Newcastle disease virus, may be involved in the etiology of infectious mononucleosis. A further suggestion that the serologic reactions reported here are not explained by Sendai virus infection *per se* lies in the fact that many studies have been carried out in this country in which attempts at isolation of virus from patients with non-bacterial respiratory infections, viral pneumonitis, and infectious mononucleosis have employed chicken embryos and mice in a manner that would be expected to result in isolation of Sendai virus if it were present. Sendai virus is also cultivated quite readily in several lines of tissue culture cells (16) frequently used in such studies in recent years. Failure to isolate this virus suggests that one, or perhaps several, much more fastidious agents may be responsible for the observed antigenic stimulation.

Summary. Measurement of antibodies in acute and convalescent serums from college students hospitalized because of acute, non-bacterial respiratory infections revealed 5 cases of influenza-like illness and 6 cases of viral pneumonia with 4-fold or greater increases in reactivity with Sendai virus measurable by hemagglutination-inhibition and neutralization *in ovo*. Thirteen of 110 patients hospitalized with the diagnosis of infectious mononucleosis were found to develop increases in serum reactivity with Sendai virus during their illnesses. Efforts to isolate a virus from throat washings of 17 of these patients were unsuccessful. The possible role of Sendai virus in the etiology of these illnesses and the significance of the antibody responses were discussed.

1. Kuroya, M., Ishida, N., *Yokohama M. Bull.*, 1953, v4, 217.

2. Fukumi, H., Nishikawa, F., Kitayama, T., *Jap. J. M. Sc. and Biol.*, 1954, v7, 345.

3. Sasahara, J., Hayashi, S., Kumagai, T., Uamamoto, Y., Hirasawa, N., Munekata, K., Okaniwa, A., Kato, K., *Virus*, 1954, v4, 131.

4. Gerngross, O. G., *Problems in Virology*, 1957, v2, 71.

5. Gorbunova, A. S., Gerngross, O. G., Gnorizova, V. M., Bukrinskaia, A. G., *ibid.*, 1957, v2, 76.

6. Jensen, K. E., Minuse, E., Ackermann, W. W., *J. Immunol.*, 1955, v75, 71.

7. DeMeio, J. W., Walker, D. L., *J. Immunol.*, 1957, v78, 465.

8. Gardner, P. S., *Brit. M. J.*, 1957, v1, 1143.

9. Committee on Standard Serological Procedures in Influenza Studies, *J. Immunol.*, 1950, v65, 347.

10. Reed, L. J., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

11. White, G. B. B., Gardner, P. S., *Brit. M. J.*, 1957, v1, 381.

12. Sommerville, R. G., Carson, H. G., *ibid.*, 1957, v1, 1145.

13. Chanock, R. M., Parrot, R., Cook, K., Andrews, B., Bell, J., Reichelderfer, T., Kapikian, A., Mastrota, F., Huebner, R. J., *New England J. Med.*, 1958, v258, 207.

14. Burnet, F. M., Anderson, S. G., *Brit. J. Exp. Path.*, 1946, v27, 236.

15. Evans, A. S., Curnen, E. C., *J. Immunol.*, 1948, v58, 323.

16. Parker, H., Walker, D. L., unpublished observations.

Received April 17, 1958. P.S.E.B.M., 1958, v98.

Influence of Various Agents on Mast Cells Isolated from Rat Peritoneal Fluid.* (24075)

GUSTAV ASBOE-HANSEN† AND DAVID GLICK

Histochemistry Laboratory, Department of Physiological Chemistry, University of Minnesota Medical School, Minneapolis

There is considerable evidence that mast cells contain and are able to release acid mucopolysaccharides, histamine, and, in certain species, serotonin(1). Mast cell degranulation with loss of metachromatic material is effected by histamine-liberator substances *in*

* This study was supported by research grants from N.I.H., U. S. Public Health Service, and Louis W. and Maud Hill Family Fn.

† Present address: Connective Tissue Research Laboratory, Univ. Inst. of Medical Anatomy, Copenhagen, Denmark.

vivo(2). However, Riley *et al.*(3) were not able to demonstrate conformity between liberation of histamine and heparin in such experiments. In cheek pouches of living hamsters Asboe-Hansen and Wegelius(4,5) observed release of mast cell granules not only in response to histamine liberators, but to histamine itself, serotonin, certain physiological fluids, hyaluronidase, and experimentally induced edema. They advanced the hypothesis that degranulation may be a physiological response to edema, and that acid mucopolysaccharide from mast cells binds extracellular water to form mucinous ground substance. The present investigation was designed to supplement such *in vivo* studies by observations on the *in vitro* influence of these agents, along with others, on mast cells isolated from rat peritoneal fluid.

Methods. Using the procedure of Glick *et al.*(6) to obtain suspensions of pure mast cells from peritoneal fluid of adult albino rats, aliquots containing 960-5200 cells/μl were subjected to the influence of various agents which are either known to influence the morphology and function of these cells, or would be expected to. To 15 μl of a mast-cell suspension in Hanks' balanced salt solution, pH 7.4, 14 μl of agent in solution was added, and after 20 minutes at room temperature, 24°C, 30 μl of 0.01% toluidine blue in Hanks' solution was added. After 10 minutes the cells were examined microscopically in a blood cell counting chamber; percentage of cells degranulated was recorded. The agents used were the following: Heparin sodium (Nutritional Biochemicals), 100 units/mg, in Hanks' soln., pH 7.4. Histamine hydrochloride (Hoffmann-la Roche) in Hanks' soln., pH 7.4. Serotonin creatinine sulfate (Aldrich Chemical) in Hanks' soln., pH 7.4. Hyaluronic acid, human umbilical cord, in Hanks' soln., pH 7.4. Bovine testicular hyaluronidase (Parke Davis) 200 VRU (viscosity reducing units)/mg, in 0.05 M phosphate buffer with 0.05 M NaCl, pH 7.0. Protamine sulfate (Squibb) in Hanks' soln., pH 7.4. Compound 48/80 (Burroughs Wellcome) a condensation product of p-methoxyphenethylmethylamine with formaldehyde, kindly supplied by Dr. Edwin J. de Beer, Tuckahoe, N. Y. *Escherichia coli* endotoxin, Boivin type lipopolysaccharide, prepared and kindly supplied by Dr. Wesley Spink, Dept. Medicine, Univ. Minnesota.

Results. The results are summarized in Table I. The finding that protamine and 48/80 prevent metachromatic staining of mast cells confirms the results of Padawer and Gordon(7) on air-dried and methanol-fixed smears of peritoneal fluid. In our experiments these agents not only prevented staining by toluidine blue, but removed the dye from previously stained mast cells. In both instances the granules were retained. The decolorizing effect is probably due to binding of acid mucopolysaccharide by these agents, as indicated by Mota *et al.*(8). The retention of intact granules is in accord with the finding of Ottoson and Glick(9) that 48/80 produced no demonstrable change in total dry mass per cell as measured by x-ray absorption.

Substances causing edema in mammalian tissues such as histamine, serotonin(10), and *E. coli* endotoxin(11) did not influence the mast cells *in vitro*. This is in agreement with the conclusion of Asboe-Hansen and Wegelius(4,5) that mast cell degranulation *in vivo* may result from tissue edema.

Free extracellular granules, observed in all suspensions of the mast cell, may be due to some mechanical disruption during isolation. In these experiments, as well as in the *in vivo* studies cited, a certain degree of degranulation was observed in buffer or Hanks' solution without agent. A minimum of degranulated cells was observed in the hyaluronic acid solutions.

Testicular hyaluronidase left a spongy or honeycomb-like non-metachromatic structure in isolated mast cells. The heated enzyme lacked this property. When rats were pretreated with an intravenous injection of 2 mg *E. coli* endotoxin 20 minutes before sacrifice, their mast cells had almost no metachromatic granules after hyaluronidase treatment, in contrast to controls. *In vitro,* however, endotoxin did not influence the cells. It should be borne in mind that heparin, not only is unaffected by hyaluronidase, but is an inhibitor of the enzyme. The results lend some evidence to a standing discussion on different

TABLE I. Influence of Various Agents on Mast Cells Isolated from Rat Peritoneal Fluid.

Agent	Final conc. (%)	Influences
Heparin (100 units/mg)	.5	Disintegration of all cells
	.05	*Idem*
	.005	Disintegration of some cells
	.0005	Same as in control Hanks' solution
Histamine	.5	*Idem*
	.05	"
Serotonin	.5	Cell clumping
	.05	*Idem*
Hyaluronic acid	.5	8% of cells partially degranulated
	.05	10% *Idem*
Testicular hyaluronidase (200 VRU/mg)	1.0	76% of cells totally degranulated, no cell disintegration
	.25	60-73% *Idem*
		E. coli endotoxin treated rats: 89-90% of cells totally degranulated, no cell disintegration
Bovine testicular hyaluronidase (65°C, 48 hr)	1.0	36% of cells partially degranulated, same as in phosphate-saline control
Protamine	.5	Cell clumping, no staining, degranulation or disintegration
	.05	*Idem*
Compound 48/80	.5	"
	.05	"
E. coli endotoxin	.5	Same as in control Hanks' solution
	.05	*Idem*
Phosphate buffer, .05 M with .05 M NaCl, pH 7.0		35% of cells partially degranulated
Hanks' solns., pH 3 to 9		31-50% *Idem*

morphological findings on fixed tissue mast cells(12-15), *e.g.*, Morris and Krikos(14) reported no effect by bovine testicular hyaluronidase preparations at pH 5-7 on metachromasia of mast cells in sections of hamster cheek pouch. At low pH the enzyme solutions, whether or not heat inactivated, prevented the metachromatic staining. In mast cells resistant to hyaluronidase, heparin may be exerting its inhibitory effect on the enzyme (1).

Influence of pH was ruled out in our experiments; no morphological changes differing from controls were observed with any of the agents in suspensions of mast cells in Hanks' solution adjusted to pH values from 3 to 9.

Summary. *In vitro* studies on mast cells isolated from rat peritoneal fluid revealed: Metachromatic granules were partially removed by testicular hyaluronidase, but when rats were pretreated systemically with *Escherichia coli* endotoxin, 2 mg iv., this effect of hyaluronidase was much more extensive. *E. coli* endotoxin, 0.5%, did not influence the mast cells *in vitro*. Metachromatic staining of the cells was prevented, and if previously stained the color was abolished, by compound 48/80 or protamine sulfate. These agents and serotonin, 0.5%, neither degranulated nor disrupted the cells, but they produced cell clumping. Heparin, over 0.005% caused cell disintegration, whereas 0.5% hyaluronic acid or histamine did not appear to affect the cells adversely. Hyaluronic acid seemed to protect them to some degree against degranulation.

1. Fulton, G. P., Maynard, F. L., Riley, J. F., West, G. B., *Physiol. Rev.*, 1957, v37, 221.

2. Fawcett, D. W., *Anat. Rec.*, 1955, v121, 29.

3. Riley, J. F., Shepherd, D. M., Stroud, S. W., West, G. B., *Nature*, 1955, v176, 1123.

4. Asboe-Hansen, G., Wegelius, O., *Acta Physiol. Scand.*, 1956, v37, 350; *Nature*, 1956, v178, 262.

5. Wegelius, O., Asboe-Hansen, G., *Exp. Cell. Res.*, 1956, v11, 437.

6. Glick, D., Bonting, S. L., den Boer, D., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 357.

7. Padawer, J., Gordon, A. S., *Anat. Rec.*, 1955, v121, 411.

8. Mota, I., Beraldo, W. T., Junqueira, C. U., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 455.

9. Ottoson, R., Glick, D., *Exp. Cell Res.*, 1958, in press.

10. Benditt, E. P., Wong, R. L., Arase, M., Roeper, E., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 303.

11. Thomas, L., *Ann. Rev. Physiol.*, 1954, v16, 467.

12. Compton, A. S., *Am. J. Anat.*, 1952, v91, 301.

13. Hill, M., *Experientia*, 1957, v13, 395.

14. Morris, A. L., Krikos, G. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 527.

15. Velican, C., Velican, D., *Acta haematol.*, 1958, in press.

Received May 7, 1958. P.S.E.B.M., 1958, v98.

Influence of Cooling and CO_2 Content of Blood on Bleeding Time.* (24076)

W. O. CRUZ AND A. C. OLIVEIRA

Laboratory of Hematology, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

Application of a physiological method using hind-limb preparation for studies of bleeding time(1), revealed that certain procedures, such as shorter or larger aeration or more careful and longer refrigeration of blood samples, had an effect on this phenomenon. A systematic study of these effects is here presented.

Methods. As details of the method employing hind-leg of dog for study of bleeding time are in press, including hematocrit, hemoglobin, platelet and clot retraction determinations, we present here a summary of this method. *Hind leg preparation*: Incision of approximately 12 cm on inner side of thigh, beginning at crural arch and following inner edge of sartorial muscle. Dissection of artery, vein and femoral nerve. Isolation of the saphenous branch of the artery and femoral vein, tying off collaterals. Attach 2 threads proximal and distal, to the saphenous branch. Intravenous injection of Liquémine (325 UI Heparin), 0.65 ml/kg of body weight. Plastic tubes were inserted into femoral artery and vein of the heparinized dog and the leg isolated by tourniquet at base of thigh, leaving free the artery, vein and femoral nerve. Arterial blood (citrated) to be tested was forced from a flask by air under variable pressure into the arterial inflow tube and collected as it dripped from the venous outflow. Flow rate was kept constant and controlled by varying flask pressure to maintain an average drop rate of 50 ± 10/minute. Bleeding time was determined on shaved thigh by making 2 shallow razor cuts below incision and wiping away the blood continuously with absorbent paper until bleeding stopped. Blood gas determinations were made with manometric apparatus of Van Slyke(2). For pH determination a direct pH reading meter was used (Electronic Instruments, England). Tests for accuracy were made several times during each experiment with appropriate buffer solution. Blood was aerated by shaking in open flask for half hour. Blood samples previously aerated were restored to normal CO_2 content by agitation 3 hours in flasks containing air plus 9% CO_2. When not in use blood samples were under petroleum-jelly. Blood was refrigerated in environment of water and ice cubes and kept between 1° and 2°C, with occasional stirring, in refrigerator for a half hour; reheating was gradual, leaving blood at room temperature for about half hour, then in water-bath at 37°C, 3 hours. Refrigeration of blood from heparinized dog (1593) and reinjection of this blood after previous rewarming was done as follows: 100 ml of blood was taken from the carotid and refrigerated under petroleum jelly at 1° to 2°C for 15 minutes, then reinjected into saphenous vein after passing a glass coil immersed in water-bath at 40°C. This procedure was repeated every 15 minutes until approximately a whole blood volume of the dog was thus treated.

Results. Cooling Experiments. Normal citrated blood samples from 11 dogs (normal

* This work was aided by Nat. Research Council and by grant from Dr. Guilherme Guinle.