

INVESTIGATION OF PLASMA HISTAMINASE ACTIVITY IN WOUND HEALING*

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INTRODUCTION

In an earlier paper increased plasma histaminase activity in certain clinical conditions of allergic aetiology was reported (7). A higher plasma histaminase activity in women than in men was also observed. Even though the exact role of histamine and the significance of histaminase activity has not been established, changes in the formation or content of tissue histamine are quickly reflected by changes in plasma histaminase activity. Several workers (2, 18) have reported changes in mast cell count and tissue histamine content during the healing of skin wounds. In the present work plasma histaminase activity has been studied to further extend the information on histamine-histaminase relationship during wound healing. An attempt has been made to correlate the changes in enzyme activity with changes in content of mast cell constituents by estimating blood clotting time and tissue histamine content.

MATERIALS AND METHODS

Plasma histaminase activity was estimated according to the method described by Kapeller Adler (5).

Wistar rats bred in Madurai Medical College Animal House weighing 120—160 g were used. They were used in groups of twenty. In etherised rats linear incised wounds were made under aseptic conditions on the dorsal skin and apposed with interrupted silk sutures. Investigations were made for one week. Two to five rats were killed daily during the post-operative period. An uniform strip of dorsal skin with the wound in the middle was excised and extracted with 10% trichloroacetic acid for the estimation of histamine content as described by Parrat and West (12). Blood was obtained by cardiac puncture for estimation of plasma histaminase activity and leucocyte count. For clotting time, blood was obtained from tail.

Similarly a study of plasma histaminase activity, clotting time and leucocyte count was made during the post-operative period in adult male patients (to avoid sex difference) who had undergone selective abdominal operations for peptic ulcer and who had no other apparent associated diseases. These patients who were admitted for treatment in the surgical wards of the Government Erskine Hospital, Madurai, had routine post-operative care and a course of Streptopenilin injections for a period of 7 days from the day of operation. Healing was normal in all the cases selected for study and there were no post-operative complications. Blood was drawn from the cubital vein and plasma histaminase activity, clotting time and leucocyte count were estimated on alternate days. Estimations were made in several patients and mean values ob-

tained for each post-operative day were noted and compared with mean values obtained from healthy adult males.

The effect of a single injection of Streptopenicillin in healthy adult males was studied. Even though there was no significant change in clotting time or plasma histaminase activity 3 hr and 24 hr after injection, to assess the effect of prolonged post-operative Streptopenicillin therapy on the parameters, two groups, each of 20 adult Wistar rats weighing 130–150 g were subjected to dorsal skin wounds and studied for 9 days post-operatively. One group received daily intramuscular injections of procaine penicillin 500 U/100 g and the other group, injections of streptomycin sulphate 10 mg/100 g from the day of wounding for 8 days. Plasma histaminase activity, clotting time, leucocyte count and tissue histamine content were estimated in

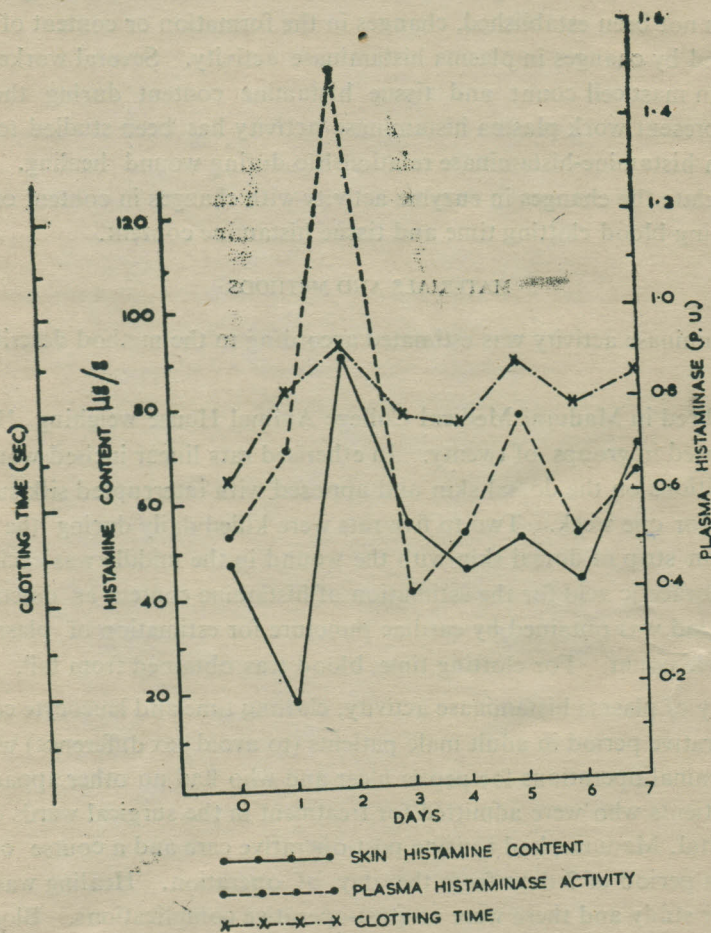


Fig. 1

Effect of injury in rats on changes in skin histamine content, plasma histaminase activity and clotting time. Calibration for histamine content and clotting time is common

groups of 2 or 3 animals at a time sacrificed on alternate days. Four such estimations were made and mean values obtained and compared with those obtained for untreated animals.

Histamine content was assayed on the isolated guinea pig ileum as described by Parrat and West (12) and expressed as $\mu\text{g/g}$ (base) of fresh tissue. Clotting time was estimated by capillary tube technique. Total leucocyte counts were made in Fuchs Rosenthal Chamber and absolute eosinophil count calculated from differential count.

RESULTS

Wound healing in untreated Wistar rats :

The data are given in Fig. 1. Skin histamine content in the dorsal skin area was significantly ($p < 0.05$) reduced during the first 24 hr ($n=4$). In the next 24 hr it overshot the normal range and thereafter returned to normal levels. The plasma histaminase activity exhibited a rise and the peak occurred between 24 and 48 hr ($p < 0.05$) and then returned to near normal ($n=4$). The clotting time increased within 24 hr after injury and reached a maximum ($p < 0.05$) in about 48 hr. Though the peak level was not maintained, it remained fairly high throughout the week ($n=4$).

The absolute eosinophil count remained within the normal range (60-200/cu/mm) throughout the observation period, suggesting no apparent correlation with changes in plasma histaminase activity ($n=4$).

Wound healing in post-operative patients :

Plasma histaminase activity showed an initial peak in the first 24 hr as in the rat but remained significantly ($p < 0.05$) above normal during the major part of the post-operative period. It showed a tendency to return to near normal levels only in the later period (Fig. 2). Clotting time remained within the normal range (197-207 sec) throughout the period of study. Streptopenicillin had no effect on these parameters (Table I; > 0.05).

TABLE I

Effect of Streptopenicillin on plasma histaminase activity and clotting time in normal men

Investigation	Before injection	2hr after injection	24hr after injection
Mean plasma histaminase (P.U./ml)	0.132 ± 0.22 (25)	0.12 ± 0.15 (5)	0.18 ± 0.15 (5)
Mean clotting time (Sec)	207 ± 15.14 (15)	207 ± 108.26 (5)	197 ± 59.54 (5)

Figures in parentheses indicate the number of observations.

Wound healing in Wistar rats treated with streptomycin and or penicillin:

Change in histamine content of dorsal skin in wounded rats treated with penicillin or streptomycin were similar to those obtained in untreated rats (Table II). But plasma histaminase activity was relatively lower in the penicillin and streptomycin treated rats when compared to that in untreated wounded rats on all the days. It is well known that some antibiotics inhibit diamine oxidase activity (22). Single estimations for each post-operative day of investigation on pooled blood from 3 animals confirmed the reports of earlier workers (Table III). There was no difference between the treated (63—87 sec) and untreated (63—87 sec) rats in the clotting time.

TABLE II
Effect of antibiotics on skin histamine levels in rats after injury

Group	Mean skin histamine level ($\mu\text{g/g} \pm \text{S.E.}$)					
	Before injury	1	3	5	7	9
Control*	44.5 ± 12.848	17.233 ± 5.164	89.325 ± 22.102	51.25	68.3	40.0
Penicillin (single estimation from pooled tissue)	44.5	20.0	26.25	30.0	26.25	26.25
Streptomycin (single estimation from pooled tissue)	44.5	15.0	15.0	22.5	30.0	41.25

*n=4

TABLE III
Effect of antibiotics on plasma histaminase activity in rat after injury

GROUP	Mean plasma histaminase activity (P.U./ml \pm S.E.)					
	Before injury	1	3	5	7	9
Control*	0.45 ± 0.26	0.7 ± 0.515	1.5 ± 2.18	0.78	0.628	0.4
Penicillin (single estimation from pooled blood)	0.45	0.1	0.2	0.1	0.0	0.0
Streptomycin (single estimation from pooled blood)	0.45	0.5	0.2	0.0	0.2	0.2

*n=4

DISCUSSION

The immediate fall in the skin histamine level soon after injury observed in this study is in agreement with the previous reports. Nilzenn (10) has reported that histamine is released from the site of injury. Kameswaran and West (6) and Sanyal and Bhatt (17) also reported a fall in the skin histamine level following injury. Wichmann (21) in a quantitative study of mast

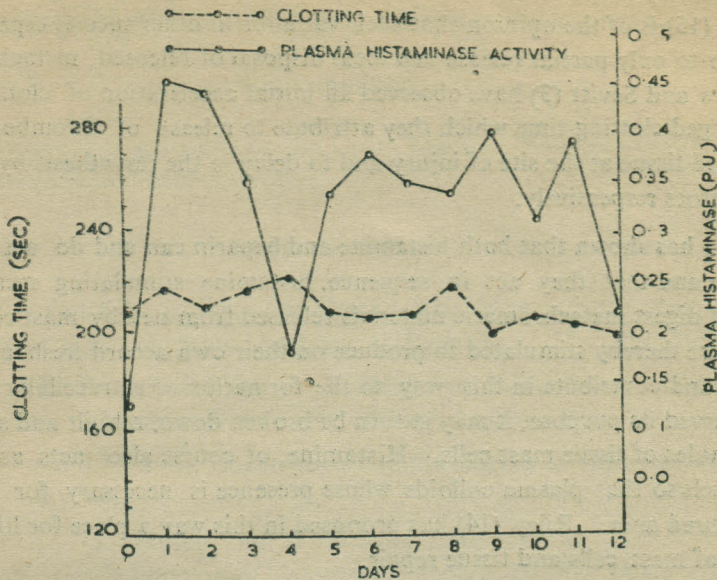


Fig. 2

Changes in plasma histaminase activity and clotting time in post-operative patients

cells during wound healing in rats found a decrease in their number during the first 24 hr followed by an increase during the second day which reached a peak on the 8th-10th day and then gradually returned to normal by the 32nd day. These studies suggest that the fall could be due to degranulation of mast cells and release of histamine in response to tissue injury (1, 19, 20). It had also been suggested that this fall may be due indirectly to the result of endogenous secretion of cortisone which is known to reduce the synthesis of histamine in rat skin (16).

The increased plasma histaminase activity observed in the immediate post-operative period in humans is similar to that observed in rats during wound healing. In a similar study in human wound tissue, uninjured tissue only rarely showed a measurable histamine forming capacity while human wound tissue regularly showed a high histamine forming capacity (quoted from Kahlson and Rosengren, 8).

These reports support the immediate increase in plasma histaminase activity in the present study which may or may not be related to the histamine released from the injured site. However, this increase in plasma histaminase activity does not appear to be associated with an increase in absolute eosinophil count (7, 9).

Increased clotting time in rats after injury suggests increased heparin activity. It is likely that this increased heparin activity is due to degranulation of mast cells following tissue injury. However, no such change is observed in human patients. Such a release of heparin either following or during histamine release is reported only in dogs, but not in other species

(13). Although Riley (14) attributes this unique feature in dogs as being due to certain species variation, West (15) is of the opinion that such variation in other species especially in rats may probably be due to only partial release and local disposal of released metachromatic granules. In humans, Innes and Sevitt (3) have observed an initial acceleration of clotting followed by a period of prolonged clotting time which they attribute to release of thromboplastic substances from the damaged tissue at the site of injury and to delay in the resynthesis by the liver of depleted clotting factors respectively.

Riley (14) has shown that both histamine and heparin can and do act primarily on the connective tissue and that they act in sequence, histamine stimulating mesenchymal cells to phagocytose and digest metachromatic materials released from nearby mast cells. The connective tissue cells are thereby stimulated to produce on their own accord fresh and specific mucopolysaccharides and contribute in this way to the formation of extracellular ground substance. Once this has served its purpose, it may in turn be broken down, rebuilt and stored in sulphated form in the granules of tissue mast cells. Histamine, of course also acts as a vasodilator of small blood vessels so that plasma colloids whose presence is necessary for phagocytosis (4), leak into the injured area. Riley (14) has proposed in this way a place for histamine and heparin in the cycle of mast cells and tissue repair.

The data on wound healing in Wistar rats treated with antibiotics indicate that penicillin and streptomycin have no significant influence on the skin histamine level and heparin activity during wound healing. Ovchinni-Kova (11) has studied the interrelationship between the antibiotics of different chemical structure and heparin. He has reported that antibiotics of polypeptide nature have the ability to fix or form complexes with heparin. This may partially account for the lesser increase in clotting time in post-operative human patients than in rats. The low plasma histaminase activity in the penicillin and streptomycin treated group of rats also suggests a probable direct or indirect influence on the activity of the enzyme. However, post-operative patients even when treated with Streptopenicillin, exhibit a rise in plasma histaminase activity. The effect of antibiotics on the plasma histaminase needs further study.

SUMMARY

An attempt has been made to study the changes in plasma histaminase activity during wound healing, after injury.

1. During healing of clean incised wounds in rats, immediately after injury there was a fall in tissue histamine content at the site of injury, a rise in plasma histaminase activity and increased clotting time. The effects were reversible within the first week after wounding.

2. In post-operative patients after elective abdominal surgery, a similar rise in plasma histaminase activity could be detected. This rise did not appear to be affected by injection of Streptopenicillin, used throughout the experimental period.

3. In rats, intramuscular injection of penicillin or streptomycin separately during the period of wound healing was associated with lower levels of plasma histaminase activity when

compared with operated controls. Whether high doses of these drugs have an inhibitory influence on the enzyme activity directly or indirectly remains to be elucidated.

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