Technical Papers

Gastric Mast Cell Diapedesis[1] in the Albino Rat[2]

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Mast cells have been observed by us in the stomach wall and in the gastric secretion of the albino rat. Twenty-two young adult rats (11 females, 11 males) of the Sprague-Dawley-Holtzman strain were killed by neckstroke, after having been fasted for 36 hr. The stomachs were opened by cutting along the larger curvature. After gently removing food traces, two smears each were taken from the gastric secretion covering the glandular mucosa. One was stained with Wright's stain, the other with a 1% aqueous solution of methylene blue, which is known to stain mast cells metachromatically in fresh, unfixed tissues (*1*).

The stomachs of the rats were cut according to our standard procedure (*2*), fixed in 80% ethanol, and embedded *in vacuo*. Paraffin sections were stained with hematoxylin and eosin, and with 0.5% aqueous solution of toluidine blue 0.

The following results were obtained with all animals. Microscopically, the smears of the gastric juice showed a strikingly rich bacterial flora. Columnar cells, polymorphonuclear leucocytes, and sheets of squamous cells were seen in varying numbers. The amount of mucus also varied. Large mast cells with distinctly metachromatic granules were seen (Fig. 1), varying from 0 to 4/microscopic field, with cellular material. In the cellular debris, free nuclei and disintegrating mast cells showing pseudopods, vacuolation, and various degrees of disintegration were observed.

Metachromatic mast cells were observed in the submucosa of the forestomach and of the glandular stomach in all zones (*2*). They also could be seen lying in the muscle layer and in the subserosa. Their nuclei were usually masked by their granules. Vacuolation of mast cells in varying degrees was noted.

Except for the presence of mast cells in it, the cytological picture of the gastric secretion was similar to that described by Hollander *et al.* (*3*). The presence of leucocytes in the juice supported the observations of Tomenius (*4*) on gastric leucopedesis. No recording of the presence of mast cells in the gastric juice was encountered in the literature (*1, 3–5*). No mast cells were found in the stomach wall or in the gastric secretion of 3 rhesus monkeys and 10 guinea pigs (*6*).

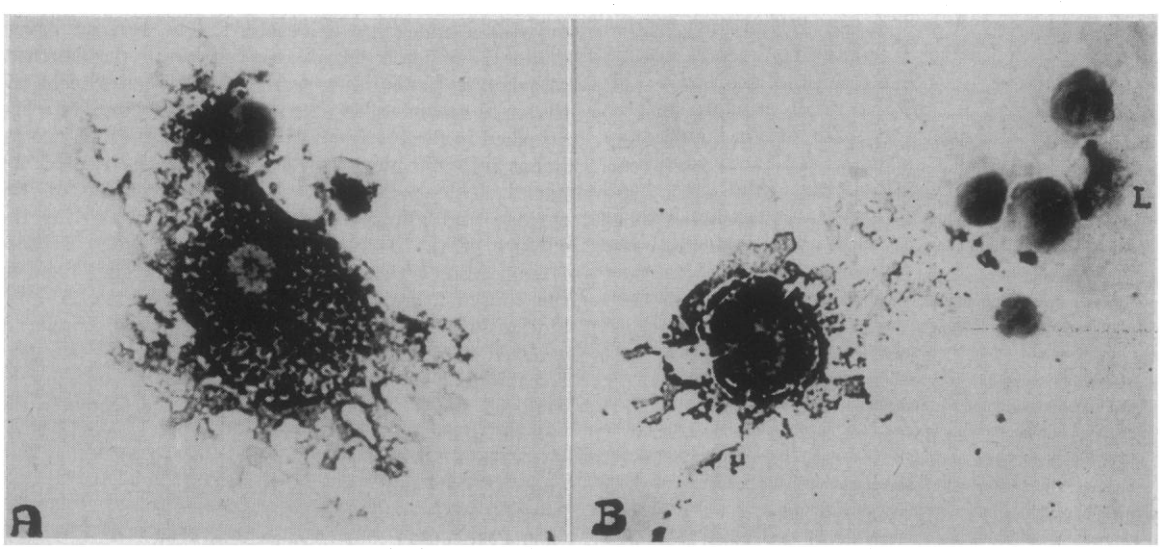

FIG. 1. Mast cells in gastric smear stained with methylene blue. *A*: Mast cell with distinctly metachromatic granules around the nucleus and hazy granules in the peripheral, hazy pseudopods. One of these at the upper pole of the cytoplasm seems to be englobing a cell. *B*: Disintegrating mast cell. *L*: Leucocyte. × 500.

Because the ameboid movement of mast cells (*7, 8*) and gastric leucopedesis (*4*) have been established, the conclusion can be drawn from our findings that gastric mast cell diapedesis occurs in the albino rat. During and at the end of this migration, the mast cells shed a part or all of their metachromatic granu-

[1] Diapedesis (from the Greek *dia*, 'through'; and *pedesis*, 'a leaping') has been used to describe the passage of blood cells through the unruptured vessel walls into the tissues. Tomenius and previous authors employ "leucopedesis" and "leucocytic diapedesis" to describe the passage of leucocytes through the stomach wall. We felt justified, therefore, in using the expression gastric mast cell diapedesis in referring to mast cell migration through the stomach wall.

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lar substance into the tissues or the gastric juice, as evidenced by the extracellular granules and cytoplasmic vacuolation previously described. This supports the point of view of Paff and Bloom (*8*) concerning the secretory life cycle of the mast cell.

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A New Brain Slicing Technique for Tissue Respiration Studies

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The problem of preparing brain slices for respiration studies quickly and accurately, with a minimum of damage to the tissues, has never been adequately solved. Brain tissue lacks firm consistency and homogeneity and is therefore subject to more maceration and damage when using free-hand methods of cutting than are some of the other tissues of the body. This problem is magnified in the small animal, such as the mouse or the rat, where the organ is not only gelatinous but is also small. Under these conditions Umbreit (*1*) has recognized that "the free hand method of slicing even by experts leaves much to be desired." In the course of work requiring serial sections of mouse brain tissue for respiration studies using the conventional Warburg respirometer, the senior writer has developed a new technique, rapid and consistent in results, which does not require any prolonged training in manipulation. Further, there is minimal damage to the cut surfaces of the tissue, since no pressure is exerted in cutting, and the tissue is not subjected to the wiping action of a wide blade. In general, the procedure consists of embedding the brain in agar and cutting it with a thin, flat nichrome wire knife, mounted on a microtome developed for this technique.[1]

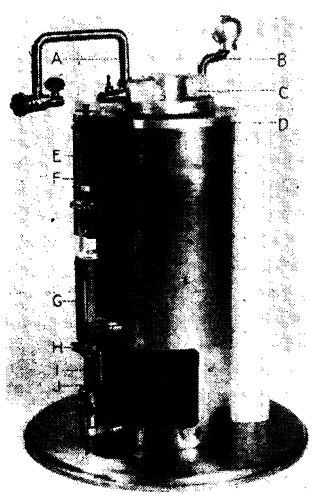

FIG. 1. Microtome with a partially cut embedded brain: *A*, blade frame; *B*, handle; *C*, head; *D*, cylinder cap; *E*, syringe; *F*, mounting block; *G*, brass frame; *H*, split block; *I*, drive shaft; *J*, helical gear; *K*, rubber drive belt.

The microtome (Fig. 1) is mounted on a steel cylinder frame 25.5 cm high having a diameter of 19 cm, which is permanently fixed to a flat, wide base for stability. A shaft 12 mm thick, fixed to the head holding the blade frame and handle, passes through the cylinder cap and ends in a centered well in the bottom plate. The blade rotates on a horizontal fixed plane passing through the embedded tissue, which moves upward a constant distance with each sweep of the knife. The tissue is held in a 10-cc syringe barrel cut off about 2 cm from the tip. The mechanism actuating the plunger of the syringe is a helical gear, connected to the center shaft by a rubber belt, which raises the tissue as the knife is rotated. The instrument described is fixed to cut the tissue 400 μ thick,[2] but any thickness of tissue may be cut by altering the diameter of the drive shaft or helical gear. This may be done with pulleys of various sizes or with a cone-type drive shaft. The syringe barrel holder is a 5 × 9 cm block of bakelite permanently attached to the cylinder wall and cut vertically in the center to accommodate the syringe barrel snugly when attached to the block with two rubber bands. A groove is cut into the block to accommodate the rim of the barrel. This prevents vertical displacement of the syringe and allows the knife to pass just over the surface of the barrel. The plunger rests on a brass frame riding on each side of the helical gear fixed at the top and bottom to the cylinder frame. A split block, forming the bottom of the brass frame, is grooved to fit the gear and rides upward as the gear is rotated. The plunger may be adjusted as may be required for the tissue, or to return the frame to the bottom after cutting, by opening the screw lock on the frame block, causing the two parts to spring apart slightly and drop. After setting it to the proper depth it may again be tightened.

The U-shaped blade frame (5 × 8 cm) is patterned after a jeweler's saw. Flat jaws for holding the blade are tightened by thumbscrews, and tension may be exerted on the blade by adjusting the screw passing through the outer part of the frame. The knife blade itself is made from a 0.005″ × 0.030″ nichrome heating element wire sharpened on the leading edge with a small stone and Turkish emery. It is not nec-

[1] The authors wish to acknowledge the capable technical assistance of William Marshall in the construction of the microtome.

[2] The measurements were made on the fixed preparations with an eyepiece micrometer.