The "Periosteal Spread" Technic for Study of Mast Cells.* (28077)

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The mast cell is attracting increasingly more interest in connection with studies on release of heparin, histamine, serotonin, anaphylactic and anaphylactoid reactions, calciphylaxis, etc. This cell is easily identified, owing to its distinctive metachromatic granules, but its great fragility predisposes it to damage by fixation and cutting. To avoid such artifacts, numerous tissue-spread technics have been devised. Thin membranes (mesentery, omentum, mesometrium or even skin) can be rapidly fixed after distension over some solid plate or ring and stained for study as whole mounts without the necessity of sectioning. Thereby cell disruption is kept to a minimum and the relationships of vessels, nerves and other tissue components to mast cells can be followed uninterruptedly throughout a large area in proper perspective(1,2,3). Unfortunately, even so, the fragile mast cells tend to discharge their granules while the membrane is spread over a solid object for fixation. The calvarial periosteum of small rodents is very rich in mast cells and, being naturally attached to a solid and virtually flat base, it represents a preformed tissue spread which can be fixed in its normal position without being touched.

Materials and technics. Most of our observations were made on female albino rats of the Holtzman strain with an average initial body weight of 100 g (90-110 g), although preliminary experiments have shown that the technic is equally applicable to smaller or larger rats, as well as to the hamster and mouse. Guinea pigs and rabbits are less suitable because they possess only few mast cells. The skin over the calvarium is carefully shaved with electric clippers, preferably one or two days prior to autopsy. The animals are killed by decapitation to minimize mast cell damage by self-inflicted trauma such as occurs when the rats are killed with chloroform. While the head is held by the snout, the skin covering the calvarium is gently removed after freeing it by a circular incision. The skull cap is detached by 2 lateral incisions made with pointed scissors inserted through the foramen magnum on each side and united between the eyes just behind the nose. The calvarium is then gently lifted up by a pair of forceps applied to the occiput. This bone-plate is immediately placed in the fixative (4 parts of absolute alcohol and 1 part of 10% formaldehyde) without ever touching either its dural or its external surface. After 24 hours of fixation, 1-2 mm of the tip of the frontal bone is broken off by dorsad reflection, with a pair of flat forceps; using this fragment as a handle, the external periosteum can be peeled off as a flat sheet

FIG. 1. Preparation of "periosteal spread." *Left:* After decapitation and careful removal of the skin, the skull cap is freed by a circular incision and lifted up with forceps applied to occiput. *Right:* After fixation, tip of frontal bone is broken off by dorsad reflection with forceps and external periosteum peeled off.

FIG. 2. Typical mastocyte forms found within external periosteum. *A:* General view showing preferential arrangement of mostly fusiform mastocytes in 2 rows along sides of blood vessels, and more polymorph cells at distance from vessels. Most of the large ovoid cells on surface of periosteum are not visible (since, being in another layer, they are out of focus) but a few of them are seen near left upper corner and one (arrow) just behind the largest vessel is visible though slightly out of focus (× 60). *B:* Pear-shaped mast cell with beginning degranulation around pointed pole. *C:* Various bizarre-shaped cells with pseudopodia. *D:* Two cells in the process of shedding part of their granule-laden cytoplasm (clasmatocytosis?). *E:* Fusiform. *F:* Hook-shaped mast cells. *G* and *H:* Two mast cells with plate-like portions

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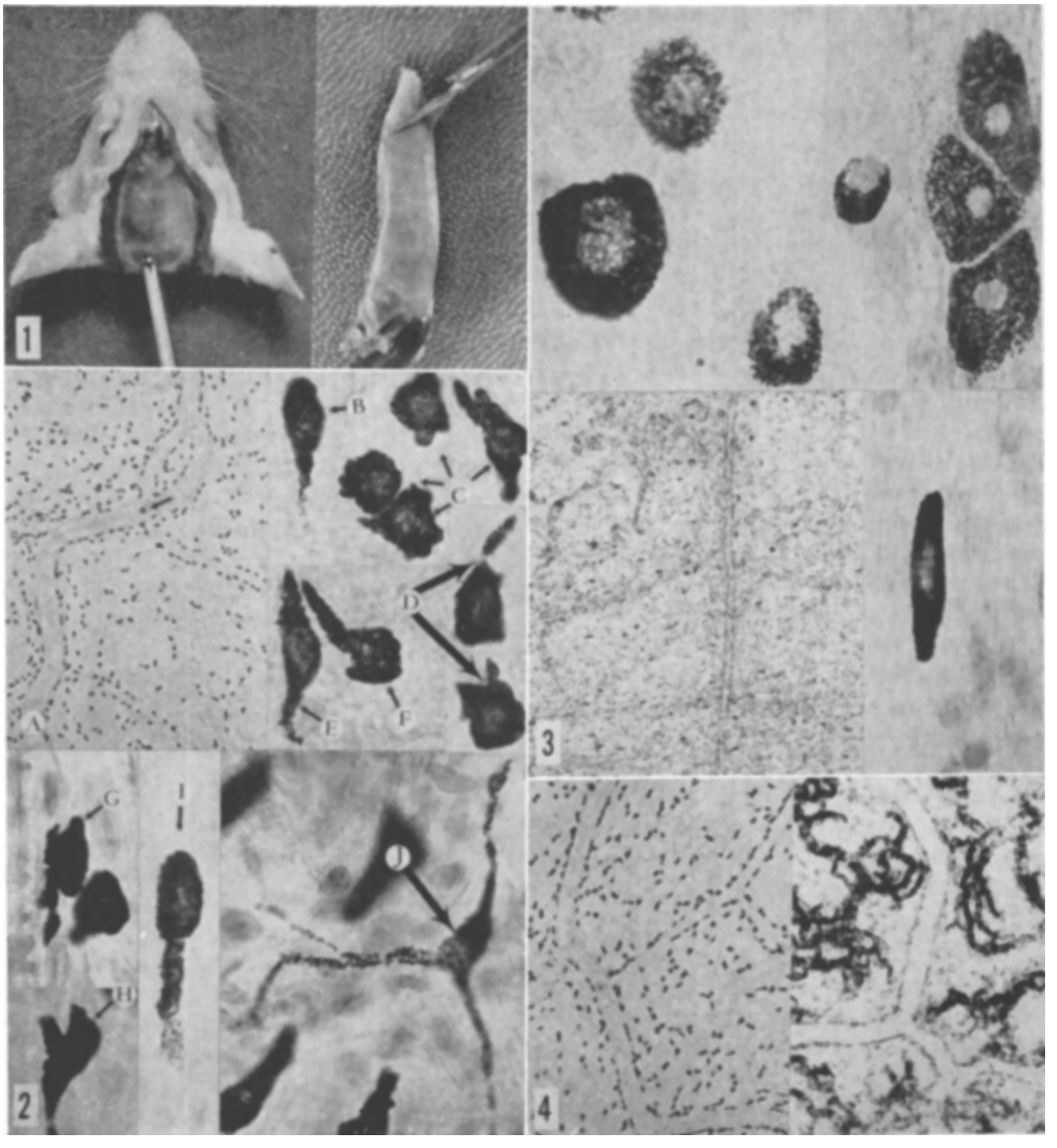

extended towards the faintly visible wall of adjacent vessel. *I:* Comet-shaped mast cell with degranulation in tail region, possibly representing wake in the course of ameboid motion. *J:* Spider-shaped mast cell with metachromatic granules in its 4 elongated processes. (*A* × 60, *B-J*, × 500 under oil immersion; all sections azure A.)

FIG. 3. Mastocytes on surface of external periosteum and in dura. *Top:* On periosteal surface all mastocytes are more or less ovoid and flat, with little or no metachromatic granules in nuclear region. Note great difference in sizes of both cells and granules themselves. At right 3 large mast cells form chain with contiguous surfaces flattened (azure A, × 500). *Bottom left:* General view of dura showing predominantly rod-shaped mast cells without obvious relationship to blood vessels (azure A, × 60). *Bottom right:* Typical appearance of rod-shaped dural mast cell (azure A, × 500).

FIG. 4. Ink fixation in vessels after topical treatment with compound 48/80. External periosteum of 2 rats which received 1 ml of 30% Higgins' American India Ink i.v. Just before this, one animal (right) received 50 μg of compound 48/80 in 0.2 ml of water s.c., above the calvarium. The specimens were fixed 24 hr after these injections. *Left:* Mastocytes are normal; no ink fixation. *Right:* All mastocytes are disrupted but their granules are still distinguishable around the vessels (here black, on slide purple), while adjacent vessels have fixed black ink particles (azure A, × 60).

without risk of subsequent shrinkage (Fig. 1). To remove the dura, the procedure is then repeated with another little frontal-bone segment broken ventrad in similar fashion. The 2 periosteal membranes are placed in dioxane for at least 2 hours, then rinsed in tap water for 2 minutes (longer exposure to water may cause connective tissue swelling), stained in azure A (1:20,000 parts of 30% methylalcohol), dehydrated without rinsing in 2 changes of dioxane, cleared in xylol and mounted between slide and cover slip. Blotting with cigarette paper or filter paper before mounting helps to smooth out small wrinkles. To obtain very thin and flat preparations, it is advisable to remove the thickest regions along the sagittal sinus and the periphery of the spreads and to use only the 2 thinnest portions covering the frontal and parietal bones on each side.

Results. When prepared in this manner, the mast cells remain especially well preserved and very few of them show degranulation. The exceptional degranulated mast cell is usually surrounded by perfectly preserved specimens; this implies that here, the discharge of granules is not due to mechanical trauma but presumably reflects a normal cell activity.

In the rat in which most of our work was performed, the external periosteum of the calvarium contains a very large number of mast cells, most of which are arranged in 2 rows (not as a complete sleeve) along the blood vessels and seen in profile as more or less fusiform elements. However, many of these cells exhibit one or more protrusions, or pseudopodia, which sometimes take on bizarre configurations. Of course, artifactual deformations cannot be excluded with certainty, but the high granule content of the cytoplasm and the absence of any loose granules around the cells makes the outlines extremely sharp so that the protrusions are unlikely to result from degranulation. The most characteristic cell forms among the perivascular mast cells may be described as fusiform, Y-shaped, pear-shaped or comet-like, in the latter case with one short and pointed or long and broad process. In addition, there are cells with many bizarre rounded protrusions, some of which may be shed (clasmatocytosis ?) without discharge of the granules from the detached piece of cytoplasm. This phenomenon has previously been seen in living mast cells and hence does not necessarily represent an artifact(4). Occasionally, the detached, but undisrupted, cytoplasmic segment forms a plate attached to an adjacent vascular wall. In addition, some extraordinarily large cells with long pointed processes were seen to be laden with metachromatic granules, although never as densely as the typical mast cells. These elements are reminiscent of the "spider-shaped mastocytes with filamentous pseudopodia" found in the mininges of *necturus*(5). Finally, at a distance from the blood vessels, and on the surface of the periosteum touching the bone, there are very regularly formed, oval, flat mast cells with little or no granulation in the region of the nucleus (Fig. 2 and 3).

The picture is entirely different in the dura. Here, almost none of the mast cells show any relationship to blood vessels and they are almost uniformly rod-shaped, without processes (Fig. 4).

In accordance with expectations, the periosteal mast cells of the rat discharge their granules and eventually disappear under the influence of such histamine liberators as compound 48/80, polymyxin or dextran; the dural mast cells are much more resistant to these agents. Essentially similar observations have been made in the mouse and hamster.

Summary. A technic is described for the study of mast cells on such "natural tissue spreads" as the external periosteum of the calvarium and the dura mater of small laboratory rodents. These membranes can be fixed as flat sheets, while still attached to their normal osseous base, without the use of the customary traumatic procedures incident to the preparation of artificial tissue mounts.

The photographs were prepared by Miss Madeleine Barath.

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Comparative Protective Action of Unsaturated Fatty Acids for Mice Against Exotoxin, Endotoxin and Snake Venom.* (28078)

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Peripheral vascular collapse in animals can be produced by bacterial exotoxins and endotoxins, and snake venoms. There is evidence that initiating mechanisms responsible for the altered hemodynamic activity differ for each of the foregoing groups of substances. The present study offers further evidence for this conclusion in that injection of unsaturated fatty acids into mice protected against staphylococcal, tetanus and gas-gangrene exotoxins, and also snake venoms, but not against bacterial endotoxins.

Doery and North(1) demonstrated that mice were protected against staphylococcal toxin following an injection of a sublethal amount of venom. We have confirmed this observation, but protection against bacterial endotoxin was not accomplished by this procedure(2). They postulated that this defense was related to the venom enzyme, phosphatidase A, which interacted at the surface of the cells of the host releasing fatty acids and lysophosphatides. The unsaturated fatty acids in turn protected against the bacterial exotoxin.

Materials and methods. Mice. Swiss-Webster white female mice weighing 15-25 g were housed in stainless steel cages in an air-conditioned room. All injections were made into the tail vein in quantities not exceeding 0.5 ml.

Exotoxins.† These included *Staphylococcus* crude toxin (lot #4925-197) with 1:10,000 merthiolate as a preservative; *Clostridium perfringens* crude toxin (lot #W347) and *Clostridium tetani* toxin (lot #T1971M), each with 1:20,000 phenylmercuric acetate as a preservative.

Endotoxins. Standardized amounts of *Escherichia coli* and *Brucella melitensis* endotoxin were used, prepared by methods described elsewhere(3).

Snake venoms.‡ Venoms from *Bothrops jararaca* and *Crotalus terrificus* were employed.

Fatty acids. Solutions of the unsaturated fatty acids, linoleic and oleic acid, were prepared by Dr. Naip Tuna of the Department of Medicine in a concentration of 0.005% in distilled water and 0.01 N NaOH. This basic solution was used in all of the experiments as a diluent for all of the substances injected into mice. No ill effects occurred when mice were injected with 0.5 ml of fatty acid solutions.

Results. Bacterial exotoxins. The results of the protection against staphylococcal, *Cl. perfringens* and *Cl. tetani* toxins, with oleic or linoleic acid are shown in Table I.

The LD_{50} of the staphylococcal toxin was 0.5 ml of a 1:20 dilution. When 0.25 ml of a 1:10 dilution of toxin was added to 0.25 ml of 0.1% of either oleic or linoleic acid, the mixture permitted to stand at room temperature for a few minutes, and then injected into groups of 20 mice, significant protection against the toxin was observed.

When 0.5 ml of titrated *Cl. perfringens* toxin was injected into 20 control mice only 2 out of 20 survived, whereas a mixture of the same amounts of toxin and oleic acid

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