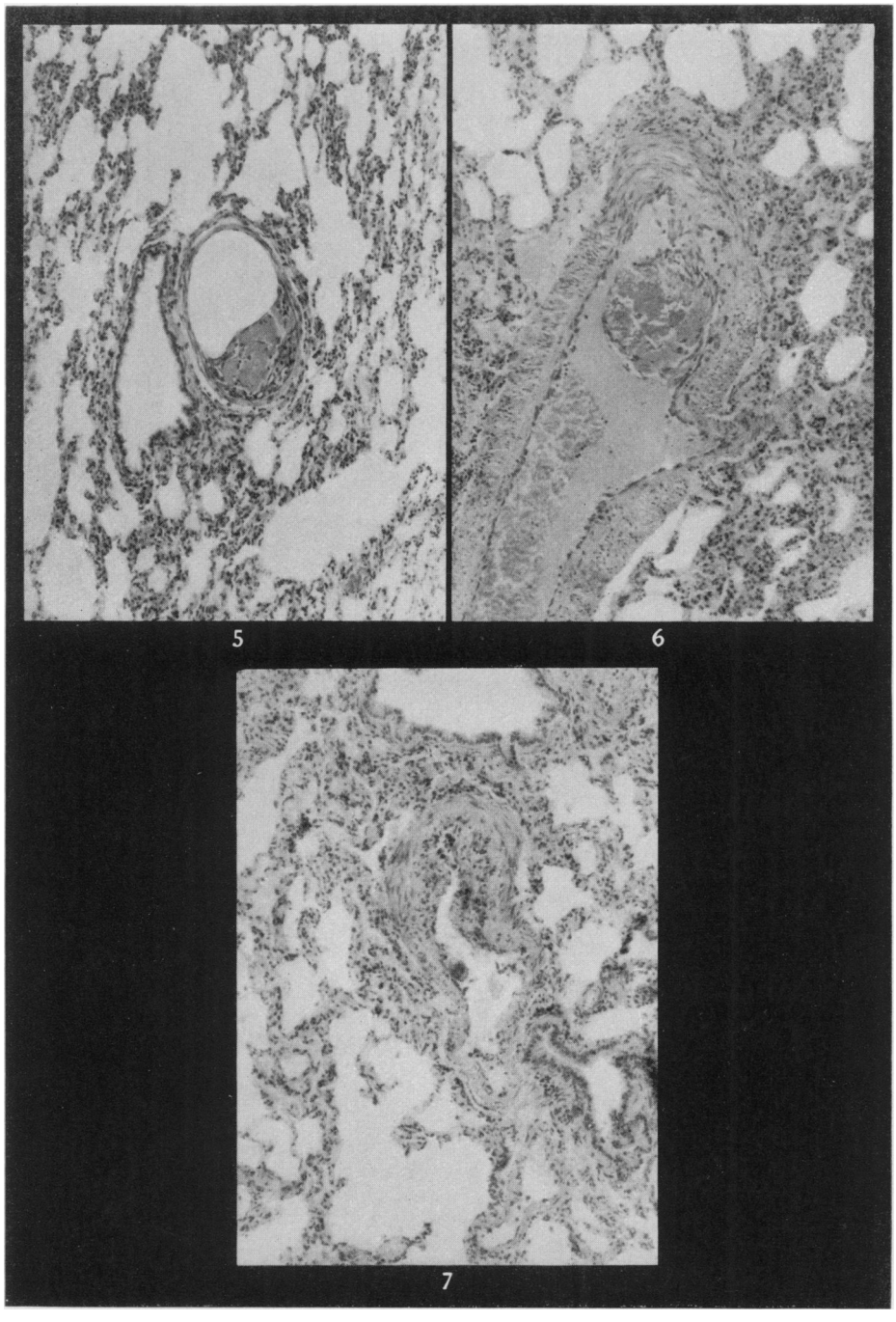
FIG. 7.—Fibrotic intimal lesion in a pulmonary artery. H. and E. ×80.

FIG. 8.—Horizontal section of kidney showing mottling of cortex. ×105.

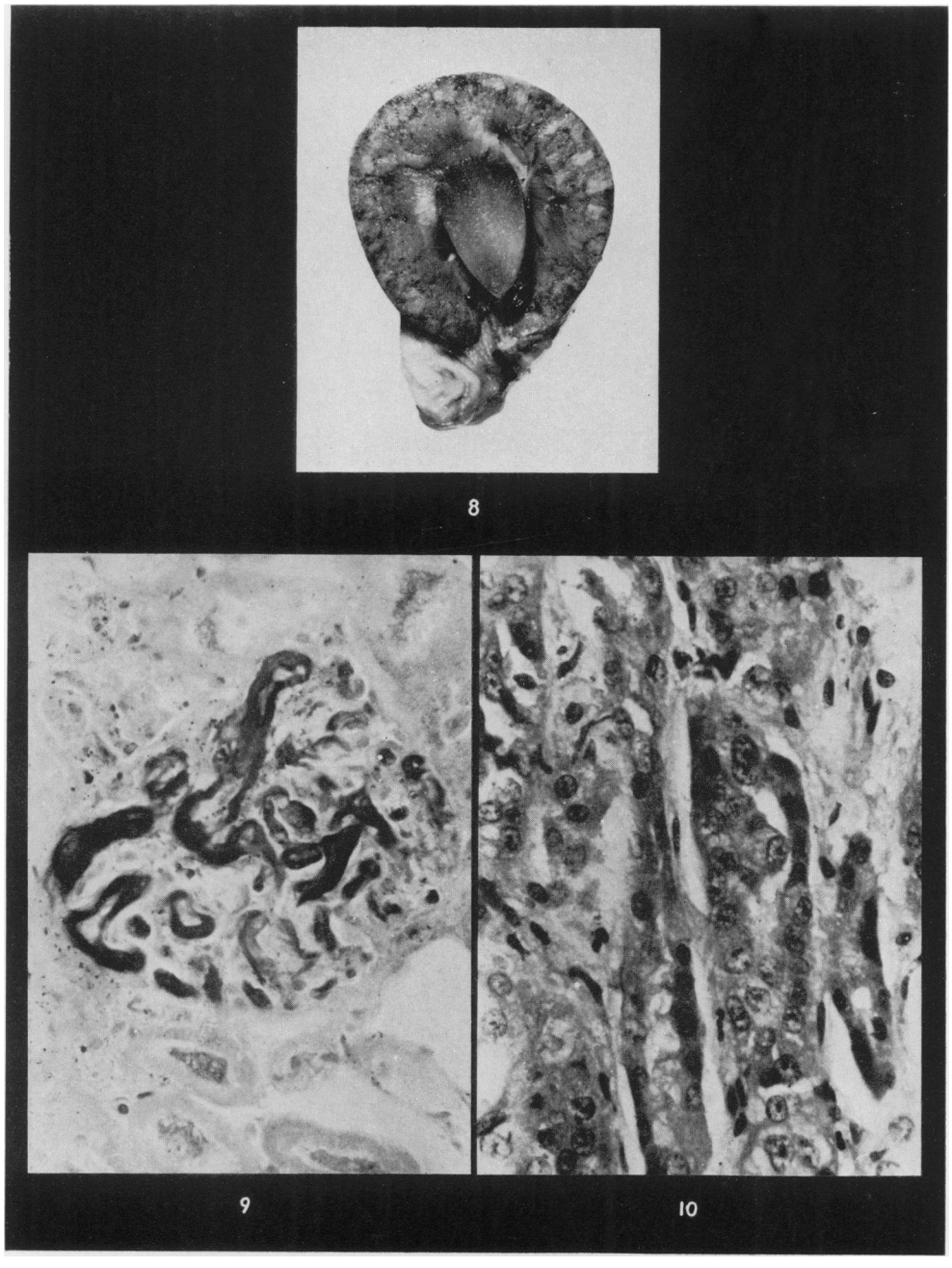
FIG. 9.—Fibrin lining glomerular capillaries. Weigert's fibrin stain. ×320.

FIG. 10.—Renal tubules showing epithelial regeneration. H. and E. ×320.


2
3
4



Scott.



8

9

10

irregular. Elsewhere they were heaped up, with prominent nuclei and nucleoli and occasional mitotic figures, a picture suggesting epithelial regeneration. However, the glomeruli, many of which showed gross distortion and dilatation of their capillaries, with large amounts of albuminous material in Bowman's capsule, were entirely free from fibrin.

The kidneys of the rabbit dying after 7 days showed bilateral renal cortical necrosis but many tubules were similar to those described above (Fig. 10). No interstitial fibrosis was present and a small number of affected glomeruli were more homogeneous and circumscribed than the rest, but did not show reticulin or collagen deposition.

Heart.—No thrombi were seen in the small branches of the coronary arteries, although very occasional sub-epicardial coronary veins contained thrombi. No thrombotic masses were seen adhering to the endocardial surfaces of the ventricles. Many hearts in the generalized but not the localized reactions showed acute focal necrosis of the myocardium which was maximal 24 hr. after the second injection and these lesions healed rapidly leaving fibrotic scars. No vascular lesions reminiscent of organized thrombi were found.

Spleen and suprarenals.—Involvement of these organs was inconstant. Masses of fibrin were sometimes present in the splenic sinuses and in the veins of the suprarenal medulla. With the passage of time, no residual lesions could be indentified in either organ.

Distribution of thrombi

The number of thrombi in the organs mentioned above was assessed in generalized and localized reactions, both " uncrossed " and " crossed ".

The liver was the organ most consistently involved and the numbers of thrombi identified were essentially the same in the generalized and localized reactions produced with either endotoxin and in the " crossed " generalized reactions. By comparison however, hepatic involvement was greatly reduced in the " crossed " localized reactions.

In the localized reactions no correlation could be detected between the severity of the skin reaction and the number of thrombi found in the liver and elsewhere, although severe reactions were sometimes accompanied by massive thrombus formation.

In the lungs, moderate to large numbers of thrombi were encountered in the generalized and localized reactions as well as in the " crossed " generalized reactions, while very scanty thrombi were present in the " crossed " localized reactions. Massive pulmonary involvement was never associated with minimal hepatic involvement although the converse occasionally occurred.

Renal involvement occurred in generalized reactions produced by both endotoxins separately. Eight out of a total of 44 animals developed renal lesions, while of 12 animals in which " crossed " generalized reactions were produced, 6 developed renal lesions. Statistical analysis showed these findings to indicate that " crossing " the endotoxins increased the incidence of renal involvement.

On the other hand, only one of 12 animals, in which localized reactions were produced, developed renal lesions and of 12 in which " crossed " localized reactions were produced, none showed renal involvement.

Apart from the one rabbit which developed bilateral renal cortical necrosis with thrombosis 7 days after a second intravenous injection of endotoxin A, renal

involvement always occurred in the presence of moderate or massive hepatic involvement.

Splenic involvement was unusual in the generalized and localized reactions and in " crossed " localized reactions, but was markedly increased in the " crossed " generalized variety, the same applying to a lesser extent to the suprarenals.

DISCUSSION

Sanarelli (1924) described haemorrhagic lesions in the intestines and kidneys of rabbits receiving an intravenous injection of cholera organisms, followed, 24 hr. later, by a similar injection of a filtrate of either *Esch. coli* or *Bacillus proteus*.

This phenomenon has become known as the generalized Shwartzman reaction because of certain similarities between it and the Shwartzman phenomenon of local tissue reactivity (Shwartzman, 1928, 1937).

This so-called generalized Shwartzman reaction has been reproduced using a variety of bacterial agents either alone or in combination with non-bacterial substances. Thomas and Good (1952*a*) used two intravenous injections of a meningococcal filtrate, while Thomas, Denny and Floyd (1953) preceded an intravenous injection of this by one of group A haemolytic streptococci. Other bacterial agents have included the endotoxins of *Serratia marcescens* (McKay and Shapiro, 1958 ; Graber, Tumbusch, Rudnicki and Vogel, 1960) and *Esch. coli* (McCluskey, Zweifach, Autopol, Benacerraf and Nagler, 1960).

Brunson, Davis and Thomas (1955) followed the intravenous injection of meningococcal filtrate by one of a high molecular weight acidic polymer, while Thomas and Good preceded the injection of this filtrate by the administration of cortisone (1952*b*), trypan blue or Thorotrast (1952*c*).

In the generalized Shwartzman reaction the material deposited in small vessels is fibrin derived from the circulating blood, but the phenomenon of local tissue reactivity or localized Shwartzman reaction, as originally described, did not include systemic thrombi.

Thus the production of thrombi in the localized reactions herein described may be due to the relatively large intradermal dose, enough endotoxin escaping from the injection site into the general circulation to act as preparer for the generalized reaction. This possibility is strengthened by the absence of thrombi in rabbits in which localized reactions were produced (McCluskey *et al.*, 1960), using smaller doses of one of the lipopolysaccharides of *Esch. coli* employed in these experiments. The localized reactions herein described are therefore modified generalized ones in which the preparing dose has been reduced.

While the localized Shwartzman reaction may be one of local tissue reactivity or sensitivity, the use of the terms preparer and provoker in connection with the generalized reaction need not imply that the latter has a basis of tissue sensitivity in the strict immunological sense. This is strengthened by the use of apparently antigenically inert substances in conjunction with bacterial derivatives in the production of the generalized reaction. These two types of substances sometimes have common properties, Stetson (1951) demonstrating that meningococcal filtrate and agar both produce clumping of leucocytes *in vitro* and leucopenia *in vivo*, the latter effect also occurring with the endotoxin of *Serratia marcescens* (McKay and Shapiro, 1958).

The greater number of thrombi formed after the second intravenous injection

compared with the first and the parallel increases in the coagulability of the blood (McKay and Shapiro, 1958) may be due to a purely cumulative effect and the endothelial damage demonstrated by Pappas *et al.* (1958), to the direct action of endotoxin.

The severe glomerular and tubular changes, without fibrin deposition, seen in the kidneys of one animal after 3 days as well as the small foci of acute necrosis, sometimes seen in the liver and heart, not obviously related to thrombus deposition, shows that endotoxins, in the quantities used in these experiments, are capable of damaging endothelial and parenchymatous cells.

The formation of fibrin thrombi in animals receiving such substances as cortisone (Thomas and Good, 1952*b*), Thorotrast and trypan blue (Thomas and Good, 1952*c*) followed by the intravenous injection of meningococcal filtrate may be due to inhibition or blockage of the reticulo-endothelial system, possibly depressing anti-coagulant factors in the face of increased coagulability and endothelial damage, a process which may form the basis of the generalized reaction.

The production of this reaction in a rabbit population known to be infested with coccidiosis raises the possibility that this condition may act as preparer. The presence of only very occasional thrombi in livers of rabbits after one intravenous injection, indicates that mild chronic coccidiosis does not act in this way to any significant extent, if at all.

However, the finding of moderate numbers of thrombi in the liver of one animal with acute hepatic coccidiosis, after an intradermal injection, and of another with severe chronic coccidiosis with liver necrosis after one intravenous injection, shows that these degrees of this infestation may act as preparer possibly because of the accompanying inflammation and tissue damage rather than the presence of the parasites themselves. The absence of thrombi elsewhere in these animals shows that the preparation is confined to the liver and that an organ may be prepared by the presence of active disease. There was no evidence that coccidiosis with severe hepatic necrosis appreciably altered the number of thrombi in the liver and elsewhere.

The presence of thrombi in central and hepatic veins and sinusoids of the liver but not normally in portal veins or hepatic arteries, as well as the involvement of glomeruli before renal arteries, suggests that the thrombi are formed primarily in capillaries, venules and veins, probably on damaged endothelium, and do not result from embolization, such as occurs following the intravenous injection of thromboplastin (McLetchie, 1952).

The greater frequency of thrombi in pulmonary vessels in the lower than in the upper lobes shows that haemodynamic factors play a part in determining the site of their formation, as does their predilection for capillaries, venules and veins rather than for arteries. Consequently the majority of the small unidentifiable pulmonary vessels containing thrombi are probably veins or venules and the arterial involvement limited to the small number of thrombi, embolic from the liver, seen in recognizable pulmonary arteries. The finding of thrombi in the portal veins of an occasional scarred liver suggests that structural alterations in an organ may influence the site of thrombus formation.

The higher incidence of renal and splenic thrombi in " crossed " generalized reactions than in any other, and the great reduction in or absence of thrombi in the liver, lungs and kidneys in the " crossed " and " uncrossed " localized reactions, shows that the overall pattern of thrombus deposition among the various organs

can, to a certain extent, be altered by varying the dose of the endotoxins and the combinations in which they are administered.

The apparent development of the covering endothelial cells from adherent white corpuscles and the absence of cellular reaction both around and within the walls of pulmonary arteries containing organizing thrombi is further evidence that the cells responsible for endothelialization and organization are partly of haematogeneous origin (Dible, 1958).

The failure of venous fibrin thrombi to organize in contrast to the organization of pulmonary arterial emboli, the latter surrounded by red and white corpuscles, confirms previous evidence (Filshie and Scott, 1958) that red corpuscles are necessary for organization and the speed of organization of the emboli may be due to the incorporation of the white corpuscles responsible for this process within the thrombi.

The progressive reduction in the number of thrombi after 24 hr. indicates that the majority are rapidly destroyed but the exact mechanism of this is not clear. It is feasible that fibrinolytic substances, possibly elaborated by the reticulo-endothelial system and vascular endothelium, are partly responsible. In this connection it is of interest that a single intravenous injection of meningococcal filtrate can produce numerous thrombi in the presence of blockage of the reticulo-endothelial system (Thomas and Good, 1952*b* and *c*).

The disintegration of thrombi, their almost complete failure to organize and the absence of any changes which obviously result from their presence indicates that, under normal conditions at least, the deposition of fibrin thrombi does not lead directly or indirectly to chronic morphological changes in the organs concerned.

SUMMARY

The generalized and localized Shwartzman reactions were produced in rabbits, using two endotoxins of *Esch. coli*, and the thrombotic sequelae studied to discover whether the deposition of fibrin thrombi in small vessels might be the basis of some disease processes.

The nature of the generalized reaction was discussed in the light of these and other experiments, and doubt expressed as to its possible immunological basis. The conclusion was reached that the reaction may incorporate increases in blood coagulability, vascular damage and reticulo-endothelial blockage, the latter resulting in inhibition of fibrinolytic activity.

The presence of active disease in an organ predisposed the latter to thrombus deposition. The site of thrombus formation in an organ was affected by haemodynamic factors and alterations in internal structure.

The number of thrombi in certain organs and their distribution amongst the various organs was altered by varying the dosages of endotoxins and the combinations in which they were administered.

Further evidence was produced of the probable haematogenous origin of the cells responsible for the endothelialization and organization of the thrombi. The speed with which the latter disappeared raised the possibility of massive fibrinolytic activity, particularly in view of the part played by the reticulo-endothelial system in the production of the thrombi.

Although the endotoxins themselves produced endothelial and parenchymatous damage, there was no evidence that under normal conditions the deposition of

fibrin thrombi themselves resulted in any chronic morbid anatomical changes in the organs concerned.

This study was carried out under a research grant from the Endowment Fund of the Royal Free Hospital, to whom I am indebted. My thanks are also due to Dr. N. Crowley for valuable criticism and to Mrs. H. Glenn for her technical assistance.

REFERENCES

BOOTH, E., MUIRHEAD, E. E. AND MONTGOMERY, P. O'B.—(1956) *Arch. Path.* (*Lab. Med.*), **61**, 169.
BRUNSON, J. G., DAVIS, R. L. AND THOMAS, L.—(1955) *Amer. J. Path.*, **31**, 669.
DIBLE, J. H.—(1958) *J. Path. Bact.*, **75**, 1.
FILSHIE, I. AND SCOTT, G. B. D.—(1958) *Ibid.*, **76**, 71.
GERBER, I. E.—(1936) *Arch. Path.* (*Lab. Med.*), **21**, 776.
GOOD, R. A. AND THOMAS, L.—(1953) *J. exp. Med.*, **97**, 871.
GRABER, C. D., TUMBUSCH, W. T., RUDNICKI, R. T. AND VOGEL, E. H.—(1960) *Surg. Gynec. Obst.*, **110**, 443.
HARRISON, C. V.—(1948) *J. Path. Bact.*, **60**, 289.
HEARD, B. E.—(1952) *Ibid.*, **64**, 13.
KLIMAN, A. AND MCKAY, D. G.—(1958) *Arch. Path.* (*Lab. Med.*), **66**, 715
MCCLUSKEY, R. T., ZWEIFACH, B. W., AUTOPOL, W., BENACERRAF, B. AND NAGLER, A. L.—(1961) *Amer. J. Path.*, **37**, 245.
MCKAY, D. G. AND SHAPIRO, S. S.—(1958) *J. exp. Med.*, **107**, 353.
MCLETCHIE, N. G. B.—(1952) *Amer. J. Path.*, **28**, 413.
PAPPAS, G. D., ROSS, M. H. AND THOMAS, L.—(1958) *J. exp. Med.*, **107**, 333.
SANARELLI, G.—(1924) *Ann. Inst. Pasteur*, **38**, 11.
SHAPIRO, S. S. AND MCKAY, D. G.—(1958) *J. exp. Med.*, **107**, 377.
SCOTT, G. B. D.—(1956) *J. Path. Bact.*, **72**, 543.
SELZER, G. AND PARKER, R. G. F.—(1951) *Amer. J. Path.*, **27**, 885.
SHWARTZMAN, G.—(1928) *Proc. Soc. exp. Biol., N.Y.*, **25**, 560.—(1937) ' The Phenomenon of Local Tissue Reactivity '. New York (Hoeber).
SMETANA, H.—(1933) *Arch. Path.* (*Lab. Med.*), **15**, 175.
STAMMLER, F. W.—(1959) *Amer. J. Path.*, **35**, 1207.
STETSON, C. A.—(1951) *J. exp. Med.*, **93**, 489.
THOMAS, L., DENNY, F. W. AND FLOYD, J.—(1953) *Ibid.*, **73**, 751.
Idem AND GOOD, R. A.—(1952*a*) *Ibid.*, **96**, 605.—(1952*b*) *Ibid.*, **95**, 409.—(1952*c*) *Ibid.*, **96**, 625.