

INTERACTION OF HISTAMINE WITH HEPARIN

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Histamine is now well accepted as a mediator of hypersensitivity reaction. Intravenous infusion of histamine produce anaphylaxis in laboratory animals (1). The release of histamine from mast cells by interaction between IgE and specific antigen has been demonstrated in several human cells and tissue systems (2,3,4). The histamine containing mast cell granules have a high heparin content. Histamine is stored as a heparin-histamine complex in the mast cell granules (5).

The effect of histamine on the interaction between heparin and methylene blue (MB^+) has been studied using visible adsorption spectroscopy. The concentration of histamine required to completely remove MB^+ from the heparin- MB^+ complex (i.e. the Limiting Concentration, L.C.) has been determined over the pH range 3-10 and compared with the L.C. for Ca^{++} ions (6). The value of the L.C. is a qualitative estimation of the binding affinity of the counter ion for the heparin; the lower the heparin; the

lower the L.C., the greater is the strength of binding.

The interaction between some cationic dyes and polyanions leads to a change in the absorption spectrum of the dye (metachromasia) 7-13.

These dyes, which do not obey Beer's Law, aggregate on the polyanion surface, to form a metachromatic complex and thus produce a characteristic metachromatic shift. Addition of cations can reverse this effect.

Following the work of Landsmeer on the effects of metal ions on the degree of staining of mast cells (14), semi quantitative studies were made on the influence of various cations on the quantity of dyes bound to polyanionic materials (15-16). The histamine containing mast cells have a high heparin content and are well known to form a complex both in vivo and in vitro (17-18).

The present study indicates, that variation of pH significantly alters the strength of binding of histamine to heparin; maximum binding occurring at pH 4. At pH 5.5 however, the strength of binding of Ca^{++} ions to heparin is considerably greater than histamine.

2. MATERIALS AND METHODS

Heparin (170 units/mg.) was obtained from Boots Pure Drug Company, MB^+ from E. Gurr Ltd., and histamine dihydrochloride from Merk.

Heparin- MB^+ complex was prepared by mixing aqueous solutions of heparin and MB^+ so that the final concentrations were 10^{-4} "equivalents per anionic site" and 10^{-5} M respectively. The term "equivalents per anionic site" is defined as the molecular weight of the heparin te-

trasaccharide divided by the number of anionic sites within the tetrasaccharide, and under These conditions the ratio of anionic sites - cationic dye ($S/D = 10$). Visible absorption spectra were recorded using a Bechmann recording spectrophotometer.

3. RESULTS

The visible absorption spectra of heparin- MB^+ complex in the absence and presence of increasing concentrations of histamine at pH 4 are shown in Fig.1. Similar curves were obtained at pH 3, 7 and 10, as well as in the presence of Ca^{++} at pH 5.5. The progressive destruction of metachromasia as indicated by the increase in the MB^+ adsorption at 665nm is expressed as a function of histamine

It is of interest to note that the L.C. for Ca^{++} at pH 5.5 is 0.7×10^{-3} M, i.e. less than that expected for histamine at this pH (Fig, 3), indicating that Ca^{++} has the higher binding affinity. Such an observation is of considerable significance in vivo. The mechanism of histamine release from mast cell granules is the subject of much discussion (19).

It is proposed that histamine releasing agents may cause an alteration of pore sizes and changes in the permeability of the membrane. Ca^{++} ions may then act on the histamine complexed within the granules and because of their greater binding affinity, cause the histamine to be released. It is well established that Ca^{++} ions are essential for this process (20).

concentration in Fig. 2. For the purpose of comparison, destruction of the complex by Ca^{++} ions at pH 5.5 is also included in Fig. 2, and the values of the L.C. for histamine and Ca^{++} are given in Table 1. Fig. 3 shows the effect of pH on the L.C. for histamine.

4. Discussion

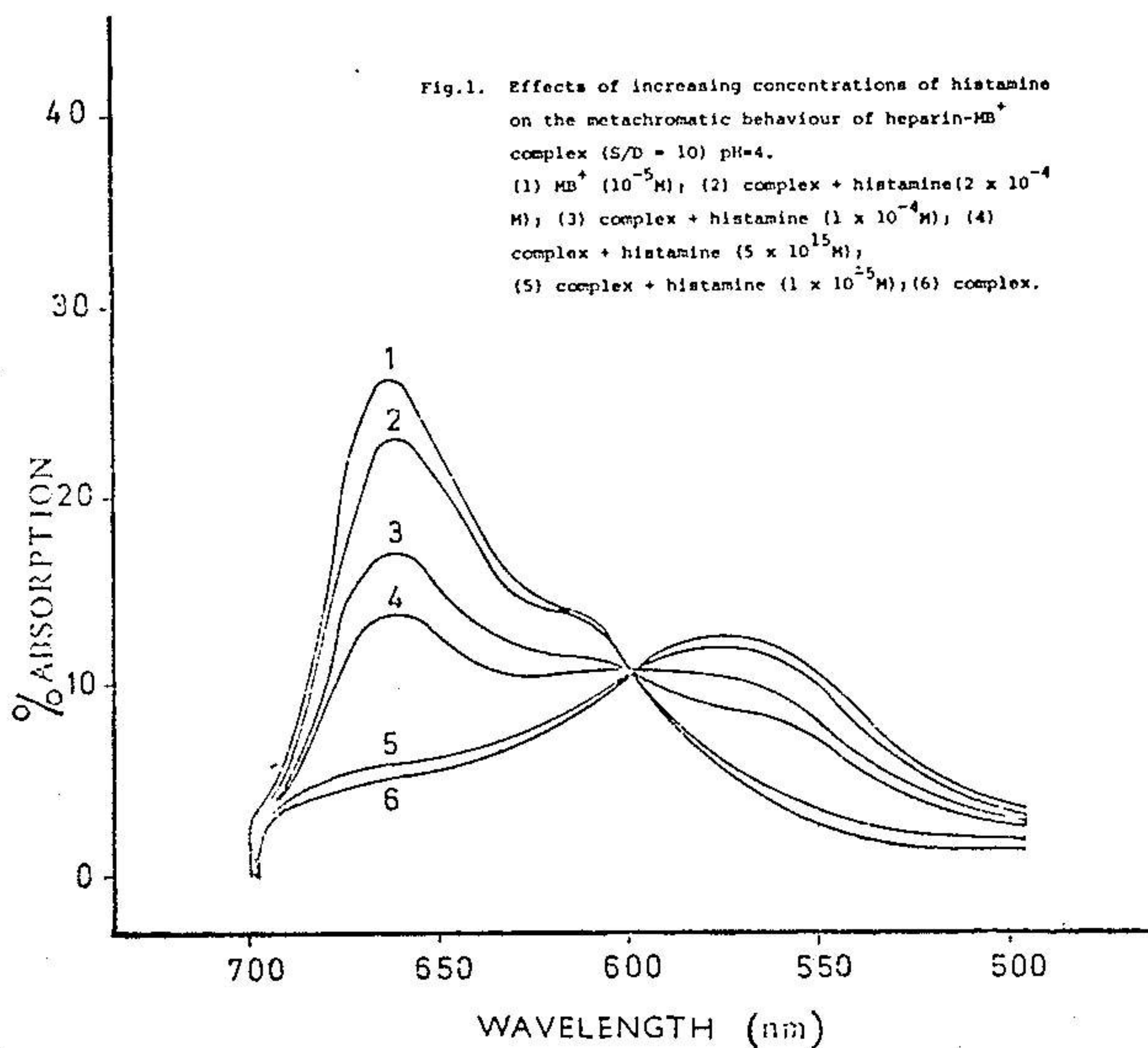
The data presented here clearly indicates that variations in pH significantly influences the strength of binding of histamine to heparin. At pH 4 the L.C. for histamine is 0.25×10^{-3} M (Table 1).

At this pH, the imidazole and side chain nitrogen atoms are fully protonated and both may bind to the anionic sites on the heparin molecule. All of the polyanionic sites (namely- NHSO_3^- , $-\text{O}-\text{SO}_3^-$ and $-\text{COO}^-$) are available for interaction with histamine, though binding of histamine apparently only occurs via the $-\text{O}-\text{SO}_3^-$ and NHSO_3^- groups, since N-desulphated heparin does not bind to histamine (5). Below pH 4, protonation of the anionic sites of heparin occurs resulting in an increase in the L.C. of histamine (Fig. 3), i.e. a decrease in the affinity of histamine for the polymer. This decrease in binding affinity may also be due to a change in configuration of the heparin molecule.

At neutral pH, heparin has a high negative charge density. Protonation of the anionic sites decreases the electrostatic charge repulsion and causes the molecule to collapse. Between pH 4-10 both the imidazole and side-chain nitrogens ionize (P_k 5.8 and 9.7 respectively) with a consequential decrease in binding affinity of the histamine, as indicated from the increase in L.C. for histamine (Table 1, Fig. 3).

Table 1. The effect of histamine and Ca^{++} on heparin-blue interaction.

	L.C. ($\text{M} \times 10^{-3}$)
Histamine (pH 3)	1.6
Histamine (pH 4)	0.25
Histamine (pH 7)	5.7
Histamine (pH 10)	10
Ca^{++} (pH 5.5)	0.7



ABSORPTION OF MB⁺ AT 665nm
 AT SOLUTION OF HEPARIN-MB⁺ COMPLEX-HISTAMINE AT 665nm

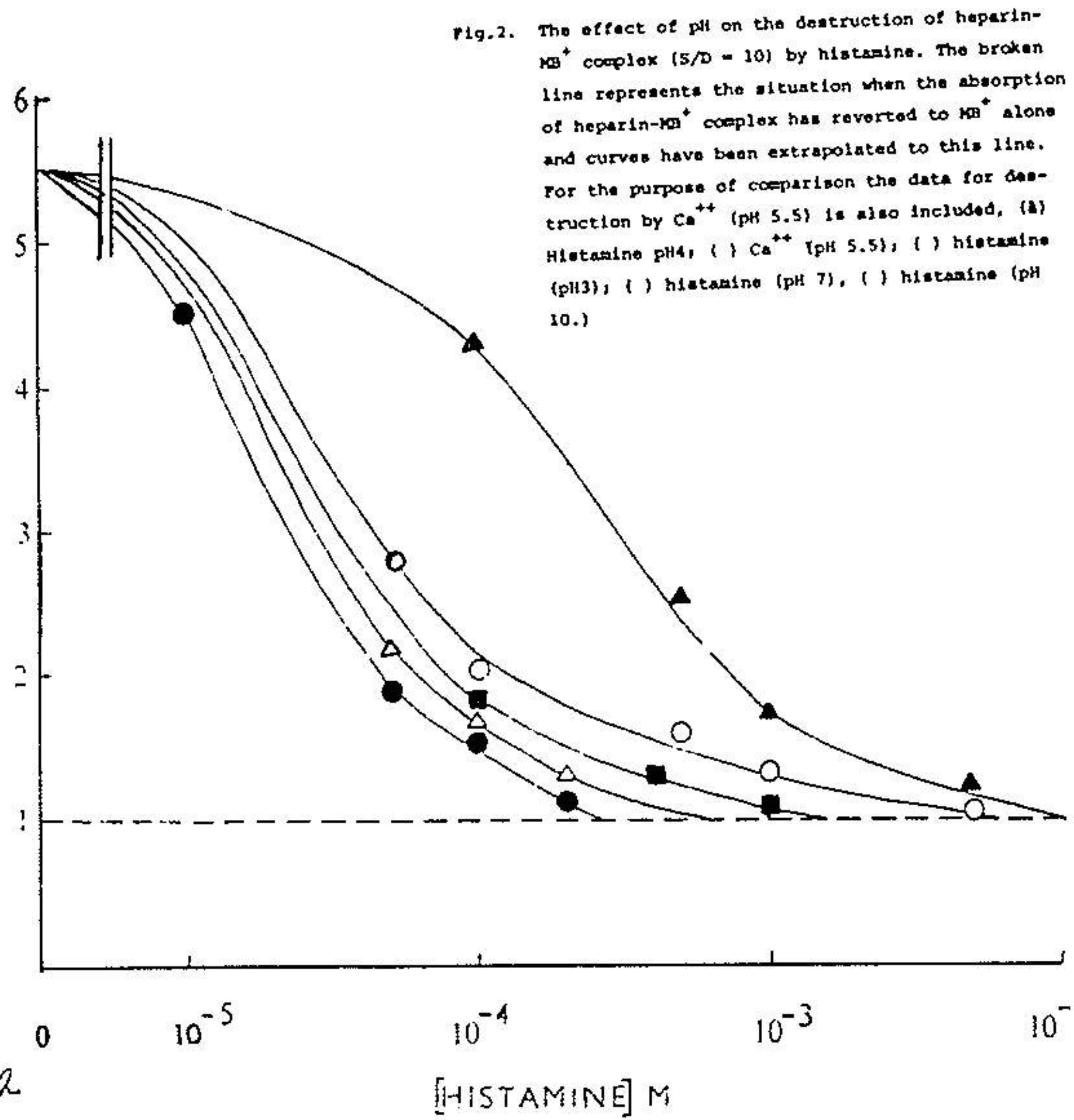


FIG-2

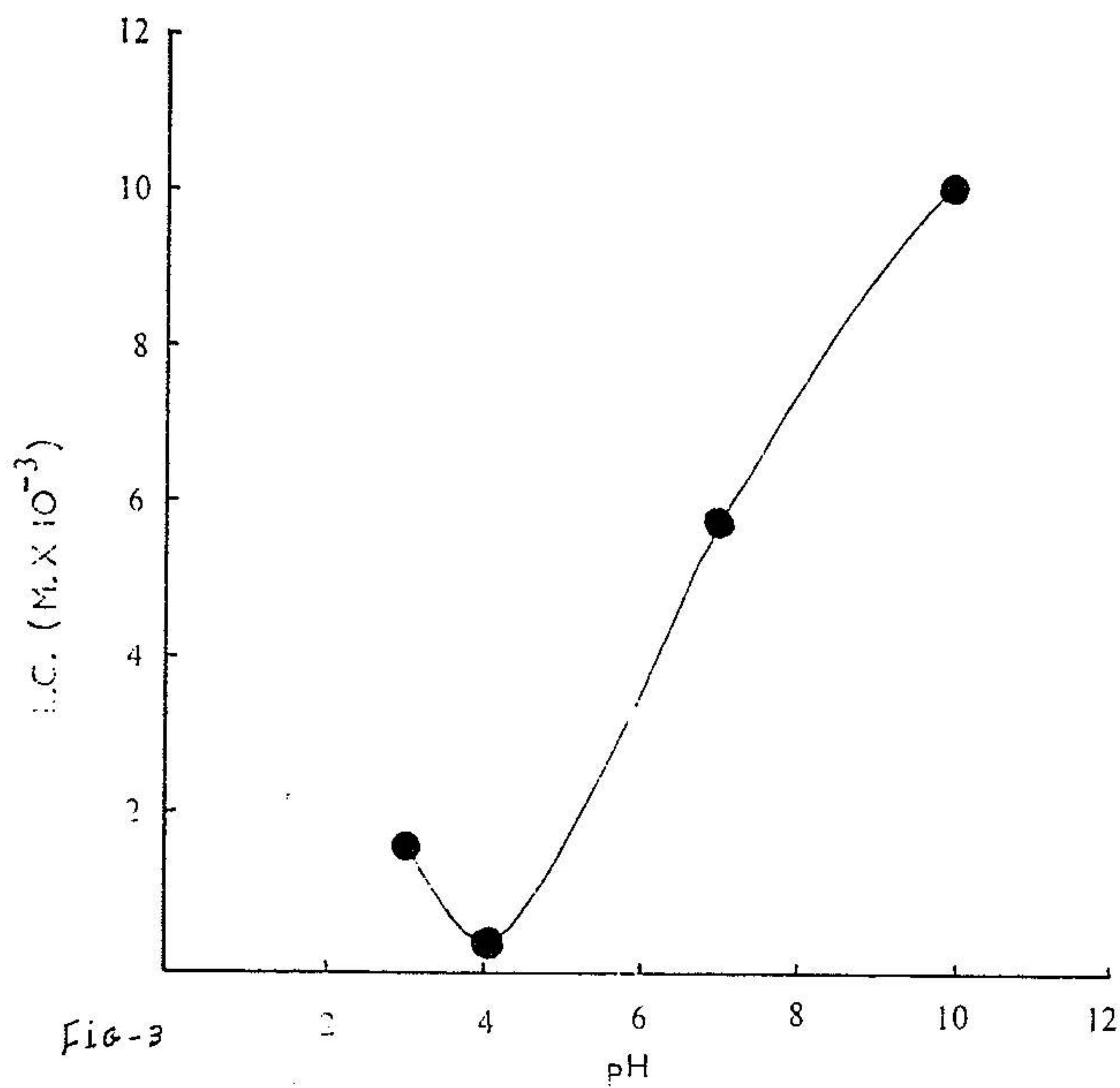


Fig-3

Fig.3. Effect of pH on the limiting concentration(L.C.) for destruction of heparin- MB^+ complex (S/D = 10) by histamine.

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