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THE FIXATION OF MAST CELLS

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Since several authors, while engaged in their study of mast cells of various animals, noticed that the granules of these cells were destroyed or aggluttinated by some of the substances used in comon histological technique such as water, glicerine, or acetic acid [1 2 3 4 5 6] a number of workers tried to find a suitable fixative for these granules.

They, however, met with the difficulty, that mast cells of different species, as well as belonging to different organs of one animal, did not at all react in the same way to the chemicals used.

Thus, the mast cells of mice, rats, guinea-pigs, show a higher degree of resistance to aqueous solutions, while those of rabbits and certain fish seem to be more easily affected[5 7 8]

Different authors, therefore, recommend a number of fixatives, which they found affective in their experiments[1 9 10] HOLMGREN and WILANDER[11] having established a relationship between heparin contend and mast cell granula tissues, were the first to try an aqueous solution of lead subacetate as a fixative, based on their knowledge that heparin is precipitated by this compound.

Their formula was later modified by CHEVREMONT and COMHAIRE[13] and by FERRI and MOTA[12] who, in their stu-

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dies on this subject used a 50% alcoholic solution of lead subacetate, most successfully.

However a survey of the literatura shows that there appears to be no systematic study on the variability of mast cell fixation in the animal series. Observations on this point are isolated and inclined to overlook the fact that mast cells from different organs or different species require a different treatment. The present paper proposes to deal with the problem.

MATERIAL AND METHODS

The material for this study was collected from 12 oxen, 3 horses, 8 pigs, 20 dogs, 8 cats, 5 rabbits, 8 guinea-pigs and 6 rats. The bovine and suilline material was obtained at the local slaughterhouse, just after death. All other animals were bled to death and all but the horses were previously anaesthetized with nembutal.

Fragments of the following organs were taken:

liver, lung and aorta	oxen, horses and pigs
liver, lung, tongue, skin and mesentery	dogs, rats and guinea-pigs
liver, lung, tongue and skin .	cats and rabbits.

Besides the fixatives of Helly, Zenker, Bouin, Gendre, Dubosq Brasil, Susa, Carnoy, 10% neutral formalin, Ehrlich (50% alcohol), Holmgren and Wilander, and the variation by Chevremont and Comhaire, several concentrations of alcoholic lead subacetate solutions (varying from 1% to 10% with or without formalin (10%) or acetic acid (0,5% to 5% — or both) were tried out, and the following selected for the further course of the experiments, as being most convenient.

"A"	50% alcohol	100,0 ml
	Lead subacetate	4,0 g
"B"	50% alcohol	100,0 ml
	Lead subacetate	4,0 g
	Formalin	10,0 ml

"C"	50% alcohol	100,0 ml
	Lead subacetate	4,0 g
	Formalin	10,0 ml
	Acetic acid	1,0 ml
"D"	50% alcohol	100,0 ml
	Lead subacetate	1,0 g
	Acetic acid	0,5 ml

From most organs 2 mm thick slices were cut and put into the fixative. The mesentery was fixed as a whole, fragments of skin were extended on cardboard. Lobes dog livers were also prepared by perfusion through branches of the portal vein. Perfusion through the carotid arteries was used in some instances for the tongues of dogs, cats and rats.

The stains used were toluidine blue or Ehrlich's tionnine in aqueous solution. The pH was brought to 4,0 by adding acetic acid.

RESULTS

Analysing the effects of varicus fixatives upon mast cells from several tissues of different animals, a great variability of response was revealed.

While in the dog, for instance, after formaldeyde fixation, a few mast cells were preserved in the skin, all were destroyed in both liver and lung tissue. In rats, mast cells of all tissues were readily preserved by any of the employed agents. Among all fixatives used in all the species studied, those containing lead subacetate proved the most effective ones. It was found, however, that aqueous solutions, such as Holmgren and Wilander's, besides having less penetrating capacity, cause grannules to aggluttinate and the metachromatic substance to appear as pericellular halo. These disadvantages are eliminated when alcoholic solutions are used.

If there is too high a concentration of the lead salt, the granula appear blurred and crowded, they may loose their metachromasia and even be dissolved when acetic acid concentration becomes too high. The presence of formalin cau-

ses aggluttination of granula and diffusion of the metachromatic substance. The addition of 0,5% of acetic acid to a 1% alcoholic solution of lead subacetate appeard to be the most suitable mixture not interferring with cell morphology and allowing for a long storage period.

A preliminary observations showed that embedding in paraffin, is very damaging and let us to work only with frozen sections.

The following differences were found for the various tissues and animals:

Cattle and **Horses** — Mast cells of all tissues were destroyed by formalin, Zenker's fluid and Susa, were poorly fixed by fluids of Bouin, Dubosq Brasil, Gendre and Carnoy, and only fair results with Ehrlich's and Helly's solutions. Aqueous solutions of lead subacetate will fix mast cells, but cause granules to aggluttinate. Of the alcoholic solutions used, mixture "D" yielded the best results.

Swine — Only compounds of lead subacetate were found suitable for the fixation of mast cells in these animals. Even Ehrlich's solution did not preserve them adequately.

Dogs — Again, best results were obtained with lead subacetate compounds. In the skin, some mast cells can be preserved by Helly, Carnoy, formalin and picric acid compounds. In the liver, tongue and mesentery, however, all but lead subacetate solutions failed to give any favourable images. It was observed that the mast cells of the skin and mesentery of dogs are, as a rule, more resistant to the action of fixative agentes than are those of the liver. The method of perfusion, used for the fixation of tongue and liver in dogs, was found to be most, suitable for the preservation of mast cells.

In the **Cat** and **Guinea-pig** mast cells were preserved only by agents containing lead subacetate. Solutions containing picric acid, mercurium bichloride or formalin will fix some mast cells in the skin, though not satisfactorily.

FIXATIVES	Bov.	Equ.
Formalin	+	+
Bouin	+	+
Helly	+	+
Carnoy	+	+
Susa	−	−
Dubosq Brasil .	+	+
Gendre	+	+
Ehrlich	+	+
Zenker	−	−
Holmgren & Wilander ..	++	++
Chevremont & Comhaire ..	++	++
"D"	+++	+++

− no fixation
+ poor fixation

TABLE I

ANIMALS					
Swin.	Dog.	Cat.	G. pig	Rab.	Rat.
−	−	+	+	−	+++
+	−	+	+	−	+++
+	−	+	+	−	+++
+	+	+	+	−	+++
−	−	−	−	−	+++
+	−	+	+	−	+++
+	−	+	+	−	+++
+	++	+	+	+	+++
−	−	−	−	−	+++
++	++	++	++	++	+++
++	++	++	++	++	+++
+++	+++	+++	+++	+++	+++

++ regular fixation
+++ good fixation

Mast cells of the **Rat**, presented a totally different response, namely, they were fixed in all organs examined by any of the fixatives used. They appear, thus, to have a much greater resistance than those of any other species.

Table I compares the action of mixtude "D" with that of all other solutions used in the present experiments.

The results described above for frozen sections, are true, to a certain extent, also for paraffin sections. However, one should bear in mind, that cell morphology is very often not preserved satisfactorily by this embedding method. No detailed study was made to determine the cause of such undesirable influence. It is probable, however, that temperature in the oven might be one of the main factors.

DISCUSSION

The mast cells of various animals show a difference of behaviour regarding their solubility and fixability in various solutions, strongly suggesting that they should have different structures. This agrees with the fact that there is also a certain variation of response to some cyto-chemical reactives.

Thus, several authors[7,11] had already noticed this phenomenon, later confirmed by SILVÉN[8] who showed that mast cell granules of rats are extremely resistant to aqueous solutions, while those of rabbits are very succeptible. Through the present study we may extend these data to other animals. In our experiments the granula of mast cells of rats, guinea-pigs and cats resisted better to the various solutions employed than those of cattle and horses; those of pigs, dogs and rabbits were most easily destroyed.

This knowledge bears great importance. Failing to recognize this variation several authors[15,16,17] reported that no mast cells were present in the tissues of rabbits, which we know today is not true. They are, we know now, destroyed by the use of aqueous fixatives. When fixed adequately, mast cells can be seen in almost all tissues of the rabbit. Besides there are differences of fixability according to the tissue of origin. Mast cells found in the skin and mesentery are more

stable than those from the liver and tongue. In addition, HOLMGREN [18] observed that age also had its influence; he was unable to preserve mast cells from human embryos with any other fixative but these containing lead-subacetate.

That mast cells from different species are not similar is also shown by some investigators [19 20] who reported that the subcutaneous mast cells of rats react positively to P.A.S. (periodic acid Schiff), while, according to Compton [21] those of the hamster react negatively. This behavior could be explained supposing that some mast cells contain a trisulphuric form of heparin and others a monosulphuric one, for, according to certain authors [19 22] trisulphuric heparin is P.A.S., positive, while the latter reacts negatively, owing to three of its oxydrils being sterified.

COMPTON [21] working with hamsters, observed that hialuronidase did not destroy the metachromatic properties of mast cells. He concluded therefore, that these cells did not contain hialuronic acid. This opposes the views of others [23 24 25 26] concerning mast cells of man and rats.

This diversity of behaviour in connection with different fixing agents not being, as yet, studied satisfactorily, has induced many erroneous interpretations, not only, as described above, from the morphological point of view, but also with regard to mast cell physiology. In the past, several investigators, observing at times a metachromatic halo around these cells, ,thought they were seeing a phase of secretion, and EHRLICH[1], and DOWNEY[27] were the first to suggest that these aspects might well be artificial. We know now that they are frequently observed in the dog, when the mast cells have not been properly fixed. In rats, it is rare to find such artificial aspects owing to improper fixation; frequently however, some cells are ruptured by rough handling and the granules shed, thus appearing to be extracellular. DEVITT et al. [28] drew attention to the occurrence of such phenomena, caused by laboratory technique. Not knowing, however, that there are substantial differences between the mast cells of

different species, they tried erroneously to extend their findings in rats to all animals.

Alterations likely to be caused by embedding processes should also be kept in mind. MICHELS [10] noted that in lower vertebrates neither paraffin was nor celloidin were suitable for the preservation of mast cells. The experiments reported in this paper allow us to state that this is true also for higher vertebrates. It was not attempted here to determine the factor or factors rendering there process inconvenient.

The results found in the present report bring out the importance of using an adequate fixative when studying the morphology or physiology of mast cells. There is no doubt that, should we review the experiments carried out up to the present, employing a more suitable fixing solution, more light would be cast upon this matter and many a doubtful point clarified.

SUMMARY

After revision of the literature, the authors present a study about mast cell fixation in several times of domestics and laboratories animals.

It was experimented several fixatives habitually used and some mixture with lead subacetate in alcoholic solution.

The authors obtained good results in freezer sections with the following mixture:

Alcohol 50°	100,0 ml
Lead subacetate	1,0 g
Acetic acid	1,0 ml

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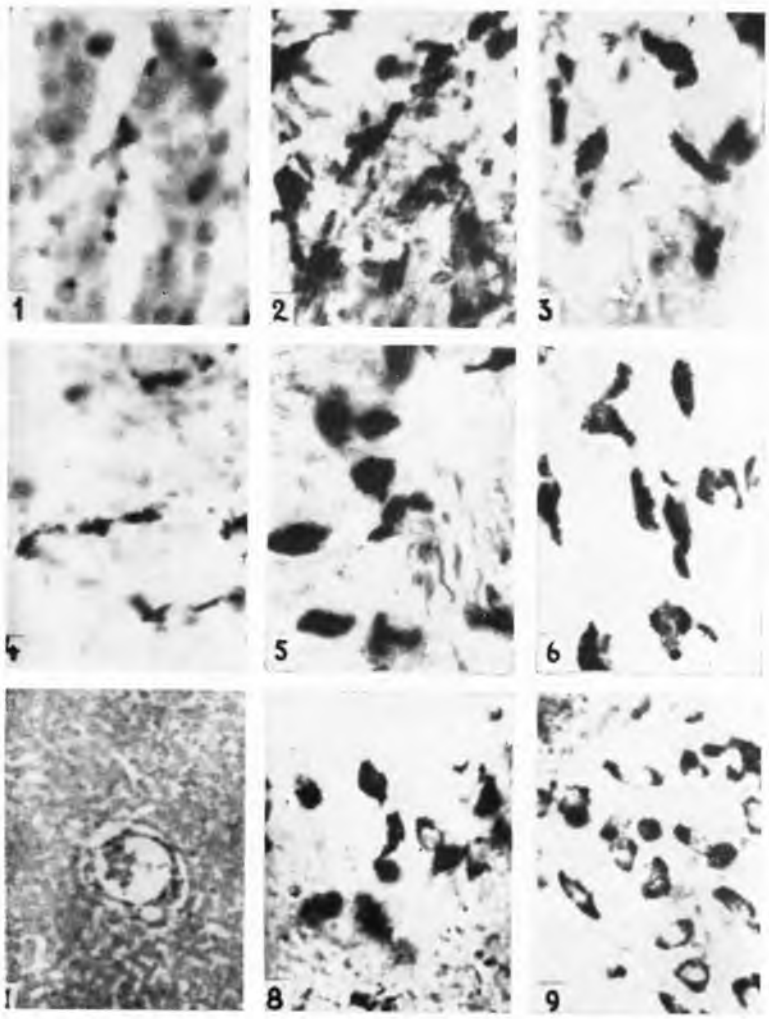

Fig. 1 — Pig's liver. Mast cells have not been preserved. Formalin. Toluidine blue. 400 X.
Fig. 2 — Pig's liver. Granules of the mast cells were bad fixed. Holmgren-Wilander. Toluidine blue — 1.000 X.
Fig. 3 — Pig's liver. Good fixation of the mast cells. Mixture "D". Toluidine blue — 1.000 X.
Fig. 4 — Bovine's liver. Mast cells were bad fixed. Formalin. Toluidine blue. 400 X.
Fig. 5 — Bovine's liver. Mast cells were bad fixed. Holmgren-Wilander. Toluidine blue. 1.000 X.
Fig. 6 — Bovine's liver. Good fixation of the mast cells. Mixture "D". Toluidine blue. 1.000 X.
Fig. 7 — Dog's liver. Mast cells have not been preserved. Formalin. Toluidine blue. 100 X.
Fig. 8 — Dog's liver. Mast cells were bad fixed. Holmgren-Wilander. Toluidine blue. 1.000 X.
Fig. 9 — Dog's liver. Good fixation of the mast cells. Mixture "D". Toluidine blue. 1.000 X.