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Haemostatic Plugs as a Histological Vital Reaction in the Skin Wounds of Guinea Pigs

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Hämostatische Pfropfen als histologische Vitalreaktion in Hautwunden von Meerschweinchen

Summary: Applying a histological staining method (Carstairs ' modification of picro-Mallory staining), haemostatic plugs could be demonstrated at points of vascular lesion in one-minute vital, or older, skin wounds of the guinea-pig on the average in 33% if the specimen was taken immediately after the reaction period, and in about 10% of wounds examined if the specimen was taken five days after death. No haemostatic plugs could be observed in postmortally inflicted skin wounds.

Zusammenfassung: Unter Verwendung einer histologischen Färbungsmethode (Modifikation von Carstairs der Picro-Mallory-Färbung) konnten haemostatische Pfropfen an den Gefäßläsionsstellen in vitalen Hautwunden von 1 Minute oder älter beim Meerschweinchen in durchschnittlich 33% beobachtet werden, wenn die Probe unmittelbar nach der Reaktionsperiode entnommen war, sowie in etwa 10%, wenn die Probe 5 Tage nach dem Tode entnommen wurde. In postmortal zugefügten Hautwunden konnten keine hämostatischen Pfropfen beobachtet werden.

Key word: Vital reaction, Haemostatic plugs.

Differentiation between an injury sustained during life and a postmortal wound is continuously one of the problems in forensic medicine when the reaction time has been short - for instance, a few minutes or less than an hour after the trauma. In addition to the inflammatory and necrotic phenomena, which have already long been utilized (WALCHER 1930, 1936), enzyme histochemical reactions (RAEKALLIO 1961, 1965) and histamine and serotonine determinations (FAZEKAS and VIRAGOS KIS 1965, RAEKALLIO and MÄKINEN 1966, BERG *et al.* 1968) have furnished useful aid in the problem. In the most recent years even observations by scanning electron microscopy have been made concerning haemostatic phenomena, in the first place as regards thrombocyte and fibrin accumulations. The former were more clearly seen in endothelial lesions of the major blood vessels (BÖHM

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and TSCHOMAKOW 1972), but rather not in cut wounds of the rat skin (BÖHM and TSCHOMAKOW 1973). In the latter, as well as in certain other injuries (BÖHM 1974), differences were observed between vital and postmortal haemostasis, primarily in the structure of fibrin and erythrocyte accumulations. According to other authorities (SCHNEIDER 1974), however, no distinct structural differences between vital and postmortal clots can be demonstrated by scanning electron microscopy. Histological and immunohistochemical methods also failed to reveal a distinct difference between vital and postmortal clots (LAIHO 1967).

Histological observations concerning the contribution of thrombocytes to haemostasis were already made in the last century (HAYEM 1882, LUBNITZKY 1885, EBERTH and SCHIMMELBUSCH 1886) and later in this century (e.g. RIBBERT 1915, MÜLLER 1931, APITZ 1942, ZUCKER 1947, HUGUES 1953, JØRGENSEN and BORCHGREVINK 1963, 1964). It was demonstrated in the studies cited that at the point of the vascular lesion a thrombocyte accumulation frequently forms, and this usually continues even at greater size on the outside of the vessel, into the haematoma. Such thrombocyte accumulations could be noted, in the first place, in vessels of larger size than the capillary level. The studies were mostly hampered by difficulties in finding these thrombocyte accumulations, for instance in lack of an appropriate staining method; as a rule the formations described were so far between that series sectioning was frequently necessary in order to detect them. Apart from the scanning electron microscopy studies already mentioned, to my knowledge no attention has been paid in forensic medicine to histological thrombocyte plugs as vital reactions. In the present work the thrombocyte plugs were histologically studied in vital and postmortal skin wounds, using common paraffin cuts and optic microscopy.

MATERIAL AND METHODS

In preliminary tests several histological staining methods for demonstration of thrombocytes were tried out. For use in the present study the modification by Carstairs of the picro-Mallory staining was chosen. CARSTAIRS (1965) has found this to be a reliable method, e.g. by comparison of the results with those obtained by the immunohistochemical demonstration method.

A total of 54 guinea-pigs were used in the study. After shaving their back, four wounds penetrating the skin and about 1.5 cm long were cut with a scalpel in ether anaesthesia. The wounds were made longitudinally on either side of the central line, two in the cranial and two in the caudal part of the back. One pair of the wounds was excised as a specimen immediately after termination of the reaction period and the other pair five days after the death. The dead animal had been kept at room temperature the intervening time.

The reaction times of vital wounds studied in this work were 1, 2, 5, 10, 15, 30 and 60 minutes and 2, 4, 8, 12, 24 hours, and 3 days. The animals were killed with a blow on the head and specimens of two of the wounds were taken immediately, without waiting for the heart to stop finally. The other two wounds were excised five days after the death to serve as specimens. The number of guinea-pigs whose

wounds were sampled after vital times of 1 to 60 min. was three each and the reaction times from 2 hours to 3 days are represented by two animals each.

The postmortal wounds were induced 1, 2, 5, 15 and 60 min. and 4, 12 and 24 hrs after stopping of heart activity following the killing with a blow on the head. Immediately after the heart action had ceased, the animal was suspended with its back downwards in order to produce hypostasis. After the wounds were cut, the animal was kept suspended on its bakc for one hour in each post mortem experiment, so that hypostatic haemorrhage of the wounds might ensue. Two wounds were then immediately sampled and the other two, five days after death. The studies of postmortal wounds inflicted 1 to 60 min. after death include three guinea-pigs each and those of wounds made after 4 to 24 hrs, two each.

The sampling procedure comprised excision of the wound together with about 1 cm of surrounding skin. The piece of skin with the wound was flattened out on a cardboard base, to which it was affixed by its margins with staples. It was then fixed for two days or longer in common 4% buffered formalin. Upon fixing, the wound with about 0.5 cm of surrounding skin was cut off from the base and embedded in paraffin in usual manner. Starting at the underside of the skin, an average of 15 cuts spaced at 10 µ were taken parallel to the skin surface, whereby the inner wound edges in their entirety were included in the cuts at the said spacing. It is thus understood that the cuts mainly contained material from the lower skin surface over the thickness mentioned, from the boundary against the subcutis, where the vascularisation is more profuse than on the outer surface.

RESULTS

The stainability of the cuts was as described by CARSTAIRS: The thrombocyte accumulations acquired a lead-blue or lead-grey stain, while fibrin and muscle were stained red, erythrocytes yellow, and connective tissue bright blue. However in specimens of short reaction time the young fibrin could also bei stained like thrombocytes and then the differentiation between these components was only morphological. For this reason a name haemostatic plug is used instead of thrombocyte plug concerning the results obtained. In the basal parts of the hair sheaths a greyish homogenous stain was often observed, but this was differentiable by its location and morphology from the haemostatic accumulations in the vascular lumina or in their vicinity. The five-days-old wounds frequently displayed bacterial accumulations, which usually took on a bluish violet stain and could be clearly distinguished from haemostatic accumulations.

Table 1, as well as Figs. 1 to 10, show the occurence of haemostatic plugs in the vital wounds examined. It is thus noted that such plugs could be observed in vital wounds as late as in the specimens taken five days after the death. However, their observation was greatly variable in the specimens examined. If the specimen had been taken immediately after termination of the vital reaction time, haemostatic plugs could be seen in about 33% of all wounds examined. But if the specimen had been taken five days after the death, plugs were only detected in about 10% of the wounds. During the period of greatest interest

Table 1. *Presence of haemostatic plugs in the vital wounds studied*

Reaction time after vital wounding	Number of wounds with haemostatic plugs /Number of wounds examined	
	Specimen taken immediately after reaction period	Sample taken five days post mortem
1 min	3/6	1/6
2	2/6	0/6
5	3/6	1/6
10	4/6	0/6
15	3/6	1/6
30	1/6	0/6
60	2/6	1/6
2 hrs	2/4	0/4
4	0/4	0/4
8	1/4	0/4
12	2/4	2/4
24	0/4	0/4
3 days	0/4	0/4

in forensic medicine, from 1 to 60 min., plugs were observed in the vital wounds in about 43% of the specimens immediately taken, and in about 10% of those taken after five days. The haemostatic plugs occurring in one-minute vital wounds were not morphologically substantially different from those in the wounds which were a few hours old. However, the plugs in the 12-hour vital wounds were already accompanied by a more abundant fibrin accumulation. Particularly on the edges of the plug in the younger plugs, too, a more tenuous fibrin accumulation or network could be seen. Occasionally the plugs also displayed accumulations of polymorphonuclear leukocytes. The blood vessels in connection with which plugs were observed were variable in size, though larger than capillary level. In the 24-hour and three-day vital wounds the inflammatory and tissue repair reactions had already progressed so far that the plugs, if any, could have been obscured by them. Abundant fibrin accumulations could also be seen in these specimens.

No haemostatic plugs could be noted in any of the 84 postmortal wounds examined, which had been caused from 1 min. to 24 hrs after death, in the blood vessels in the specimens or in their vicinity, nor elsewhere either.

DISCUSSION

Of the vital and postmortal wounds examined, only in the former observations of haemostatic plugs could be made. The phenomenon would thus seem to be appropriate

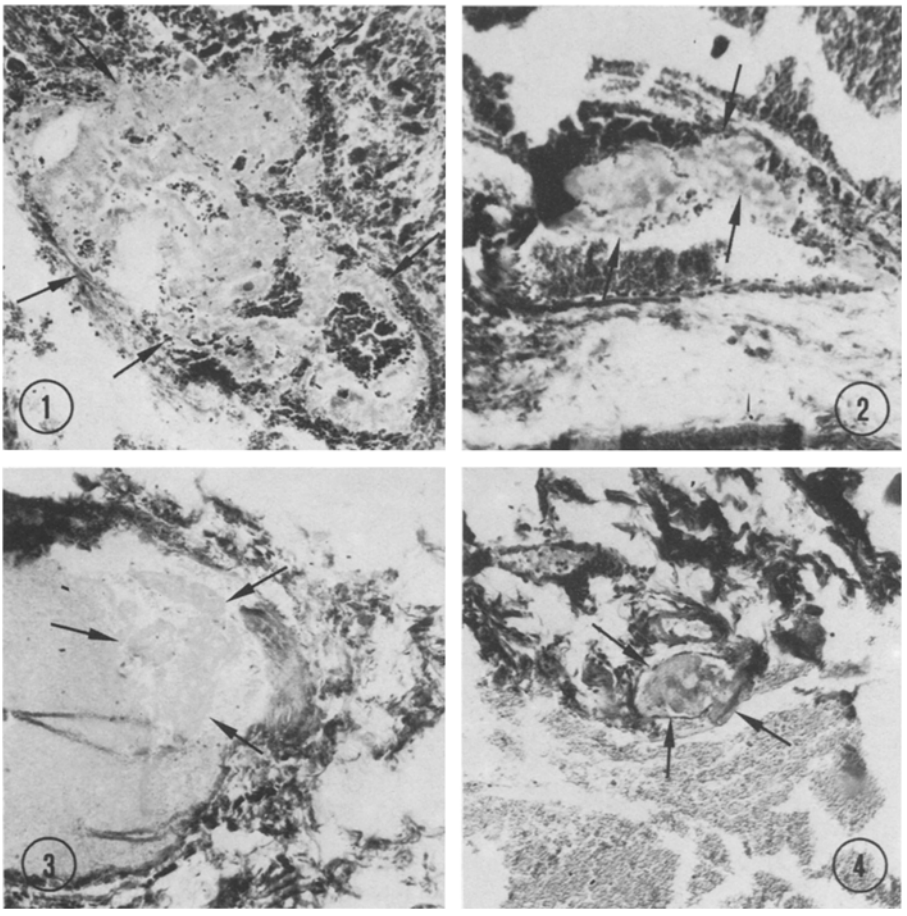

Fig. 1. One-Minute vital wound, specimen taken immediately after reaction period. Haemostatic accumulation (Arrows) in the haematoma, haemorrhagic vessel not in the micrograph. Magnification 100x

Fig. 2. One-minute vital wound, specimen taken immediately after reaction period. Haemostatic plug (Arrows) in a blood vessel, filling about half of the lumen. Magnification 100x

Fig. 3. One-minute vital wound, specimen taken five days after death. In the blood-filled vascular lumen, adjacent to the wall, a haemostatic plug (Arrows). Magnification 100x

Fig. 4. 5-minute vital wound, specimen taken immediately after reaction period. Haemostatic plug (Arrows) filling the vascular lumen and extending a small distance outside the ruptured wall into the haematoma. Magnification 100x

as an indication of vital reaction. It is further noted that the formation of the accumulations appears to be very fast: it could be histologically demonstrated after the shortest time studied in this work, one minute. According to the literature there might be chances of observation with an even shorter period

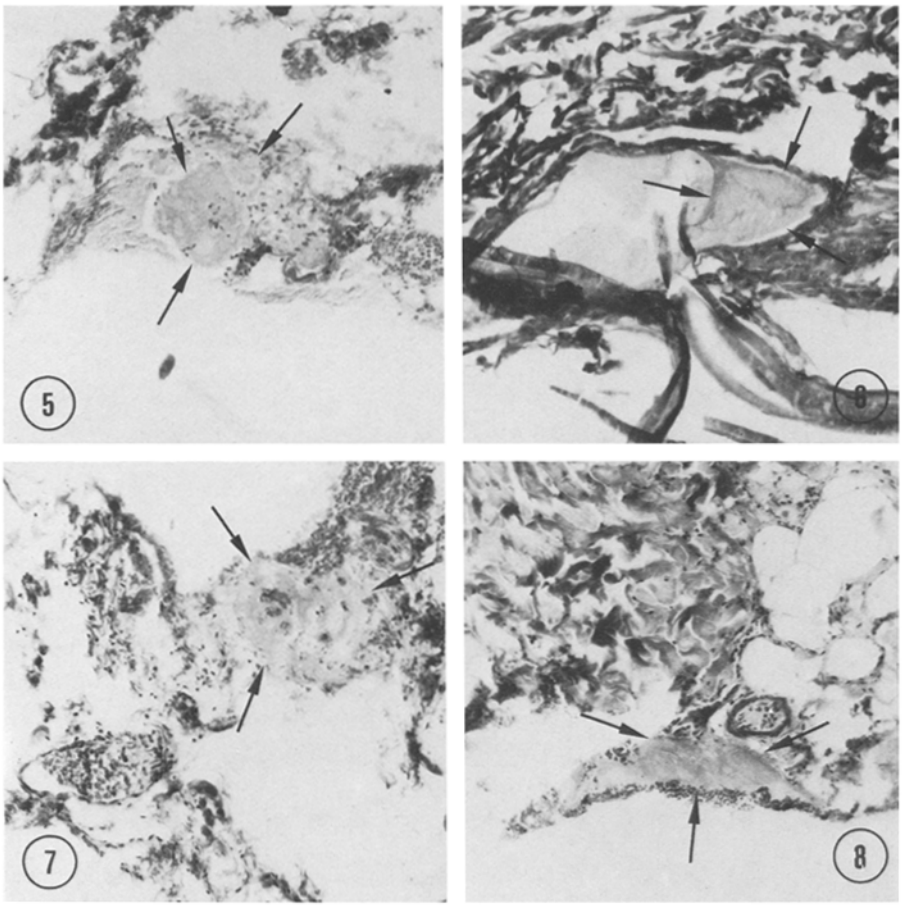

Fig. 5. 15-minute vital wound, specimen taken immediately after reaction period. Haemostatic plug (Arrows) in vicinity of blood vessel. Magnification 100x

Fig. 6. 15-minute vital wound, specimen taken five days after death. Haemostatic plug (Arrows) in vascular lumen, also containing abundant fibrin network. Magnification 100x

Fig. 7. 30-minute vital wound, specimen taken immediately after reaction period. Haemostatic plug (Arrows) in tissue close to blood vessel. Magnification 100x

Fig. 8. 60-minute vital wound, specimen taken five days after death. Haemostatic plug (Arrows) beside blood vessel. Magnification 100x

(BÖHM and TSCHOMAKOW 1972). Furthermore, the haemostatic plugs described were readily visible already at 100x or lower magnification, and they were clearly distinguished by their staining from the yellow erythrocytes in common paraffin sections.

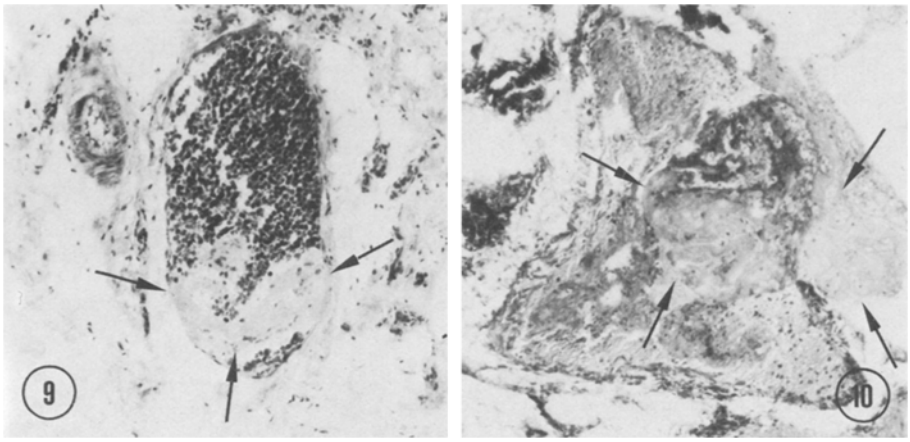

Fig. 9. 2-hour vital wound. Haemostatic plug (Arrows) in vascular lumen. Magnification 100x

Fig. 10. 12-hour vital wound, specimen taken five days after death. In vascular lumen, and continuing outside the ruptured wall, a haemostatic plug (Arrows) including an abundant fibrin accumulation. Magnification 100x

It seems to be a drawback hampering the use of haemostatic plugs as a vital reaction that in order to find them numerous sections are required, and even then the phenomenon may completely escape observation in a high proportion of wounds examined. This may be because the section does not happen to include any suitable injured vessel, or possibly the method applied has been unable to elicit them. The use of series cuts, and possibly more specific methods of demonstration, might therefore be useful. It is further noted that a prolonged postmortal period appears to diminish the chances of observing haemostatic plugs, judging from the fact that plugs could be seen in about one-third of specimens taken immediately, but in only 10% of those taken 5 days after the death.

In spite of these negative features the haemostatic plugs described add to the possibilities of ascertaining the vital character of wounds in connection with short vital periods, even with the aid of conventional histological techniques only. It should be observed, though, that the present observations were made with experimental animals. Thorough studies with cadaver material, and possibly with more specific methods, are still required.

REFERENCES

APITZ, K.: Die Dukesche Probe. Z. ges. exp. Med. *111*, 554-584 (1943)
BERG, S., DITT, J., FRIEDRICH, D., BONTE, W.: Möglichkeiten der biochemischen Wundaltersbestimmung. Dtsch. Z. ges. gerichtl. Med. *63*, 183-198 (1968)

BÖHM, E.: Zur Unltrastructur von Blutungen nach widerholter Traumatisierung. Z. Rechtsmedizin *74*, 197-206 (1974)
BÖHM, E., TSCHOMAKOV, M.: Ein Sekundenphönomen der vitalen Reaktion demonstriert an Stichverletzungen von Arterien. Z. Rechtsmedizin *71*, 235-242 (1972)
BÖHM, E., TSCHOMAKOV, M.: Frühe Merkmale einer vitalen Reaktion - Untersuchungen an Schnittverletzungen der Rattenhaut. Z. Rechtsmedizin *72*, 111-118 (1973)
CARSTAIRS, K.C.: The identification of platelets and platelet antigens in histological sections. J. Path. Bact. *90*, 225-231 (1965)
EBERTH, J.C., SCHIMMELBUSCH, C.: Experimentelle Untersuchungen über Thrombose. Virchows Arch. path. Anat. *103*, 39-87 (1886)
FAZEKAS, I.GY., VIRAGOS KIS, E.: Der Gehalt der Erhängungsfurche an freiem Histamin als vitale Reaktion. Dtsch. Z. ges. gerichtl. Med. *56*, 250-268 (1965)
HAYEM, G.: Recherches sur l'évolution des hématies dans le sang de l'homme et des vertebres. Arch. Physiol. 6 (ser. 2), 201-261 (1879)
HUGUES, J.: Contribution á l'étude des facteurs vasculaires et sanguins dans l'hémostase spontanée. Arch. int. Physiol. *61*, 565-711 (1953)
JØRGENSEN, L., BORCHGREVINK, C.F.: The platelet plug in normal persons. 1. The histological appearance of the plug 15 to 20 minutes and 24 hrs after the bleeding and its role in the capillary haemostosis. Acta path. microbiol. scand. *57*, 40-56 (1963)
JØRGENSEN, L., BORCHGREVINK, C.F.: The platelet plug in normal persons. 2. The histological appearance of the plug in the secondary bleeding time test. Acta path. microbiol. scand. *57*, 427-437 (1963)
JØRGENSEN, L., BORCHGREVINK, C.F.: The haemostatic mechanism in patients with haemorrhagic diseases. A histological study of wounds made for primary and secondary bleeding time tests. Acta path. microbiol. scand. *60*, 55-82 (1964)
LAIHO, K.: Immunohistochemical studies on fibrin in vital and postmortem subcutaneous haemorrhages. Ann. Acad. Sci. Fenn. Series A, V Medica *128*, 1-85 (1967)
LUBNITZKY, S.: Die Zusammensetzung des Thrombus in Arterienwunden in den ersten fünf Tagen. Arch. exp. Path. Pharmakol *19*, 185-208 (1885)
MÜLLER, E.: Durch Benzol erzeugte Thrombopenie. Ein Beitrag zur Frage der Benzolschädigungen beim Kaninchen. Beitr. path. Anat. *86*, 273-286 (1931)
RAEKALLIO, J.: Histochemical studies on vital and postmortem skin wounds. Ann. Med. exp. Fenn. *39*, Suppl. 6, 1-105 (1961)
RAEKALLIO, J.: Die Altersbestimmung mechanisch bedingter Hautwunden mit enzymhistochemischen Methoden. Lübeck: Schmidt-Römhild, 1965
RAEKALLIO, J., MÄKINEN, P.L.: Histamin content as vital reaction. Zacchia *41*, 273-285 (1966)
RIBBERT, H.: Die Histologie der Blutungen und die extra und intravasculäre Thrombose. Virchows Arch. path. Anat. *220*, 133-147 (1915)
SCHNEIDER, V.: Über rasterelektronenmikroskopische Untersuchungen an vital und postmortal entstandenen "Thromben". Z. Rechtsmedizin *74*, 47-54 (1974)
WALCHER, K.: Über vitale Reaktionen. Dtsch. Z. ges. gerichtl. Med. *15*, 16-57 (1930)
WALCHER, K.: Die vitale Reaktionen bei der Beurteilung des gewaltsamen Todes. Dtsch. Z. ges. gerichtl. Med. *26*, 193-211 (1936)
ZUCKER, H.D.: Platelet thrombosis in human hemostasis. A histologic study of skin wounds in normal and purpuric individuals. Blood *4*, 631-645 (1949)

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