EXPERIMENTAL PLATELET EMBOLISM

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Received for publication December 20, 1961

IT has long been known that injury to blood vessels may result in the production of a white thrombus in the vessel lumen and that under certain circumstances the white thrombus may break off and travel along the vessel as an embolus (Florey, 1925). Emboli of similar appearance have been seen occasionally in the pial arteries of experimental animals during observations on cerebral blood-flow, and it has been suggested (Denny-Brown, 1960) that the emboli arise from damaged areas in the larger cerebral arteries.

The following experiments were undertaken to determine under what circumstances thrombi, and consequent emboli, could be produced by vascular injury and to determine the composition and behaviour of the thrombi.

MATERIALS AND METHODS

Observations on the cortical vessels were made in adult rabbits and in white rats. The anaesthetics used were intravenous Nembutal (30 mg. per kg.) or intraperitoneal 25 per cent Urethane (6–8 ml. per kg.). After a midline longitudinal skin incision over the sagittal suture, the skin, muscles and periosteum were reflected laterally. A trephine opening was made in the right parietal region about 3 mm. from the midline, and a rongeur was used to extend the exposure rostrally. When completed the parietal window was oval and, in the rabbit, measured 3 cm. in its long axis. During craniotomy, bleeding from diploic vessels was stemmed by smearing plasticine into the trephine cut. After removal of the bone, the dura was excised radially and reflected, and from this time and throughout the experiment the cortical surface was bathed continuously with warm saline. The surface blood vessels, illuminated by a reflected light using a green filter at the light source, were observed through a Leitz binocular microscope (magnification $\times 52$). Respiratory movements, which tended to hinder observation and measurement of vessels, were diminished by raising the head of the animal and supporting it in a clamp. Photographs were taken with an Endixa single-lens reflex camera through the objective lens of the dissecting microscope. Additional illumination was provided by electronic flash at a distance of 6 in. from the cortex (1/800 sec.). Ektachrome high-speed film was used.

Mesenteric vessels.—Observations were made on adult rabbits, guinea-pigs and pigeons. On two occasions young rabbits, previously starved for 48 hr., were used. The peritoneal cavity was opened by a mid-line incision and a loop of small intestine delivered. The mesentery was laid over a D-shaped sheet of clear perspex,

to which it was secured with small pegs. The vessels were illuminated by transmitted light and were viewed through a dissecting microscope. The bowel was covered with gauze packs and the mesentery was bathed throughout the experiment by warmed saline. In adult animals, but not in young animals, a quantity of fat usually invests the vessels and hinders visibility. The fat was incised directly over the vessels and reflected to each side.

Normal appearances

Cortex.—A rich network of veins and arteries spreads over the pink background of the cortex. About two-thirds of the area exposed through the parietal window is supplied from below by branches of the middle cerebral artery and the remainder from above by branches of the anterior cerebral. Anastomoses between the small arteries are frequent. The largest cortical arteries measure about 300 μ in diameter and are pulsatile. After branching and reaching a size of about 10–20 μ they disappear by dipping down abruptly into the cortex. The veins, which are much more numerous, are easily distinguishable by being larger and darker in colour, having a more sinuous course and a flatter contour. One or two of the larger veins appear to be filled partially with arterial blood and when two veins join to form a larger vein, the streams from the two tributaries remain distinct. The movement of the blood corpuscles can be discerned in all vessels, but most easily in the smaller veins.

Mesentery.—The arteries running in the mesentery are larger than those of the cortex (usual size 250–350 μ) with fewer branches and a straighter course. On reaching the attachment of mesentery to intestine the arteries break up into smaller branches running under the serous coat, and at about size 50 μ they disappear from view by entering the outer muscular layer.

Reaction of normal cortical vessels to mechanical injury (rabbit)

When a cortical artery is nipped gently between the ends of needle forceps and then released, no bleeding occurs, but local contraction lasting 1–5 min. may follow. Later the injured area is visible as a slight fusiform swelling. If the injury is more forceful a small tear may occur in the vessel wall and bleeding ensues. At the same time the artery usually contracts vigorously in the area of the injury, the lumen being reduced to about a quarter of normal. Localized contraction lasts for periods ranging from 1 to 5 min., depending on the size of the artery and the extent of the injury. Bleeding from the injured vessels, often profuse and pulsatile at first, gradually ceases and as it does so a white plug can be seen accumulating on the inside and on the outside of the vessel at the point of injury. In the present study, the shortest interval between injury and appearance of a plug was 5 sec. The inner plug grows across the vessel until it completely fills the lumen, when it is shaken violently by the arterial pulsation. During formation small pieces may be detached and carried rapidly downstream ; sometimes a stream of white material resembling a snow-plume may be seen leaving the injured area ; usually, however, the plug remains intact while growing, and finally obstructs the vessel. At this time the whole or part of the plug usually begins to move distally down the vessel from the original area of injury towards the next bifurcation. Alternatively, the plug may remain occluding the vessel for some minutes before being disturbed. The plug completely fills the vessel and obstructs the flow of blood.

Distal to the plug the blood may be darker in colour and the column of stagnant blood may show clumping of red cells.

The embolus may be followed down the artery to the point where it impacts at a bifurcation, sitting astride the junction and appearing to bulge the arterial wall a little. There is no evidence of spasm. After a time the embolus disappears down one or other branch, gradually moulding itself to fill the vessel, or breaking into two. There is no increase in size after the embolus leaves the site of injury ; in fact, it tends to become smaller as pieces break off and are swept away downstream. It finally disappears from view at the point where the small arteries turn downwards into the cortex, or it may sometimes be seen to disintegrate into a great many small white pieces (Fig. 1).

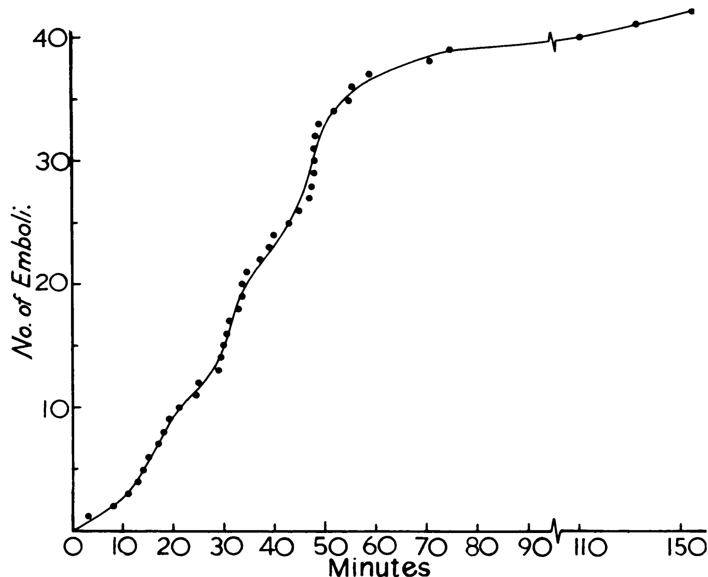


FIG. 2.—Time sequence of emboli produced by a single injury to a cortical artery in a rabbit. The vessel was observed continuously for 150 min.

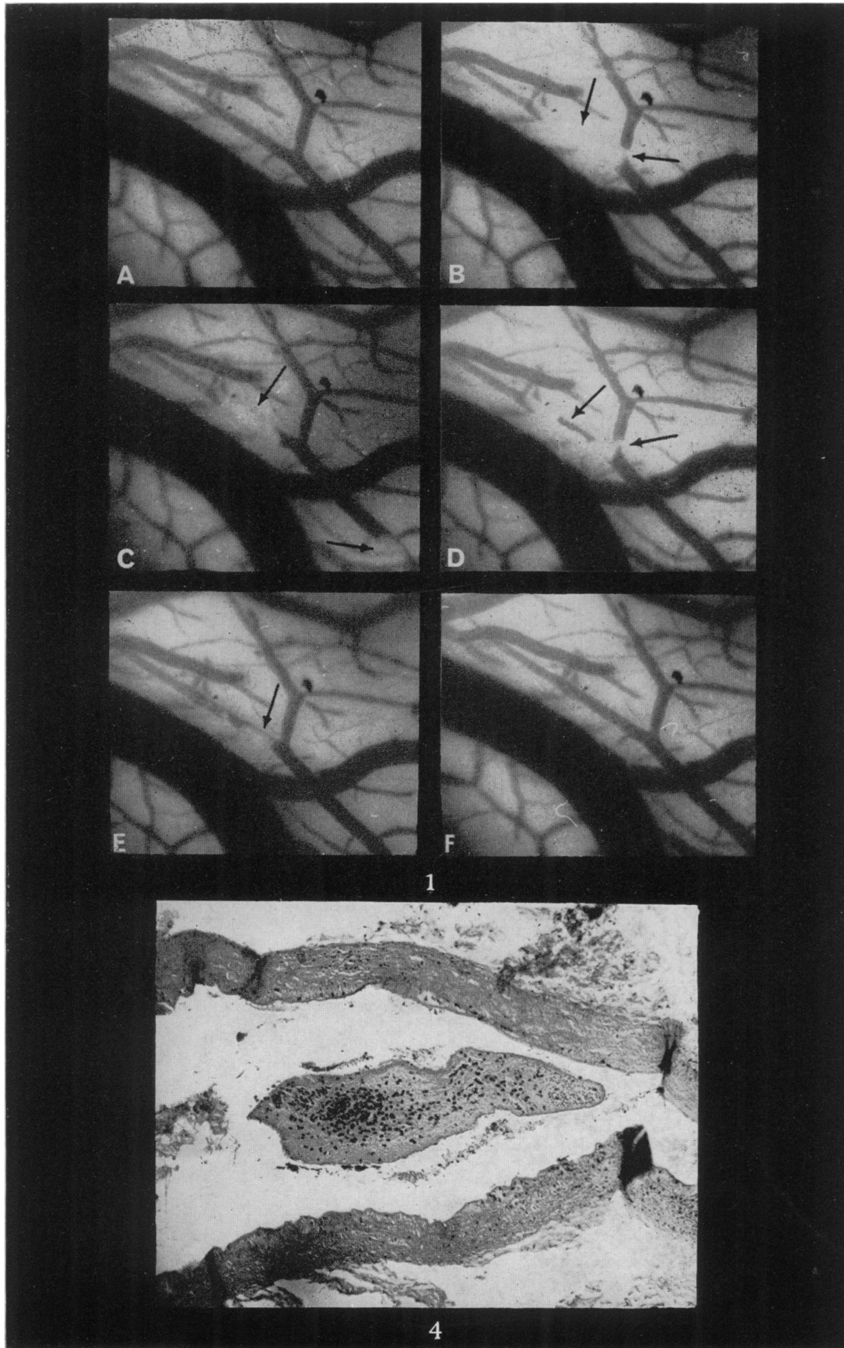
Slowing of the blood flow in the regional veins may be seen while an artery is occluded, but no white thrombi appear unless the vein itself has been injured.

As soon as one embolus has broken off from the injured vessel wall, more white material accumulates at the same site and grows into another vessel plug, finally filling the vessel and breaking off as before. A constant succession of emboli may be produced in this way over the course of 2–3 hr. by a single injury (Fig. 2 and 3). Finally, production of emboli ceases ; a small white plaque may remain at the original point of injury or the vessel wall may appear quite normal. The emboli

EXPLANATION OF PLATE

FIG. 1.—This figure illustrates the impaction of platelet emboli, at the bifurcation of a cortical artery, and subsequent break-up (rabbit). Emboli indicated by arrows, approximate time between photographs 30 sec. Blood flow diagonally from right to left. ×23.

FIG. 4.—Section of damaged right common carotid artery showing necrosis of all coats with loss of cell outline. Eosinophilic mass with enmeshed polymorphs seen in lumen adjacent to damaged intima. ×30.



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from the same area of injury frequently take a stereotyped course into the same small branches. Two or more emboli may accumulate in a small branch and coalesce to form a larger body ; they may remain discrete, separated by a small segment of stagnant blood which is darker in colour than the arterial blood.

In the mesenteric arteries of the rabbit, the formation and embolization of white thrombi after injury follow a similar pattern to that observed in cortical vessels.

In the mesenteric arteries of the other species examined (guinea-pig, rat and pigeon) although a white plug is formed at the site of injury and grows until the

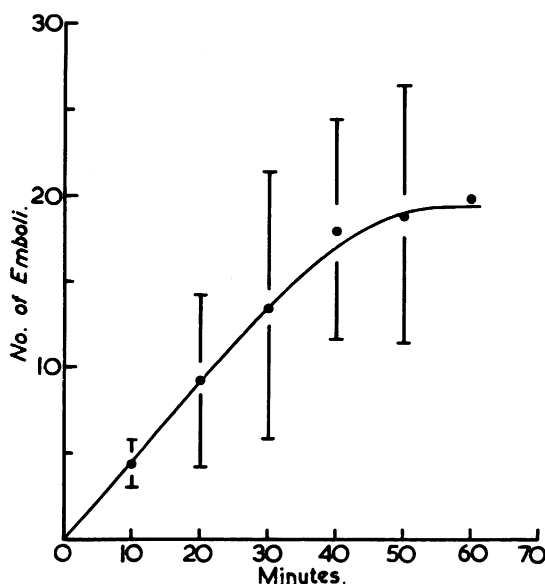

FIG. 3.—Number of emboli produced in 10 separate control experiments (cortical artery of a rabbit). Closed circles represent the means with one SD plotted.

lumen is occluded, there is less tendency for the whole plug to detach as an embolus. Instead small fragments break off from the main mass and are carried rapidly out of the field of observation without impacting at a vessel junction.

Effect of various stimuli (rabbit cortex)

Observations were made on the effectiveness of the various forms of stimuli on the artery in producing white thrombi.

(a) Pinching the artery gently between the ends of a pair of needle forceps so that no bleeding occurred was almost always ineffective, although local contraction was frequent. Repeated applications of gentle pinching in the same spot occasionally produced a small number of thrombi which embolized before reaching a size large enough to block the vessel.

(b) Partially cutting the artery with a razor-blade produced profuse haemorrhage followed regularly by the deposition of a white plug in and outside the vessel. Puncturing the artery with a fine needle had a similar effect.

(c) Complete section of the cortical artery with a razor-blade produced rapid constriction of the two ends, with shortening of the vessel. Profuse initial haemorrhage occurred, but was decreased by the annular constriction and finally stopped by the formation of a white plug at the cut end of the artery, partly in the lumen and partly sprouting from the cut end. Ligation of a cortical vessel with fine silk thread or traction on a vessel was ineffective in producing white thrombi, even if the flow was entirely stopped.

(d) A thermal injury was applied to a cortical artery by touching it lightly with a fine silver diathermy point. The temperature at the end of the electrode (80°) was sufficient when touched lightly on human forearm skin to give a local red reaction followed by a wheal and a flare. Local spasm lasting 5 min. occurred in the cortical arteries. In the 15 min. after the stimulus three white emboli were produced in the artery and several (10–15) smaller emboli also appeared in adjacent veins. White material appeared to encroach on the arterial lumen concentrically from all parts of the intimal surface rather than from a localised area of injury on the wall. Thirty-five min. after injury both artery and vein appeared normal.

A cold injury was inflicted by direct application of a small flake of dry ice to cortical vessels for repeated periods of 5 sec. A single 5-sec. stimulus was sufficient to produce a wheal and flare on human forearm skin. An artery and a vein were both included in the area of cortex treated and after five applications several small white emboli formed and detached in the vein, but none was seen in the artery which continued to pulsate normally and appeared unaffected.

(e) The cortical arteries and veins were observed during application of chloroform to the cortex. The flow of warm saline was stopped and three drops of chloroform were dropped onto the cortex. Immediately all arteries in the field of observation constricted for about 10 sec. This constriction was followed by vasodilatation and an increase in the number of visible small veins. Within 30 sec. of this application great numbers of white bodies formed on the intimal surface of arteries and veins and quickly embolized. After $2\frac{1}{2}$ min. no further white bodies were produced, but on applying a further 3 drops of chloroform the same process was repeated. Injection of a small quantity of chloroform under a cortical artery produced a great number of white emboli in the artery and in an adjacent vein after 30 sec., and continuing for $7\frac{1}{2}$ min. Some small veins draining the area were almost filled by white material.

In another experiment the right common carotid artery of an anaesthetized rabbit was exposed and dissected free for a length of 2 cm. A segment of this artery 1 cm. long was isolated between two small bulldog clamps and the arterial wall was infiltrated with xylene (0·5 ml.). Clamps were then released and the normal pulsations observed to return to the artery. The right cerebral cortex was exposed 7 min. later and the whole of the cortex under the parietal window was kept under continuous observation for 75 min. (magnification $\times 36$). Nothing abnormal was seen for 33 min., but at that time a large white embolus appeared in a large branch of the middle cerebral artery, slowly breaking up as it progressed through the smaller branches. Over the next 35 min. three more large emboli were seen in the branches of the middle cerebral artery. Apart from their large size, the appearance and behaviour of these emboli were identical to those produced by injuring the cortical vessels. At the end of this time the animal was killed. Histological examination of the damaged carotid removed immediately after death showed necrosis of all coats of the artery, with loss of cell outline and nuclear

staining. In the lumen, adjacent to the damaged intima, there was an eosinophilic mass with a number of enmeshed polymorphs (Fig. 4).

This experiment was repeated in another rabbit, using chloroform (0·5 ml.) instead of xylene for the carotid infiltration. Twelve emboli were seen in cortical branches of the middle cerebral artery during the next 25 min. of observation.

Alterations in coagulation

(a) *Heparin.*—Two rabbits of similar size (2·5 kg.) were anaesthetized and craniotomy performed. One animal received 7,000 units of Heparin intravenously after the dura was opened. The other received a similar volume of normal saline intravenously (0·7 ml.). The observers were unaware which animal received

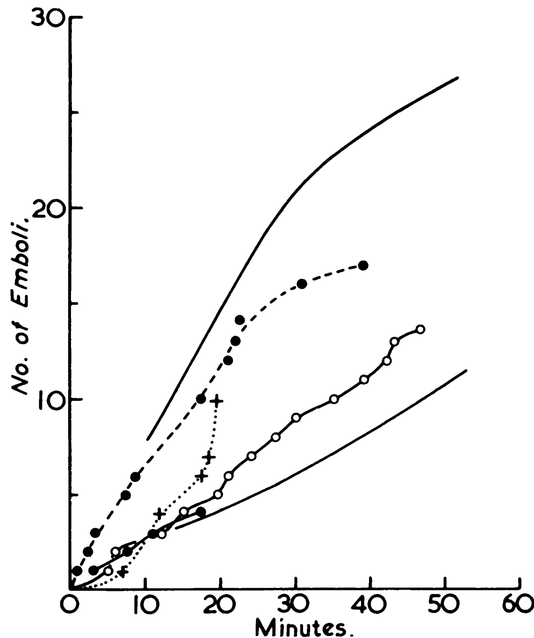

FIG. 5.—Effect of Heparin on production of emboli from a single injury (four experiments). SD of control animals indicated by continuous lines. Cortical arteries, dose of Heparin 7000 I.U.

Heparin. Clotting time was estimated after 30 min. by Dale and Laidlaw's (1911) method on blood from an ear vein. In the case of the control animal the clotting time was 2 min. 30 sec., and in the Heparin-treated animal no coagulation was evident after 30 min. No white bodies were observed in the vessels of either animal before injury or immediately after injection. In the two animals, cortical arteries of similar size were injured by nipping with forceps, haemorrhage occurring on both occasions. In the control animal 14 white emboli were observed to form and break away during 45 min. of observation. In the Heparin-treated animal 10 emboli were produced in 30 min. (Fig. 5). The behaviour of the emboli was similar in the two animals, although the size of emboli was consistently less in the Heparin-treated animal.

In another experiment, observations on a Heparin-treated rabbit (7000 units

intravenously) were continued for $2\frac{1}{2}$ hr., a fresh injury being inflicted every 30 min. to different cortical arteries. The formation and propagation of emboli continued unchanged throughout the period of observation, although clotting time by Dale and Laidlaw's method was again prolonged (more than 24 min. throughout the experiment). The results of 4 separate experiments with Heparin are given in Fig. 5.

In another experiment a guinea-pig (weight 1010 g.) was used and observations made on the mesenteric vessels. Three arteries were injured by nipping and a white plug formed quickly in each in the usual manner. It grew across the artery and occluded the lumen and a few small pieces broke off and travelled up the vessel as emboli. The whole plug did not embolise. Heparin (5000 units) was given intravenously and a further injury inflicted to another artery 5 min. later. This produced a platelet plug which grew and embolized in an exactly similar fashion to those formed before administration of Heparin.

(b) *Experimental thrombocytopenia.*—Observations were made on one rabbit before and after treatment with intravenous " agar-serum " (Bedson, 1922) to reduce the number of circulating platelets. The " agar-serum " was prepared by mixing 8 ml. of rabbit serum with 2 ml. 0·5 per cent agar in normal saline, incubating at 37° for 2 hr., standing overnight and spinning off the agar.

After exposure of the cortex a medium sized artery was injured with forceps. Five emboli were produced in the course of 12 min. A blood sample from an ear vein at this time contained 260,000 and 250,000 platelets per c.mm. (direct count after lysis of red cells with ammonium oxalate).

Twenty minutes after the initial injury, 5·5 ml. of " agar-serum " mixture was injected intravenously without change in the general condition of the animal. The injured artery was observed continuously for 64 min. and during that time 13 further emboli detached themselves. Another similar injury was then inflicted and 15 emboli were produced in a further 82 min. of continuous observation. The platelet count fell to 150,000/c.mm. 1 hr. after the " agar-serum " injection, and thereafter rose to 200,000/c.mm. after 90 min.

(c) *Russell's viper venom.*—In a large rabbit (3·45 kg.) clotting times were measured on blood from an ear artery (in plain tubes times were 6 min. 45 sec. and 7 min. 15 sec. ; in silicone-coated tubes 21 min. 30 sec. and 23 min.). The method of estimating clotting times in this experiment was that of Lee and White (1913), using both plain and silicone-coated tubes. With the latter tubes, minor coagulation defects may become much more obvious. Platelet count was 332,000/c.mm. (direct count).

The cortex was then exposed and a cortical artery injured. Six white plugs formed and embolised in 20 min. of observation. 4·5 mg. of Russell viper venom were then administered subcutaneously in a number of depots in both flanks. The formation and propagation of white emboli in the cortical vessels continued as before. Eighteen minutes after venom injection, the clotting time on arterial blood was 2 min. (silicone-coated tube only) and 170 min. after injection the clotting time (plain tube and silicone-coated tube) was less than 2 min. The number of platelets was unaltered (325,000/c.mm.).

The cortical vessels were observed for 170 min. after injection and two further arteries were injured. The formation of plugs in the vessel and the rate of embolus formation seemed normal. In one instance 4 emboli were produced in 10 min. immediately after a vessel injury.

From 2–2½ hr. after injury, however, several white emboli were seen in arteries which had not been injured.

After 3½ hr. the animal was killed. No macroscopic thrombi were visible at post mortem and there was no evidence of infarction of any organ. On section of the pulmonary artery, a small white amorphous mass surrounded by a few polymorphs was seen apparently floating freely in the pulmonary artery blood.

Identification of the emboli

(a) *Methods*.—The white thrombi and emboli were examined histologically in three ways :—

(1) The white plug forming on the outside of the injured vessel was seized with needle forceps and smeared between two coverslips, one of which had previously been coated with cresyl blue and allowed to dry. The preparation was examined immediately.

(2) The site of injury with a vessel plug *in situ* was fixed by pouring 10 per cent formol saline, cooled by dry ice, directly on to the cortex. At the same moment the animal was killed by cutting the abdominal aorta. As soon as the animal was dead a small section of cortex, together with the overlying vessels containing the plug was excised and placed in cold formol saline. The preparation after fixation was blocked in paraffin, sectioned, and stained. The same technique was used to obtain sections of an embolus which had impacted at an arterial bifurcation. Cooling of the brain surface with cold formalin just before death was found to stop the slow flow of blood in the cortical vessels which otherwise continued for some minutes after death and which displaced the emboli. In the mesentery the segment of artery containing the plug was isolated by placing two silver clips, one on each side of the plug. The vessel, still filled with blood, was removed together with the clips and placed in formol saline. After fixation the clips were removed and the vessel was sectioned and stained as before.

In preparing specimens for electron microscopy the mesenteric vessels were used. Care was taken to obtain material only from a recent injury (after 2–3 min.). The vessel was removed between clips and placed on a piece of cardboard in 2–3 drops of ice-cold fixative and immediately cut into a series of cubes about 1 mm. square. These were put into freshly prepared ice-cold osmic acid or potassium permanganate fixative.

(b) *Results*.—In smear preparations examined with 1/12 objective the white thrombus had a spongy consistency. No red or white blood cells were identified, but a large number of small granules taking up the cresyl blue stain could be seen scattered irregularly throughout the main substance. In a dried smear stained by Wright's method, the granules appeared dark red.

In fixed preparations of the injured vessel stained with haematoxylin and eosin, the plug was a pink mass with the same reticular structure, with Mallory's phosphotungstic acid haematoxylin a number of black granules were visible. It occupied most of the lumen and extended outside the vessel through a break in the endothelium (Fig. 6). Occasional leucocytes and red blood cells were trapped in the mass and a collection of polymorphonuclear leucocytes were present in the blood adjacent to the thrombus. In transverse sections of an artery containing an impacted embolus, the lumen was entirely occupied by the white body (Fig. 7).

The preparation and examination of the vessel plugs by electron microscopy

was carried out by Dr. R. Barer, Department of Human Anatomy, University of Oxford. Further studies are in progress, but we are indebted to him for a preliminary report. The bulk of the white body was seen clearly to be composed of platelets packed tightly together. No processes or pseudopodia were visible joining the cells. Many of the platelets contained dark granules, but in some areas granules were absent and cell outlines were indistinct. Occasional red cells were included in the mass, but no strands or connective material resembling fibrin could be identified either in the mass or at the edges of the plug connecting it to the intima (Fig. 8). Some of the endothelial cells at the area of vessel injury were distorted and in places the basement membrane was exposed.

Fresh sections of white thrombus were examined histochemically by Dr. S. Bradbury, Department of Human Anatomy, University of Oxford. The refractive index of the body was 1·53–1·54 and the granules had a lower index, about 1·5–1·52. The body stained blue with mercury bromphenol blue, suggesting protein components. There was no detectable ribonucleic acid, pyronin-methyl green giving a faint pink colour unchanged after ribonuclease digestion. Slight positive reaction with PAS and alcian blue and positive metachromasy indicated some acidic mucopolysaccharide. The diazo test for argentaffin substance was weakly positive in the granules.

DISCUSSION

The formation of white plugs in injured vessels was studied more than a century ago by Jones (1850) in the web of the frog. He gave a lucid description of the formation, growth and detachment of granulomatous substance at the site of mechanical or chemical injury. The substance, thought to be composed of colourless corpuscles agglomerated by tenacious matter, or possibly by fibrin, was seen to break up and pass through the capillaries. Virchow (1863) considered that the white masses he observed in blood vessels were composed of white blood cells, disintegrating fibrin and red blood cells which had lost their haemoglobin, and it was Bizzozero (1882) and Hayem (1882) who first suggested that the white thrombi were formed entirely of blood platelets with only a few white blood cells.

The formation of a platelet plug is a fundamental feature of haemostasis. In some invertebrates (*Limulus polyphemus*) aggregation of amoebocytes is the only haemostatic mechanism and no fibrin is present in the blood. In birds and amphibians, where the spindle cells are nucleated and much larger than mammalian platelets, a white vessel plug forms in a manner similar to mammals and a mass of these cells can be seen round the point of injury (Fig. 9). In all species cellular plugging precedes coagulation (Silberberg, 1938).

In man, clumps of platelets have been shown in damaged arterioles when a fresh puncture wound is excised and examined histologically (Zucker, 1949), and it seems likely that platelet plugging is the most important factor in the immediate arrest of haemorrhage from small vessels or in the bleeding time used

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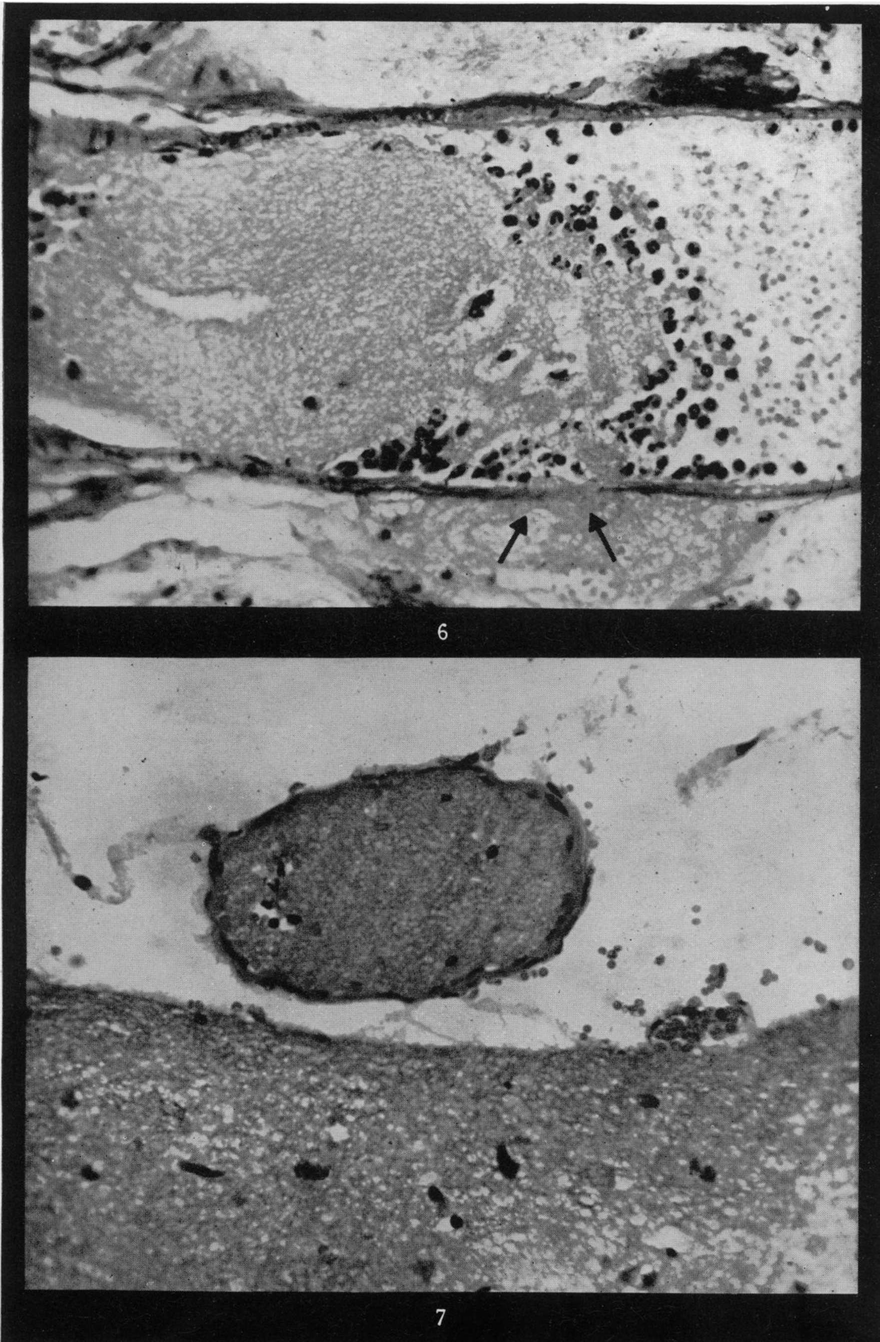
EXPLANATION OF PLATES

Fig. 6.—Formation of a platelet plug in a cortical artery of a rabbit. Note extension through arterial wall at point of injury indicated by an arrow. ×150.

Fig. 7.—Cross section of a cortical artery of a rabbit containing platelet embolus. Approximately 5 min. after impaction. ×125.

Fig. 8.—Electronmicrograph of a platelet thrombus in a mesenteric artery of a rabbit. ×9,000.

Fig. 9.—Mesenteric artery of a pigeon showing aggregation of nucleated thrombocytes around point of injury. Site of injury indicated by an arrow. ×175.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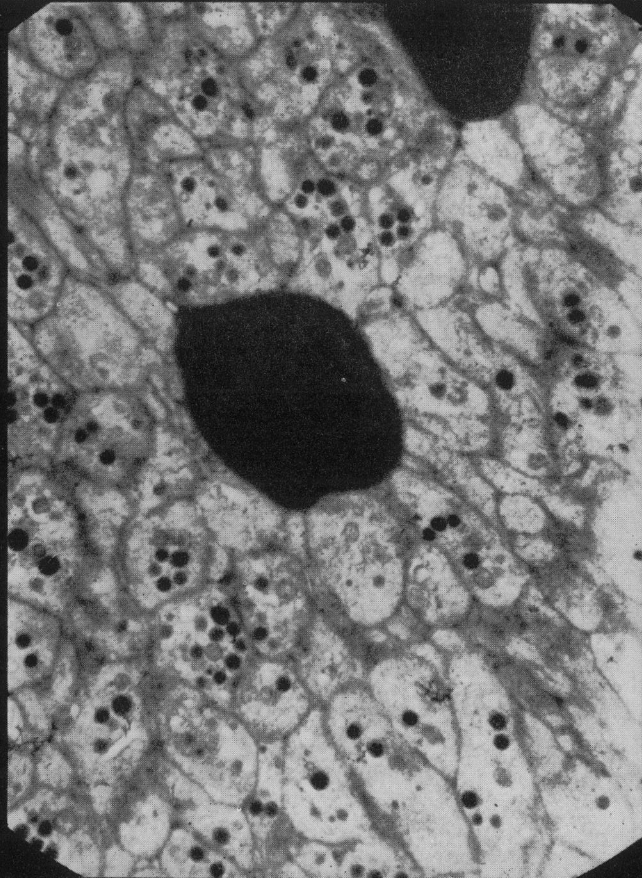

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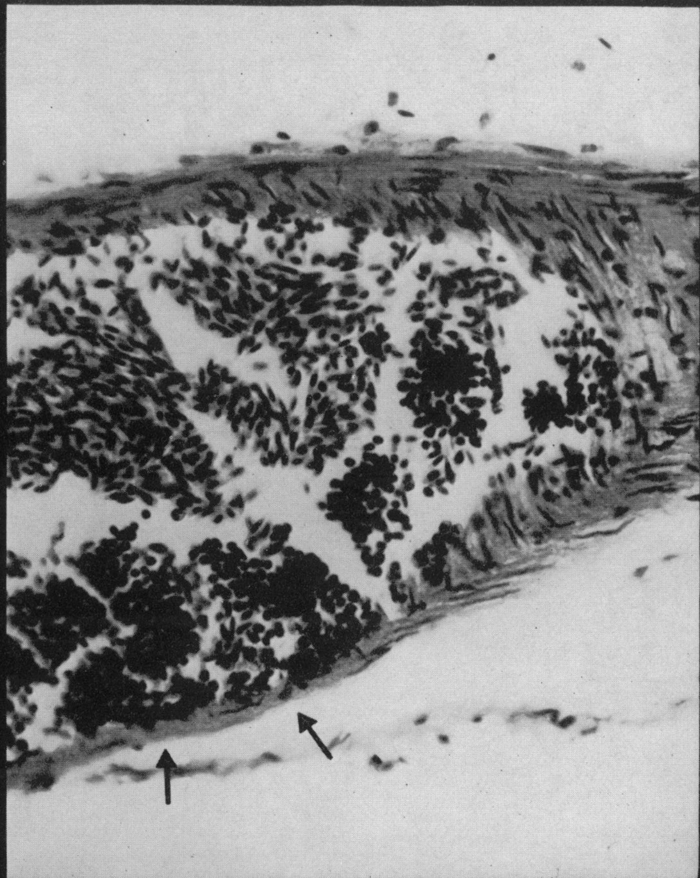

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in clinical work. Thus patients with prolonged clotting times due to congenital or acquired defects in coagulation factors, or with afibrinogenaemia, usually have a normal bleeding time (Pinniger and Prunty, 1946 ; Borschgrevink and Waaler, 1958), and only a slight prolongation of bleeding time can be demonstrated after heparin treatment at normal therapeutic levels (Borschgrevink and Waaler, 1958). Study of the formation of white thrombi in glass arterio-venous shunts has shown that the platelets adhere to a scratch or surface irregularity in the same way as to a damaged vessel, and that this behaviour is again unaffected by treatment by heparin or dicoumarol in ordinary doses (Best, Cowan and MacLean, 1938 ; Solandt and Best, 1940 ; Zucker, 1947).

On the other hand direct observations of platelet aggregation in platelet-rich plasma and measurement of platelet adhesiveness to glass beads *in vitro* have clearly shown the influence of coagulation factors, a red cell factor, and calcium ions (Sharp, 1958; Bounameaux, 1955; Hellem, 1960), but it is uncertain how far these results can be applied to platelet behaviour *in vivo*. Morphological alterations in the platelets began immediately after shedding, the first change being from an oval to a dendritic stage when a number of fine processes radiate from the centre. The processes are gradually withdrawn as the granules tend to aggregate in the middle of the cell in the transitional stage and finally the resting or enlarged stage is reached when the hyalomere is much spread out. The granules of the chromomere are grouped together to form a pseudonucleus (Bessis and Burnstein, 1948). At this stage the platelets tend to aggregate.

The present study shows that platelet aggregation begins a few seconds after injury, certainly before fibrin could have time to form. It also appears that the white thrombi, as far as can be discerned by light and electron microscopy, are composed entirely of platelets without admixture of fibrin and that the majority of the platelets are in the resting stage. The emboli tend to fragment and pass through the capillaries and it is not known whether normal platelets are reconstituted, although this seems unlikely.

The factors influencing adhesion of platelets to the injured vessel wall *in vivo* have received comparatively little attention. An early hypothesis (Loeb, 1906) was that the platelets undergo a physical or chemical change in the ectoplasmic layer as a result of mechanical injury and thereafter become adhesive, but this does not explain the observation that the platelet embolus never increases in size after leaving the injured area. The experiment of Samuels and Webster (1952) on chemical and mechanical injury to veins showed that the platelet thrombi tended to occur on the intercellular lines between the endothelial cells, but this cannot be the result of an adhesive exudate since aggregation was also induced by a scratch on a glass tube in the experiments of Best *et al.* The theory that platelet adhesion may be induced by fibrin (Wright, 1945) or by thrombin and other cofactors (Bounameaux, 1958) fails to explain the short interval between injury and plug formation. The fact that platelet emboli do not increase in size after leaving the injured area, shows that the stimulus to platelet aggregation is a disturbance originating at a site of vascular injury, propagated through the mass as it forms and ceasing when the plug is detached. Injury to the intima appeared to be the important factor in initiating the clumping process. The only occasion on which platelet emboli were produced without vessel injury was after the administration of viper venom and this may possibly have been due to general toxic injury to the endothelium.

Although not directly concerned with platelets, the experiments of Sawyer, Pate and Wilden (1953) may be relevant. They showed that arterial intima is polarised negatively with respect to adventitia and that when the polarity was locally reversed a thrombus formed at this site. When two external electrodes were used, the thrombi formed only on the wall under the positive electrode. The thrombi were said histologically to resemble naturally occurring thrombi. They also showed that after crushing or transecting the vessel, the intima changed from negative to positive with respect to adventitia, but they failed to show reversal of polarity after slighter injury.

Washed platelets are said to carry a negative charge and migrate to the positive pole of an electrophoretic cell (Bigelow and Desforges, 1952). A local area of positively charged intima might provide the correct physical or chemical environment and might be the natural stimulus for aggregation. In the experiments of O'Brien (1961) platelet adhesion to glass beads or to a layer of amnion cells were found to be inhibited by drugs which may form a monolayer on a negatively charged surface.

The phenomenon of platelet embolism may have important clinical implications. Increasing use of arteriography in man has shown that temporary or permanent ischaemic symptoms in the limbs or in the brain may be associated with obliterative disease in large proximal arteries, while the small arteries nearer the affected part may appear to be patent. Intermittent symptoms are usually ascribed to a temporary failure of collateral supply due to a fall in systemic blood pressure, but could also be due to repeated embolism from the damaged area, the emboli producing only a temporary block and finally disappearing without trace. In the present experiments numerous emboli were seen in cortical arteries after a chemical injury to the common carotid artery in the neck. An embolus producing transient local ischaemia would be most likely to produce symptoms in tissue which are easily damaged by anoxia and which have a poor collateral blood supply. Thus an embolus passing through the central retinal artery and causing temporary blindness might well pass unnoticed through a muscular artery. Recent observations on the retinal circulation in patients with carotid disease have suggested that friable emboli may be passing through the arteries during the attacks of visual loss (Fisher, 1959). These emboli are similar in appearance and behaviour to those produced experimentally in the cerebral vessel of the rabbit and there is some evidence that they are also composed of platelets (Russell, 1961). Should platelet embolism of a degree sufficient to cause clinical symptoms prove to be a common accompaniment of or prelude to arterial thrombosis, more attention will need to be paid in diagnosis to alterations in platelet numbers and adhesiveness and in treatment to agents capable of affecting platelet aggregation. Under the conditions of the present experiments in rabbits no definite action could be attributed to heparin in the production and embolism of white thrombi although the dose was considerably in excess of normal therapeutic levels in man. The number of emboli produced by arterial injury after heparin was within normal limits although the emboli appeared to be smaller and possibly more friable. The action of heparin on platelet aggregation may, however, be subject to considerable species variation (Best *et al.*, 1938) and these results do not rule out the possible therapeutic action of heparin in man.

SUMMARY

Preparations of arteries have been examined in the cerebral cortex of the rabbit and rat, and in the mesentery of the rabbit, guinea-pig and pigeon. Their response to various stimuli has been studied.

Pinching with needle forceps, burning, cooling, application of irritant substances all resulted in the formation of a white plug in the lumen of the vessel at the site of intimal injury. The interval between injury and the appearance of the plug was 5 sec. to several minutes. In cortical arteries a stream of thrombi formed over a period of 150 min. and broke off either in pieces or as a whole, to form emboli.

The rate of thrombus formation and embolization from it was not affected by pre-treatment of the animal with Heparin or Versene or by reducing the platelet concentration in the circulating blood by one half. The administration of Russell's viper venom reduced the clotting time, but did not alter the response of the vessels to stimulation, although it may have induced spontaneous thrombus formation in other arteries.

Microscopical examination of a thrombus showed an eosinophilic mass containing many small granules staining with Cresyl Blue. Only occasional white blood cells and red cells were seen trapped in the mass. Electronmicroscopy showed the body to be composed almost entirely of platelets tightly packed together with no processes or pseudopodia visible joining the cells.

The mechanism of the observed aggregation of platelets is discussed and attention is drawn to the possibility of a similar pathological basis to some clinical conditions of transient ischaemia.

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