ADDENDUM

PERIVASCULAR MAST CELLS AND THEIR GRANULAR CONTENT IN RELATION TO EXPERIMENTAL HYPERTENSION IN LABORATORY ANIMALS.

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Several reports suggest that mast cells from different species differ markedly in their responsiveness to mediator release (Rothschild, 1966; Assem and Monger, 1970; Schmutzler et at, 1978; Pearce and Ennis, 1980).

Vasodepressor effect of histamine liberated from perivascular mast cells was first discovered by Dale and Laidlaw (1910). It has been suggested that histamine has a biphasic and dual role on blood pressure - a constrictor action on large arteries and a dilator action on the capillaries (Dale and Laidlaw, 1912; Feldberg, 1927).

Naranjo and Naranjo (1958) interpreted that pressor response of histamine is mediated by release of endogenous epinephrine but Lüscher (1994) demonstrated that histamine played its dilator action by the activation of the protective metabolic process in the endothelium.

But it remains to be confirmed whether this two exactly opposite type of effects on blood vessels are really present or not. Even if it becomes a fact, it does not sound physiological.

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It has been reported further that serotonin after its release on activation of mast cells causes vasodilatation of arterioles via $S_1$ receptor, but in venules and capillaries it causes vasoconstriction via $S_2$ receptor (Buccino et al, 1967; Sole et al, 1970).

That serotonin elicits vasodilatation both directly and indirectly by release of vasodilator principle from endothelial cells or vasoconstriction associated with amplification of noradrenaline response, has been reported (Saxena et al, 1987).

It has been further suggested that serotonin effects are complex and activation of one type of receptor (presumably $S_2$) results in increase of blood pressure and heart rate while activation of other type of receptor (presumably $S_1$) results in a decrease in arterial blood pressure and heart rate (Gillis et al, 1988).

Thus it appears that the effect of serotonin on the blood vessels are not only conflicting but also confusing.

Heparin is also found in association with lipoproteins in the secretory granules of the mast cells. The effect of
heparin on blood pressure is still not clear. Susic et al, (1982) reported that heparin lowered the blood pressure in rats with developed Goldblatt's hypertension which may be due to a decrease in packed cell volume whereas Vasdev et al, (1994) revealed that high dose low molecular weight heparin normalised the elevated platelet, cytosolic free calcium, aortic calcium uptake and systolic blood pressure in spontaneously hypertensive rats.

It is evident therefore that the findings as above have no solid foundation because many of them are contradictory to each others particularly in relation to the action of the principles elaborated by mast cells on blood vessels. It has been stated that the mechanism of action of heparin on blood vessels is far from clear. Similarly, serotonin that is liberated has been shown to act via receptors but their actions varies from one report to another. Therefore, it was thought pertinent to investigate the role of mast cells in relation to hypertension produced in Goldblatt's preparation and the aim of the present study pivots on this point and as such the present experiment has been planned.

Regarding query on the release of vasoactive substances from mast cells or from nerve terminals ;-

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It has been well documented that mast cell mediators are derived from three different sources (Middleton et al., 1981). The first of these being preformed primary mediators loosely bound to the granular matrix—histamine, eosinophil chemotactic factor of anaphylaxis (ECF-A), intermediate weight ECF-A, and neutrophil chemotactic factor of anaphylaxis (NCF-A).

While the second source is newly formed mediators generated in response to the immunological reaction—prostaglandins, slow-reacting substances—anaphylaxis (SRS-A), kallikrein and bradykinin.

The third being granule associated mediators released slowly with granular dissolution—heparin, superoxide dismutase, peroxidase and aryl sulfatase-A.

Schwartz (1994) has demonstrated further that mast cell activation leads to release of mediators in the form of biogenic amines like histamine, serotonin, proteoglycans like heparin, oversulfated condroitin sulfates and neutral proteases. Leukotriene C₄, prostaglandin-D₂ and cytokines have been shown to be amongst the newly generated mediators.
48/80 compound, a histamine releaser has been found to cause steroid secretion with degranulation of mast cells (Hinson, 1989).

Schwartz et al (1994) had implicated mast cells with cardiovascular homeostasis and such implication was made in view of release of various vasoactive and chemotactic principles stored in an active form in acid medium inside the secretory granules.

Even though it is a fact that histamine, serotonin and the like substances release in small quantities from the nerve terminals, an increase in mast cell population under the present experimental situation are indicative of the involvement of them. It is true that in the present experiment no attempt has been meant to study the release of vasoactive substances from the nerve terminals but the very fact that the mast cells are involved in the process, it is quiet logical to derive conclusion to the effect that the release of histamine, serotonin and heparin are very much concerned with regulation of blood pressure by acting on the peripheral blood vessels.
Regarding query in connection to conclusion:

It is true that, based on the observations under the present experimental situation, the conclusion had been made (that some of the vasoactive and chemotactic principles liberated from perivascular mast cells are involved in the alteration of blood pressure) was biased with contention. But it is clearly evident from this observations presented in the thesis that Goldblatt hypertension preparation is associated with alteration of the perivascular mast cell population and of their granular contents.
SUMMARY

1. Mast cell count in different perivascular tissues like kidney cortical and medullary tissues, lung and mesentery showed no significant alteration after 7 days of production of experimental hypertension followed by an increase after 14 days which maintained up to 21st day of study (except spleen tissue) in comparison to the control group.

2. Histamine concentration in serum as well as in lung, spleen and mesentery was elevated throughout the experimental period in comparison to the control group. In kidney cortical and medullary tissues, significant rise found on 14th day and 21st day following production of hypertension in respect to the corresponding control group.

3. Diamine oxidase level rises both in serum and tissues of the experimental group throughout the experimental period as compared to the control group.

4. Serotonin level is found to be elevated in kidney medullary tissue and mesentery of experimental group up to 14th day of experiment followed by a decrease in its level up to 21st day after production of hypertension with respect to control group. It's concentration remain elevated in serum throughout the experimental period but in spleen tissue of experimental group, it shows

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insignificant change up to 14th day followed by significant increase at 21st day after production of hypertension with respect to control group.

5. Heparin in serum and different tissues rises significantly throughout the experimental period in experimental group in respect to the control group except mesentery which showed significant decrease on 14th day after production of hypertension in comparison to the control group.

6. Noradrenaline content of serum as well as different tissues like kidney, lung, spleen and mesentery was found to be elevated throughout the experimental period in the experimental group in respect to the control group.

7. Serum cholesterol and triglyceride levels increase in the experimental group following production of hypertension in comparison to the control group.

As obtained from the observations made from the present investigation in the experimental group, the elevation of blood pressure may be attributed to

(a) The biphasic action, i.e. both pressor and depressor action, of histamine liberated from perivascular mast cells.

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(b) Besides, as there is a rise in DAO activity and DAO being the enzyme responsible for degradation of histamine, it is quiet justified to suggest that rise in DAO has some influence in elevating blood pressure level by diminishing the depressor action of histamine.

(c) Moreover evidences suggest that heparin can stimulate the release of DAO. Thus it can be assumed that increase in heparin concentration causes an increase in DAO release that catabolises histamine (vasodilator) and thereby it causes an indirect reflection in the blood pressure level i.e. an elevation.

(d) However, evidences exist to support that histamine and serotonin can potentiate noradrenaline release. Thus, it can be surmised that increase in noradrenaline concentration may be due to an increase in histamine and serotonin level. Noradrenaline by its vasoconstrictor action increases peripheral resistance and thereby increases blood pressure.

(e) The increased triglyceride and cholesterol contents in serum may also be responsible for elevating blood pressure level since deposition of triglyceride and cholesterol can produce atherosclerotic changes which inturn indirectly cause hypertension.
However, the role played by renin angiotensin system in creating hypertension cannot be eliminated though there are interesting reports which suggest that serotonin and histamine released from mast cells can potentiate the vasoconstrictor effects of angiotensin II.
(1) Summary is added.

(2) Specificity of lead acetate and toluidine blue for mast cell staining.

Mast cells are not easily identified in human tissue sections unless special fixatives are used to preserve the granules. 4% aqueous basic lead acetate used for mast cell fixation because it preserves the granules of mast cells. The mast cells were identified by their metachromasia. Certain single dye like toluidine blue react with tissue components to stain these components in different colour from that of the dilute dye solution that is toluidine blue staining give mast cells a red purple colour (metachromatic staining). The metachromatic reaction is due the presence of sulfated acid mucopolysaccharide or nucleic acids. (Sheehan and Hrapchak, 1980).

(3) NCF-A (Neutrophil chemotactic factor - anaphylaxis)  
B CF-A (Basophil chemotactic factor - anaphylaxis)  
These are also included in the body of the thesis.

(4) The compound 48/80 is a drug which acts as mast cell degranulating agent and enhanced the overflow of endogenous histamine and serotonin (Fuder et al 1989).

(5) Correction done in the body of the thesis.

(6) All typographical errors are corrected in the body of the thesis.

(7) The word "accepted" is replaced by 'included' in the body of the thesis.
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