J. Physiol. (1948) 107, 280–288 612.116.21

THE MECHANISM OF HAEMOSTASIS IN PERIPHERAL VESSELS

By TING I. CHEN and CHIAO TSAI

From the Institute of Physiology, National Central University, Nanking, China

(*Received* 4 *August* 1947)

As early as 1897, Bier called attention to the importance of activity of the severed blood vessels in haemostasis. Küttner & Baruch (1920) described segmental spasm in the traumatized large artery, in consequence of which the injured free end became pulseless. Magnus (1923) reported that in the amputated limb the free ends of the large arteries became completely constricted and did not leak blood when the ligature was removed from the proximal stump. Stegemann (1924*a*, *b*), using the pancreas and mesentery of the living rabbit for observation, concluded that clotting played only a subordinate role in the spontaneous cessation of haemorrhage. Tannenberg & Herrmann (1927) thought that the retraction and contraction of the vessel wall were the chief factors in the spontaneous stoppage of bleeding caused by cutting the vessel. Chen & Tsai (1944) concluded from a series of experiments on the arteries of the rabbit's ear, brain and mesentery that the haemostasis of arterial haemorrhage consisted of two phases, an initial constriction phase and a subsequent coagulation phase. They pointed out that, without the initial constriction, the arrest of bleeding by coagulation was almost impossible because of the high pressure in the artery.

As to the haemostasis in the capillary, most authors think that constriction is the main factor. By means of micro-incision, Magnus (1924) found that the capillaries in the frog's foot and in human skin disappeared completely and remained invisible for a considerable time after cutting. He concluded that capillary haemorrhage was checked by the activity of the capillary itself. Heimberger (1926) observed contraction of human skin capillaries in response to blunt pressure and reported that occlusion may last for a day. He believed this reaction to be due to the contraction of the pericytes. MacFarlane (1941) studied the response of the capillaries of the human finger-nail bed, and revealed that the capillary loop underwent constriction in response to puncturing.

When investigating haemostasis in veins, Hayem (1882) observed the formation of a mass of platelets in the incised jugular vein of the dog and believed it to be important in checking the bleeding. Recently Zucker (1947) re-emphasized the significance of the formation of a platelet plug in venous haemostasis. She described the phenomenon in the venules of the meso-appendix and large branches of mesenteric veins of rats as follows: 'Within one minute a colorless refractile body about 6 μ. in diameter could be seen at the tip of the bleeding stump, but not within the lumen. Subsequent experiment demonstrated the body was formed of platelets.'

METHODS

For the study of arterial haemostasis the vessels of the intact ear and of the exposed brain and mesentery of the rabbit and those of the frog's web and mesentery were used. In the experiments with brain and mesenteric arteries of the rabbit the animals were anaesthetized with chloral hydrate (0·6 g./kg./rectum). The vessels were observed under a low-power microscope (× 48 or 80). A small cutting needle, held by a fine clamp and moved by ball-socket joints, was used for puncturing. The needle was replaced by a fine glass rod with a blunt end when pressure was to be applied. The arteries and arterioles chosen for this study were mostly 0·05–0·5 mm. in diameter.

In order to determine whether the constriction was neurogenic or myogenic in origin, the ear vessels were denervated under local narcosis (procain) by extirpating the superior cervical ganglion and resecting the great and dorsal auricular nerves according to the method of LeCompte (1941). The reaction of the vessels to mechanical injury was tested 2–3 weeks after the operation. Complete degeneration was verified by intra-vitam staining with methylene blue according to the method of Clark, Clark & Williams (1934).

The effect of hypocoagulability of blood was studied in rabbits by giving dicumarol (20 mg./kg./day for 4 days) orally or heparin (880 units/kg.) by intravenous injection prior to observation.

Similar methods were employed for the study of the marginal ear vein of the rabbit and mesenteric veins of the rabbit and frog. In many cases it was necessary to wash the blood off the wound. This was done by applying a tiny stream of warm saline solution.

For experiments with capillaries, we used anaesthetized rabbits, spinal frogs and toads, and toads or frogs whose spinal cords had been previously completely destroyed. Capillaries of 7–35 μ. in diameter were chosen. They were observed under the microscope with a magnification of 480 and punctured by a fine needle with a tip of about 3–5 μ. in diameter. A fine glass rod was employed for applying pressure. The needle or the rod was carried by a small clamp and operated through a series of ball-socket joints.

RESULTS

Arterial haemostasis

Puncture of the rabbit's ear at any spot by a sharp cutting needle may cause a transient blanching of the whole ear lasting 10–30 sec. This is purely a reflex phenomenon since it did not occur in the denervated ear.

Hard pressure on the central artery or on any of its branches by a fine glass rod caused a local constriction lasting 2–6 min. (Pl. 1, fig. 1). Puncture of the artery with a fine cutting needle brought forth a similar but stronger local constriction. The segment disappeared from sight within 30–90 sec. of the injury (Pl. 1, fig. 2). As a consequence of this strong local constriction, bleeding

usually stopped spontaneously within 1·5 min. The contracted vessel began to relax 8–15 min. later, but did not regain its former size until 10–20 min. afterwards. The blood was usually washed off by a fine stream of warm saline, but if the clot was allowed to remain on the spot, the constriction usually persisted longer, sometimes for nearly an hour. The constriction usually spread for 0·2–3·3 mm. in both directions from the point of injury.

Occasionally, dilatation supervened for a time during the period of constriction and the vessel appeared to undergo some rhythmic contraction which was again followed by uninterrupted constriction. If the transient dilatation occurred within 2·5–3 min. after the injury, momentary bleeding again ensued. Nevertheless, if it took place after the clot was already very firm, no bleeding was noticed.

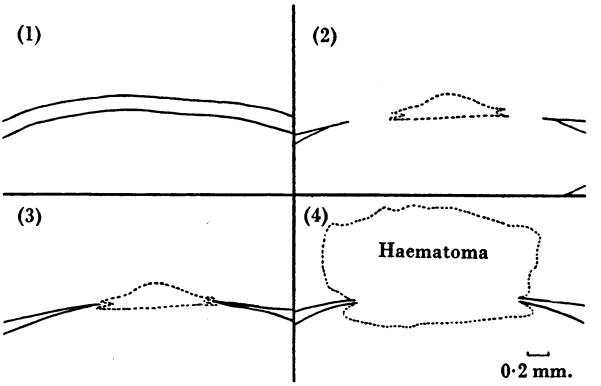
The above-described local response of the artery to mechanical stimulus also occurred in the denervated artery with no apparent delay in onset, no shortening in the duration of response and no diminution in the intensity of constriction. Since the vessels studied were all found by intra-vitam staining with methylene blue to be devoid of nerves it was concluded that the local response of the artery must be myogenic in origin.

To ascertain the relationship between blood coagulation and haemostasis in an artery, five heparinized and three dicumarolized rabbits were employed. Despite the fact that the coagulation time in the heparinized rabbits was increased to between 30 and 120 min., no serious and prolonged bleeding occurred in the artery after puncturing. On the other hand, in the dicumarolized rabbits whose blood was shown to be incoagulable for 2 hr., bleeding was so severe that it inevitably caused the death of the animal. In these animals a puncture of the artery invariably evoked a strong local constriction as in the other rabbits, which checked the immediate bleeding. However, as soon as the constriction passed off, bleeding again took place (Text-fig. 1). This continued till death, if no extraneous measures were taken to prevent it.

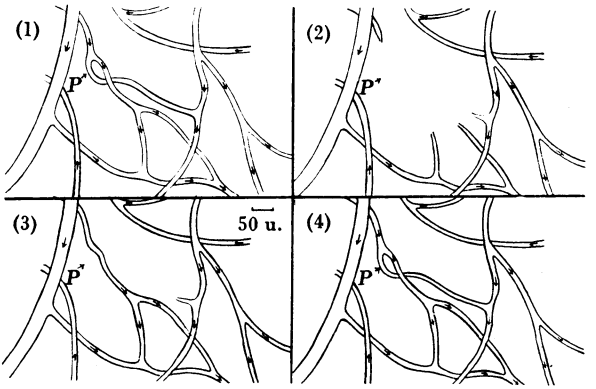
The mesenteric and brain arteries of the rabbit behaved exactly like those in the ear.

Capillary haemostasis

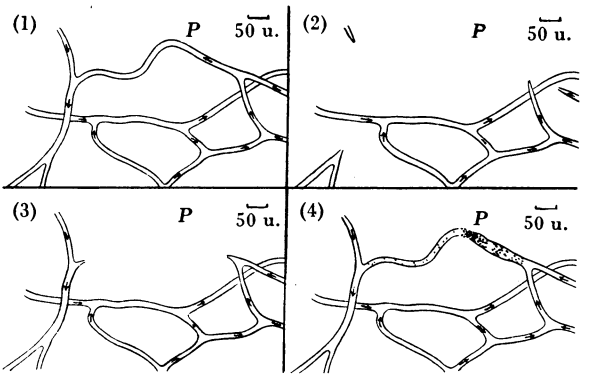
The question of capillary contractility is still in dispute. According to our own experience it differs according to species and situation. In the frog's web the capillary is contractile, whereas in the mesentery of the frog and rabbit it is not. In the former, the contraction in response to mechanical pressure or puncture usually involved a segment from 140 to 500 μ. in length (Text-fig. 2). But this was never observed in the mesenteric capillaries of the rabbit, toad and frog. In these non-contractile capillaries it was observed that parts of the capillary wall adhered to each other at the point of pressure or puncture. In the contractile capillaries adhesion also occurred but could not be seen clearly until the constriction phase waned (Text-fig. 3).



Text-fig. 1. Camera lucida tracings of the ear arteries of a dicumarol-fed rabbit after puncture. (1) Before puncturing; (2) 30 sec. after; (3) 15 min. after; (4) 18 min. after.



Text-fig. 2. Camera lucida tracings showing simple constriction of the frog's web capillaries after light pressure. Arrows indicate the direction of blood flow; *P*, point of pressing. (1) Before pressing; (2) immediately after; (3) 15 sec. after; (4) 30 sec. after.



Text-fig. 3. Camera lucida tracings showing the simultaneous occurrence of simple constriction and adhesion in the capillaries of frog's web after puncture. Arrows indicate the direction of blood flow; *P*, the point of puncturing. Adhesion is most obvious in (4). (1) Before puncturing; (2) immediately after; (3) 20 sec. after; (4) 3 min. after.

The adhesion usually took place immediately after the stimulus. It may occur at the centre (Pl. 1, fig. 3) or in the margin of the vessel (Pl. 1, fig. 4), or may occupy the entire cross-section of the vessel (Pl. 1, fig. 5) in accordance with the location and size of the injury. Some formed elements of the blood, especially the platelets, may then adhere to it from time to time. The adhesion of the wall sealed up the wound and the action was so quick that no bleeding could possibly occur. If the capillary was closed by adhesion, blood would accumulate and cause distension on both sides. The adhesion persisted for several hours.

Adhesion is easily distinguished from contraction because the former is strictly local, confined to the point of injury, and in fact not infrequently produces a negative model of the needle point, whereas the latter spreads for some distance. In order to induce bleeding in the capillaries it was necessary not to withdraw the needle but to tilt it so as to separate the walls. If bleeding occurred, it usually stopped within 30–60 sec.

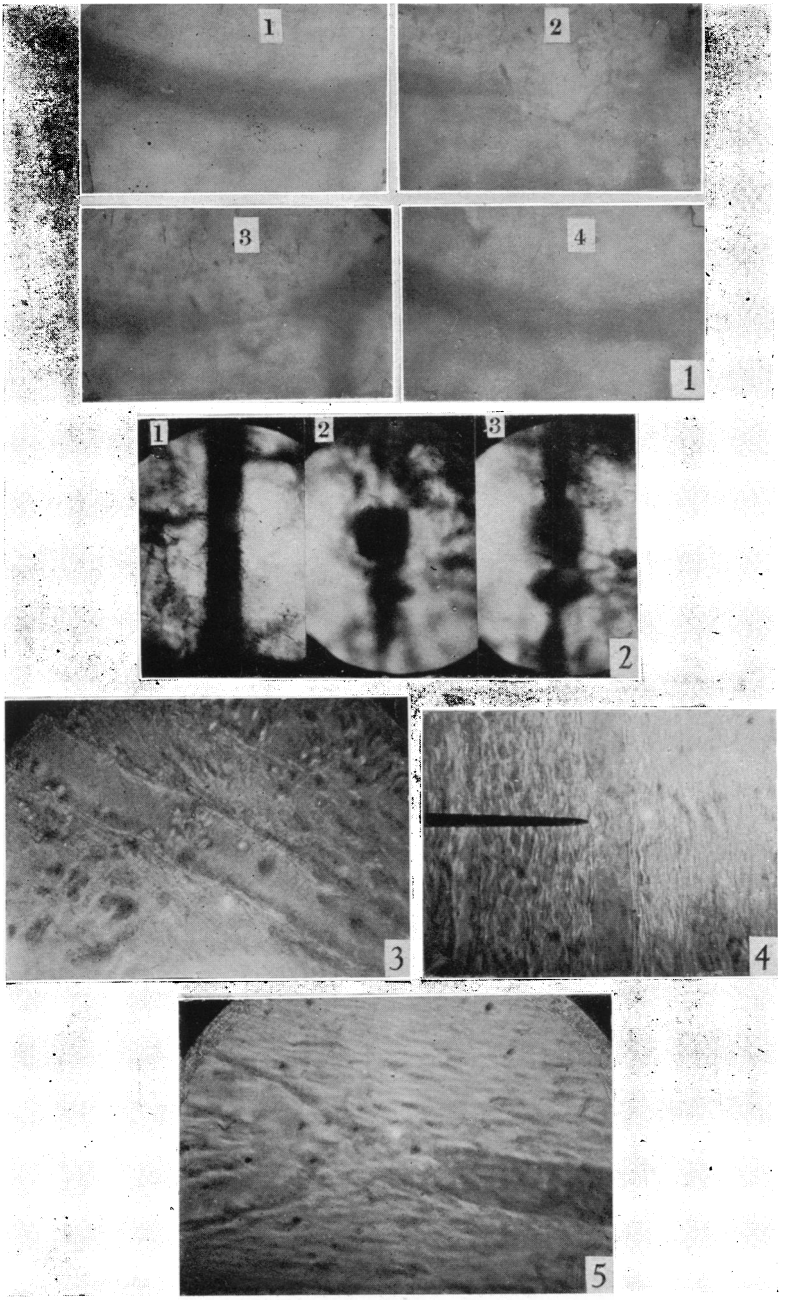
Stretching of the mesentery of the frog and rabbit so as to tease some of the vessels caused very little bleeding. Microscopic examination of the teased vessels revealed the fact that practically all the capillaries and veins were closed by endothelial adhesion at their free ends.

Venous haemostasis

The vein was punctured by a round needle and the blood which exuded was washed away by a fine stream of warm saline solution. It was found that, in most cases, bleeding ceased within 1·5–3 min. The arrest of bleeding was due to the formation of a plug inside the vein. The plug was small at first and then enlarged, until finally a large refractile body was seen lying on the surface of the wound (Pl. 2, fig. 1). If the mass outside the vein was carefully removed by micro-dissection and washing as soon as it was formed, bleeding stopped as usual, i.e. within 1·5–3 min. This was due to the plug found within the vein just below the point of puncture. Microscopic examination showed that the plug consisted of platelets.

If the size of the plug was not large enough to obstruct the entire lumen, it was subsequently fragmented and washed away by the blood stream (Pl. 2, fig. 2). On several occasions we have actually seen fragments carried away by the blood stream towards the heart. The whole plug usually disappeared within 25 min. to 7 hr. On the other hand, if the plug was so large as to obstruct the entire lumen, a small canal through the plug was usually developed within 24–36 hr. The canal, through which the blood flowed, gradually enlarged until finally the plug vanished. The whole process took about 3–4 days for its completion (Pl. 2, fig. 3).

The adhesive property of the capillary, reported above, was also demonstrated in the veins. Pressure on the mesenteric venules of the frog and the auricular venules of the rabbit with a fine glass rod or a blunt needle produced



Figs. 1-5.

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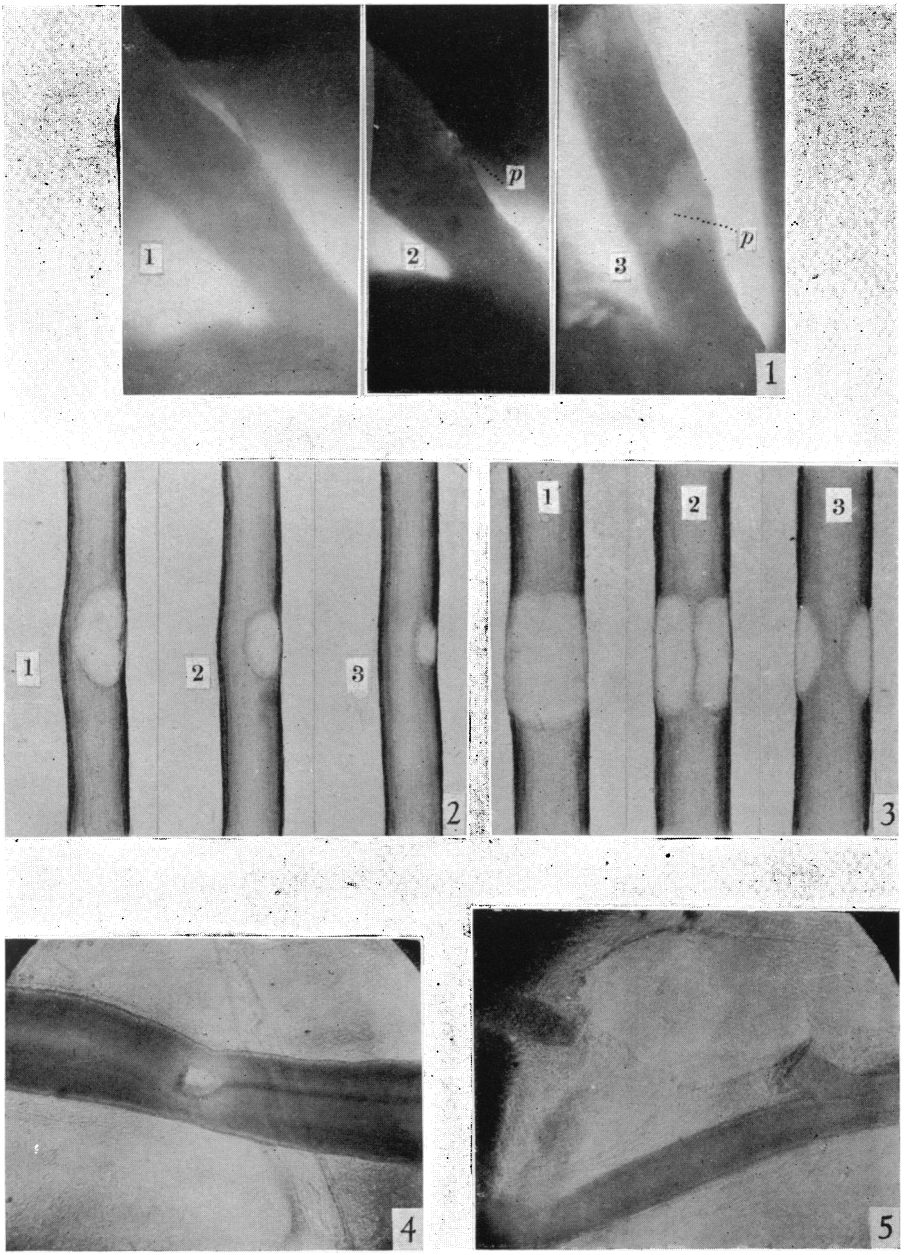

Figs. 1–5.

adhesion of the walls (Pl. 2, fig. 4). The venule can be distinguished from the capillary by its thicker wall. It is a common experience that cutting the frog's skin with a pair of scissors usually causes very little or no bleeding. Careful examination of the cut stumps of the veins disclosed the fact that the cut ends were sealed by the adhesion of the vessel walls. Pl. 2, fig. 5 shows closure by adhesion at both cut ends of a branch of the mesenteric vein of the frog. There was very little bleeding after cutting.

DISCUSSION

From the observations described above it is evident that arterial haemostasis consists of two phases, an initial constriction and a permanent coagulation. Without the initial constriction of the vessel the formation of a firm clot, strong enough to support the pressure in the artery, would be difficult or even impossible.

The constriction may be analysed into three components. The reflexogenic component is immediate and lasts for 30 sec. at most. It is usually followed by dilatation. It plays only a small part in haemostasis since it is too brief for the formation of a firm clot. The reflexogenic constriction overlaps the initial part of the myogenic constriction. However, the latter outlasts the former for a considerable period and does not vanish until some time after the clot has been well formed. If bleeding occurs and the clot is not washed away from the wound, the constriction may persist much longer; in several cases it lasted nearly an hour. The prolonged constriction in these cases cannot be merely due to the reaction evoked by mechanical stimulation because mere pressing or puncturing the vessel without allowing the clot to remain on the spot induces constriction with much shorter duration. According to the work of Janeway, Richardson & Park (1918), Tsai, McBride & Zucker (1944) and others, the platelets contain a very potent vaso-constrictor substance which is liberated into the serum when the blood coagulates. It is, therefore, conceivable that the later phase of prolonged constriction as a result of clotting may be due to the action of the vaso-constrictor substance liberated from the disintegrated platelets.

The importance of constriction in arterial haemostasis is clear from the second bleeding which occurs in the dicumarolized animal after the constriction has passed off. In other words, the constriction phase, though it cannot stop bleeding permanently, is necessary for the formation of a firm clot.

The absence of the second bleeding in the heparinized rabbit is difficult to explain. It may be due to neutralization of heparin by cephalin at the site of injury, but this is merely a conjecture.

Previous workers all considered capillary contraction as the chief mechanism of capillary haemostasis (Magnus, 1924; MacFarlane, 1941; and others). But according to our present investigation, emphasis must be laid on the significance of the adhesive property of the capillary endothelium. In the first place,

contraction of the capillary does not always follow mechanical stimulation or injury. For instance, in the frog's web some capillaries do contract in response to mechanical stimuli, but the contraction never lasts more than 30 sec., while many others do not contract at all. Furthermore, in the mesentery of the frog and rabbit none of the capillaries shows any contractility after mechanical stimulation. On the other hand, adhesion of the capillary wall is the most constant reaction observed. It occurs at the point of puncture or pressure and persists for as long as an hour, in which period the walls rarely separate spontaneously. In the contractile capillaries the walls adhere together in the same way, but this adhesion cannot be seen clearly until the contraction passes off. From this we may conclude that the chief mechanism of capillary haemostasis is the adhesion of the vessel's walls, contraction being unimportant. The adhesive property of the capillary endothelium is manifested only when the cells are injured and pressed against each other. This assumption is deemed necessary, otherwise one would have to demonstrate adhesion after constriction, which however never happens.

As the phenomenon of capillary adhesion after mechanical injury is so constant and persistent, it is difficult to explain why it has escaped the attention of previous workers, unless it was mistaken for capillary contraction. According to our own experience, capillary contraction and adhesion are two distinct phenomena easily distinguished from one another under our experimental conditions. In the first place, adhesion is local and persistent, while contraction spreads along the vessel and is transient.

We agree with Zucker (1947) that the formation of a platelet plug is the chief mechanism in the venous haemostasis. But we wish to add that in the venules the adhesion of walls as a result of injury is also important in stopping bleeding. The endothelium of the large vein may also possess this property, but, because of its wider lumen, a small point of adhesion can easily be broken through and the walls again separated by the flow of blood. Therefore, in large veins adhesion plays no essential role in haemostasis.

It should be pointed out that most of the veins are responsive to mechanical stimulation and injury. But the constriction is too mild and too brief to have any value in haemostasis.

The formation of a platelet plug in the injured vein is exceedingly interesting in view of its relation to haemostasis and thrombosis. It is well known that the platelet possesses an adhesive property and will attach to any rough surface with which it comes into contact. On account of the more rapid flow and higher pressure in the artery any platelet adhering to the damaged lining would tend to be washed away by the blood stream, thus leaving no opportunity for the platelets to accumulate and form a plug on the spot. On the other hand, the slow stream in the vein favours the adhesion and accumulation of platelets at the point of injury.

The mechanism of fragmentation and recanalization of the platelet plug is not clear. It may be due to the disintegration of the platelets or fibrinolytic action of the serum tryptase. Whether recanalization is due to the invasion of endothelial growth has not been studied in the present investigation.

The adhesive property of the capillary and venule may be an important factor in determining bleeding time. Its loss may result in the prolongation of bleeding time without affecting coagulation time. The clinical bearing of this point should be investigated.

SUMMARY

1. Arterial haemostasis consists of two phases, constriction and coagulation. The constriction evoked by mechanical injury may be composed of three elements: (1) reflex constriction, (2) local muscular response, and (3) response to chemical substances liberated from the disintegrated platelets. The local muscular response which is the most important is sufficient to stop bleeding till the formation of a firm clot. Reaction to vaso-constrictor substances would come later. Permanent arrest of haemorrhage depends upon the formation of a firm clot but the retention of a firm clot in place would be impossible without the initial constriction.

2. The mechanism of capillary haemostasis is mainly adhesion of the capillary wall as a consequence of injury of the endothelium. Adhesion causes a persistent closure of the vessel. Capillary contractility may be present in some capillaries such as those in frog's web, but it is insignificant in haemostasis because of its inconsistency and brief duration.

3. The formation of a platelet plug and adhesion are the two important mechanisms for haemostasis in veins but adhesion in large veins is rarely effective in stopping haemorrhage. Venous constriction plays a minor role in haemostasis.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Photographs showing constriction of a rabbit's ear artery after heavy pressure by a blunt needle. ×18. (1) Before pressing; (2) immediately after; (3) 1·5 min. after; (4) 4·5 min. after.

Fig. 2. Photographs showing constriction of a rabbit's ear artery after puncture. ×18. (1) Before puncturing; (2) 1 min. after; (3) 8 min. after.

Fig. 3. A photograph showing central adhesion in a capillary of toad's mesentery produced by a sharp puncture. ×200.

Fig. 4. A photograph showing marginal adhesion in a capillary of toad's mesentery produced by a sharp puncture. ×200.

Fig. 5. Photograph showing complete obstruction by adhesions in a capillary of toad's mesentery produced by several punctures. ×200.

PLATE 2

Fig. 1. Photographs showing the formation of platelet plug in a rabbit's ear vein after a sharp puncture. *p* indicates the platelet plug. ×18. (1) Before puncturing; (2) 2 min. after; (3) 7 min. after.

Fig. 2. Diagrams showing fragmentation of platelet plug in the marginal ear vein of a rabbit. (1) 7 min. after puncturing; (2) 15 min. after; (3) 23 min. after.

Fig. 3. Diagrams showing recanalization of platelet plug in the marginal ear vein of a rabbit. (1) 7 min. after puncturing; (2) 24 hr. after; (3) 48 hr. after.

Fig. 4. Photograph showing adhesion in a vein in frog's mesentery after a sharp puncture. ×30.

Fig. 5. Photograph showing adhesion in a vein in frog's mesentery after cutting by scissors. Note the adhesion at both cut ends. ×30.