in control subjects. 3. Neither age nor the presence or absence of obesity appeared to be related to the level of pressor activity in the urine in any of the groups studied.

1. Heineman, A. C., Jr., Danowski, T. S., Am. J. Med. Sci., 1960, v239, 167.
2. Hagedorn, H. C., Jensen, B. N., Biochem. Z., 1923, v135, 46.
3. Wallace, J. M., Harlan, W. R., Am. J. Med., 1965, v38, 531.
4. McKendry, J. B. R., Canad. Med. Assn. J., 1957, v76, 572.
5. Sackner, M. A., Balian, L. J., Am. J. Med., 1960, v28, 135.
6. Yonet, H. M., Ballard, H. S., New York J. Med., 1961, v61, 1939.
7. Schwartz, J. F., J.A.M.A., 1961, v176, 106.
8. Cushman, P., Jr., Am. J. Med., 1963, v35, 196.
9. Cherner, R., Croppe, C. W., Rupp, J. J., J.A.M.A., 1963, v185, 883.
10. Spurny, O. M., Wolf, J. W., Devins, G. S., Arch. Int. Med., 1965, v115, 53.
11. Bergman, H., Acta Med. Scand., 1965, v177, 287.

Received June 17, 1965. P.S.E.B.M., 1965, v120.

Mast Cell Degranulation in Fixed Preparations.* (30523)

JACQUES PADAWER (Introduced by W. Etkin)
Department of Anatomy, Albert Einstein College of Medicine, New York City

Degranulation of mast cells has been reported to occur in response to a variety of pathological and experimental conditions. On the one hand, it was said that it is a *sine qua non* of massive histamine release(1). On the other hand, it has been maintained that histamine release can occur without mast cell degranulation(2,3).

Much of the literature dealing with mast cells is based on studies of histological preparations where it is assumed that fixation precludes subsequent cytological changes. Whereas this may be essentially so for many cell types, we have found that it is not for mast cells after methanol fixation as commonly practiced.

Materials and methods. Rat peritoneal fluid preparations were made either by allowing a spread drop to dry on the slide or by a brush technique(4). When dry, they were fixed in methanol for 10 minutes as commonly done in hematology. Methanol fixation up to 14 days was also used to insure complete fixation. The cells were again air-dried and a coverslip was applied on the dry preparation. The cells were then examined microscopically at magnifications of 400 or 600 times with phase or with interference contrast (Nomarski optics) and individual cells were kept under observation while various reagents were introduced under the coverslip. Basically, the following conditions were examined: a) rehydration of the fixed cells with various aqueous media and b) substitution of the aqueous medium of a) with either aqueous toluidine blue or a saline solution of the histamine liberator Compound 48/80.† Direct measurements of cellular diameters were made with an ocular filar micrometer. Observations on individual granules also were made. Time-lapse cinephotomicrographic recordings were obtained‡ from which rapid changes in individual cells and granules could be followed reliably. The measurements of diameters are not readily translatable into volumes because of the extreme flattening of the cells in these preparations.

Results. Rehydration with distilled water, saline, or dilute aqueous toluidine blue almost

* Supported by grant from Am. Heart Assn.

† I thank Dr. S. W. Singleton, Burroughs Wellcome and Co. (USA), Tuckahoe, N. Y. for supplies of Compound 48/80, a polydisperse condensation product of N-methylhomoanisylamine with formaldehyde.

‡ I thank Dr. R. Robineaux, Centre de Recherches d'Immuno-Pathologie de l'Association Claude Bernard, Hôpital St. Antoine, Paris, for receiving me in his laboratory, where the time-lapse sequences were obtained during part of my sabbatical leave (Dec. 1963-July 1964).

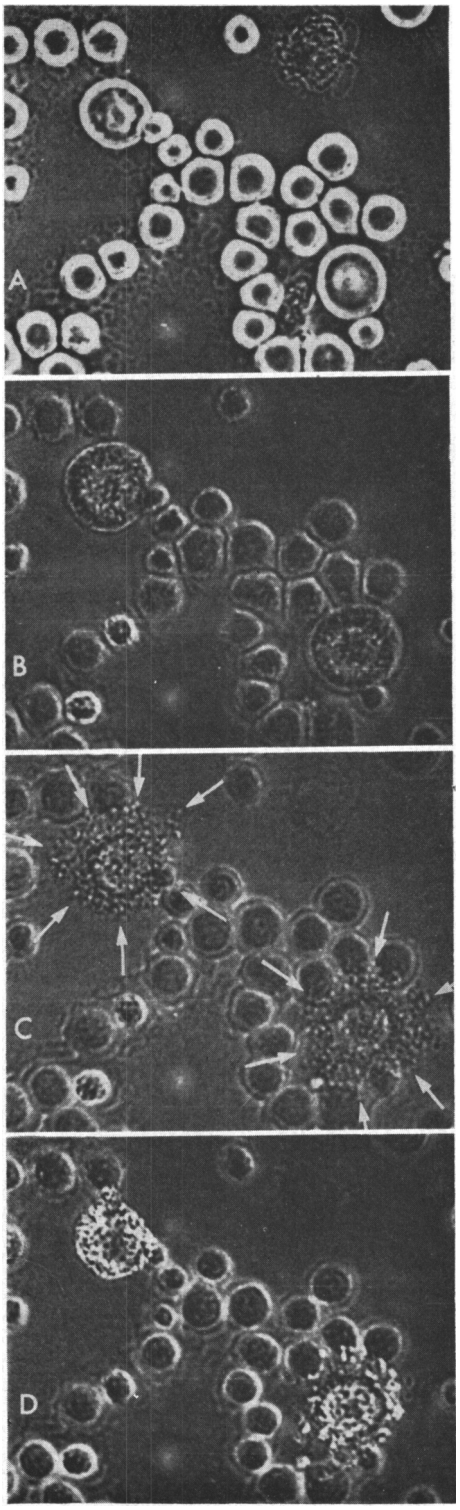

FIG. 1. Two air-dried, methanol-fixed mast cells as seen A) in air, B) in saline within 30 sec after immersion; the increase in size is more than can be accounted for by the change in refractive index of the medium, C) 6 min after immersion in saline; note severe degranulation of both mast cells (arrows), and D) within 30 sec after substitution of saline by Cpd 48/80 in saline. Cpd 48/80 causes an increase in refractility of the granules and some shrinkage of the swollen cells. One mast cell (upper left) reapproximates its initial size and shape; the other remains clearly degranulated. No further change occurred during the ensuing 10 min. From a time-lapse motion picture recording; phase microscopy × 660.

TABLE I. Swelling of Fixed Mast Cells During Exposure to Water. Example from visual observation.

Conditions	Measured diameter* Cell No. 1	Cell No. 2
Dry, in air	1.27	1.81
Distilled H_2O, 30 sec	1.45	1.92
idem , 10 min	1.60	1.99
" , 15 "	1.63	1.98

* Arbitrary units (filar micrometer).

invariably causes rapid swelling of the mast cells and of their granules (Table I). The cells swell, up to 18% increase in diameter within 20-30 seconds and, at 10 minutes some mast cells are as much as 65% larger in diameter (Fig. 1A-C). As the mast cell granules swell, they are crowded outwards beyond the original boundary of the cell. Many of the granules exhibit Brownian movement, and some occasionally become detached from the cell and drift out of the field of observation.

When cells that have been swollen by distilled water or saline are subsequently immersed in aqueous toluidine blue or in aqueous 48/80, a rapid shrinkage occurs (Table II). In some, the granules are retracted and the cells regain their initial outline. In those cases where the granules become completely retracted, the mast cells again appear relatively normal in morphology (Fig. 1D, left). Other cells do not return to their original shape (Fig. 1D, right). Where granules are not retracted, the final appearance is that of a typically degranulated cell. Mast cell granules shrink when exposed to toluidine blue or 48/80, independently of retraction.

The degree of delicacy with which the aqueous medium is applied to the preparation also plays a role in determining the final

TABLE II. Shrinkage of Preswollen Mast Cells upon Exposure to Cpd 48/80. Example derived from Movie Sequence.

Conditions	Measured diameter* Cell No. 1	2	3	4
Rehydrated in saline	44	47	55	70
Cpd 48/80, 1 mg/ml saline, 5 sec	44	43	51	63
idem 10 "	41	39	49	58
" 15 "	39	34	46	58

* In millimeters on projection screen. Error, calculated from replicate measurements of a given frame (Time and Motion Study projector) is, at most, 4% of recorded value.

cytological appearance. For instance, if the media are introduced gently under the coverslip, the cells swell, or shrink as the case may be, equally all around. On the other hand, if the toluidine blue or 48/80 solution is flushed over the rehydrated cells rather than introduced gently, mast cells are often torn apart with consequent massive displacement of granules (Fig. 2). This also contributes to mast cell degranulation.

Macrophages and eosinophilic leukocytes are present in these preparations. In contrast to mast cells, these cell types, after methanol fixation, exhibit no change in size or shape and are not disrupted by any of the manipulations described above. Eosinophil granules also remain unaffected.

Discussion. Air drying followed by methanol fixation and staining with dilute aqueous toluidine blue is often used in studies of both mouse and rat mast cells. The metachromatic characteristics of mast cells in many species are not retained after aqueous fixation. However, both fixed and unfixed rat mast cells are believed to be particularly resistant to aqueous media and, indeed, possess a marked ability to retain their metachromasia when exposed to water(5,6). Metachromasia, because it is the principal identifying feature of mast cells, has received particular attention in the literature and has often been used as a criterion of good preservation. However, it is clear from this study that although rat mast cells retain their metachromasia, their cytological integrity is nevertheless severely compromised during exposure to aqueous media. It follows that the size of the cell as well as the variable size of granules, as seen in histological preparations, has little physiological significance and that staining intensity, even under standardized conditions, will be altered significantly.

Alternate swelling and shrinkage of *live* mast cells exposed to either toluidine blue, protamine sulfate or Cpd 48/80 has been described(7). The similarity of behavior between the live mast cells as described by Smith and the methanol-fixed mast cells as described above deserves further scrutiny. It may reflect the presence of a methanol resistant contractile system.

Degranulation of mast cells is commonly observed histologically. In preparations made from tissues of intact normal rats, it has been my personal experience that gentle manipulation insures a minimum of degranulated cells. Indeed, it has been debated whether mast cell degranulation ever represents a

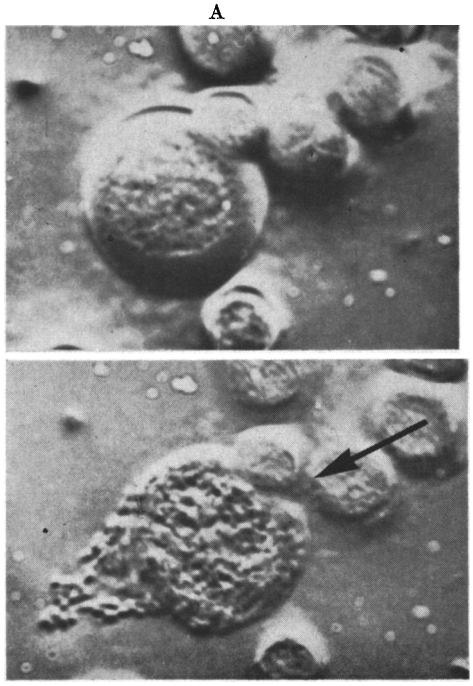

FIG. 2. Mechanical disruption of a rehydrated mast cell by Cpd 48/80. A) Cell viewed in saline shortly before contact with 48/80. The 48/80 solution was made to flood the preparation in the direction of the arrow (B). Note how refractility of granules increases and how many of them are carried away in the process. From a time-lapse cinephotomicrographic sequence. Nomarski interference-contrast optics, × 1425.

normal physiological process(8). This question is far from settled.

Under some experimental conditions, such as injection of noxious substances, *i.e.,* Cpd 48/80(9); stilbamidine(10); ovomucoid in the rat(11); Russel viper venom(12), etc. . ., mast cell degranulation is widespread in the tissues. There is thus little question that explosive changes can be elicited experimentally and that these may approximate pathological events as they may occur after snake bite or in anaphylaxis.

But is degranulation the way mast cells perform whatever function they normally subserve in connective tissues? The question remains unanswered; it is the purpose of this paper to underscore that in seeking the answer, reliance on histological preparations alone may lead to erroneous intrepretations or conclusions.

Summary. The present study indicates that significant morphological changes in mast cell size, shape, and granule size can occur following conventional fixation, and that these fixed cells are also particularly prone to damage by mechanical manipulations inherent in standard histological techniques. In all cases where mast cell degranulation has been described, these factors deserve serious consideration to differentiate fact from artifact.

1. Mota, I., Round Table Discussion, in Conference on Mast Cells and Basophils, J. Padawer, Ed., Ann. N. Y. Acad. Sci., 1963, v103, 441.
2. Smith, D. E., ibid.
3. ———, Am. J. Physiol., 1958, v193, 573.
4. Burke, W. T., Brotherson, G., Harris, C., Am. J. Clin. Path., 1955, v25, 1226.
5. Montagna, W., Eisen, A. Z., Goldman, A. S., Quart. J. Microsc. Sci., 1954, v95, 1.
6. Paff, G. H., Mergenthaler, D. D., Anat. Rec., 1955, v121, 579.
7. Smith, D. E., Science, 1958, v128, 207.
8. Round Table Discussion, in Conference on Mast Cells and Basophils, J. Padawer, Ed., Ann. N. Y. Acad. Sci., 1963, v103, 441.
9. Riley, J. F., J. Path. & Bact., 1953, v65, 471.
10. ———, ibid., 1955, v69, 269.
11. Benditt, E. P., Bader, S., Lam, K. B., A.M.A. Arch. Path., 1955, v60, 104.
12. Higginbotham, R. D., Round Table Discussion, in Conference on Mast Cells and Basophils, J. Padawer, Ed., Ann. N. Y. Acad. Sci., 1963, v103, 468.

Received June 18, 1965. P.S.E.B.M., 1965, v120.

Failure of Reserpine to Modify the Incidence of Aortic Ruptures Induced in Turkeys by Diethylstilbestrol.* (30524)

CHARLES F. SIMPSON, R. H. HARMS AND B. L. DAMRON
Department of Veterinary Science, University of Florida, Gainesville

Aortic ruptures of turkeys occur under natural conditions, and it has been reported that reserpine is highly effective in reducing mortality(1). The mode of action of reserpine in the disease has not been resolved, although it has been suggested that beneficial effects were derived from its hypotensive rather than tranquilizing property(2).

Numerous reports attest to the ability of diethylstilbestrol (DES) to induce a high incidence of aortic ruptures in turkeys(3,4). Since therapeutic studies have not been included in these trials, it seemed appropriate to determine the influence of reserpine on the incidence of aortic ruptures and hemodynamic responses in turkeys treated with DES. The results of these studies are presented here.

Materials and methods. Experiment 1. Broad-Breasted Bronze male turkey poults were raised by conventional poultry husbandry methods and fed a 20% protein commercial chick-starter mash containing 0.5% sodium chloride until 4 weeks of age. The poults were changed to a 19% protein diet containing 0.5% sodium chloride at 4 weeks, and to a 23% protein diet containing 3%

* Supported in part by a grant from Nat. Heart Inst., U.S.P.H.S. Florida Agri. Exp. Station Journal Series No. 2162.