

Influence of Compound 48/80, Protamine and Other Substances on Rabbit Blood Basophils *in vitro*.^{*} (24882)

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Basophil leucocytes and tissue mast cells have some morphological similarities and have been claimed to be functionally related(3,6,7). Both cells have been reported to contain histamine(8,13), heparin or heparin-like substances(9,10) as well as hyaluronic acid(1,4). In certain species, tissue mast cells contain serotonin (5-hydroxytryptamine)(5). Asboe-Hansen and Glick(2) showed that, *in vitro*, the histamine liberator substance 48/80 and protamine sulfate prevented metachromatic staining of rat peritoneal mast cells. These agents and serotonin caused clumping of the cells. Hyaluronidase partially removed the granules of mast cells. *In vivo*, compound 48/80 brings about a decrease in blood basophil count in rabbits(6). The present work was undertaken to study the influence of substances related to mast cell physiology on blood basophils *in vitro*.

Methods. Rabbits with high basophil counts were chosen. Blood obtained from ear vein and collected in tube containing dried anticoagulant mixture of ammonium and potassium oxalate was used. Fifty μ l of blood was added to 50 μ l of the agent dissolved in sterile Tyrode solution at pH 7.4. After mixing, blood agent mixtures were incubated at 37°C for 20 minutes. Thereafter, a sample of blood agent mixture was diluted 1:5 with modified(6) Moore and James toluidine blue diluting fluid(11). To control the possible effect of plasma protein parallel experiments were performed using blood corpuscles washed 3 times with sterile Tyrode solution. Chamber counting of all white blood cells and of basophils was performed as previously described(6). In each sample 200 basophils or more were counted. Controls were carried out using similar volume of sterile Tyrode so-

lution at pH 7.4 for incubation with blood. The agents used are listed in Table I.

Results. Control basophil and total white cell counts were considered 100%, and to this the other counts were related. There was no apparent difference between whole and washed blood. Both compound 48/80 and protamine sulfate caused a decrease in number of basophils but not in total white cell count. The same effect was observed whether incubation with either agent was performed before or after staining with toluidine blue. Histamine, serotonin and hyaluronic acid which are supposed to be contained in mast cells, did not influence the count of basophil leucocytes or of white cells. As seen in Table I, basophil and total white cell counts were lower in blood samples stained with toluidine blue after incubation with heparin. If stained with gentian violet acetic acid solution, the total white cell count did not differ from control count. Normal counts were also observed after incubation with histamine antagonists mepyramine and pyribenzamine, the serotonin releaser reserpine, and with testicular hyaluronidase.

Discussion. In some respects, the results of this experiment accord with that performed by Asboe-Hansen and Glick(2) on isolated rat peritoneal mast cells. The influence of histamine releaser compound 48/80 and of protamine on basophils, was like that on mast cells, destaining and preventing metachromatic staining of their granules. Both substances are known to have a certain affinity for acid mucopolysaccharides(12). Hyaluronidase did not break down the granular content. Heparin, an acid mucopolysaccharide with strong affinity for metachromatic stains, when added to blood sample, appeared to bind most of the toluidine blue. Consequently, the rest of the dye was unable to render all basophils or other leucocytes detectable. A control experiment involving gentian

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TABLE I. *In Vitro* Influence of Various Agents on Basophil and Total White Blood Cell Count in Rabbit Blood.

Agent	Final conc., %	Counts in % of control	
		Basophils	Total leucocytes
A. Incubation before toluidine blue staining:			
Heparin* (110 i.e./mg)	.5	76	39
Histamine hydrochloride	"	102	104
Serotonin creatinine sulphate†	"	113	90
Hyaluronic acid‡ (umbilical cord)	"	100	80
Protamine sulphate§	"	15	107
Mepyramine	"	99	89
Pyribenzamine	"	102	101
Compound 48/80¶	"	8	115
Reserpine	"	99	110
Testicular hyaluronidase‡ (100 VRU/mg)	1.0	97	103
<i>Idem</i> ‡ (3000 ")	.1	109	104
" (heated at 70°C, 40 hr)	"	86	99
B. Incubation before gentian violet staining:			
Heparin*	.5		104
C. Incubation after toluidine blue staining:			
Protamine sulphate§	.5	43	105
Compound 48/80¶	"	0	90

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violet staining substantiated this view.

Summary. Metachromatic stainability of rabbit basophils was prevented by compound 48/80 and protamine. If already stained with toluidine blue, the stain was abolished. Heparin apparently combined with the toluidine blue stain, thus accounting for a decrease in counts of basophils and other white cells. Hyaluronic acid, histamine, serotonin, as well as testicular hyaluronidase, antihistamines mepyramine and pyribenzamine, serotonin releaser reserpine, produced no change in count of basophil leucocytes or other white blood cells.

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