in localization of Be and of ATA may be related to this phenomenon. A similar potentiation of kidney damage has been observed in cadmium-poisoned rabbits treated with BAL(7). The critical importance of renal damage in animals undergoing treatment which promotes redistribution of toxic metals in the tissues warrants further investigation of the complex situation which prevails in the kidneys of animals undergoing such treatment.

Summary. The distribution of Be^7 and of C^{14}-labeled aurintricarboxylic acid (ATA) was studied autoradiographically over a period of 48 hours in the spleen, liver, lung and kidney of mice. It was found that in general beryllium and ATA were deposited in similar tissue loci. The highest simultaneous concentrations and identical patterns of deposition were seen in the red pulp of the spleen. Other organs showed reasonably good correlations in deposition of the two substances, confirming earlier therapeutic studies. An increased concentration of C^{14}-ATA was observed in areas of tissue damage produced by beryllium in the liver, kidneys and lung.

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Histochemistry XLIV. Use of Density Gradient for Isolation of Mast Cells.* (22476)

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Padawer and Gordon(1) described a differential centrifugation technic for the rapid separation of mast cells from peritoneal fluid of the rat. They flushed the peritoneal cavity with 2 or 3 ml of 0.9% NaCl, layered the cell suspension over a sucrose-gelatin-NaCl solution and centrifuged the mast cells down into the latter medium while retaining the less dense cells above the interface between the layers. Thus they were able to obtain suspensions containing more than 75% mast cells.

Effective use of density gradients for the separation of cellular particulates(*e.g.* 2-7) suggested that this principle might offer advantages in the separation of cells. The first study undertaken in this connection was the isolation of mast cells which were required for other investigations in this laboratory. Significant improvements in the method for isolation have been effected, and suspensions which were 100% pure with respect to cell type have been obtained with yields as high as 87%.

Modified procedure. Padawer and Gordon exsanguinated the rat under ether anesthesia before opening the abdomen by a mid-ventral incision. In an attempt to minimize further the contamination of the peritoneal fluid with blood, and the associated clotting which entraps many mast cells, other procedures were tested in the present study. The most effec-

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tive proved to be the use of an electrically heated wire loop or cautery knife for the incision, which reduced the red cell count in the original suspension about 7 times. Prior exsanguination seemed to have little effect when the cautery was used, but the simplest method, and one which would retain any advantage of exsanguination while eliminating anesthesia, would seem to be decapitation, blood drainage, and cautery incision.

Another change introduced was replacement of 0.9% NaCl by Hanks' balanced salt solution(8) to provide a more physiological medium. Occasional clotting, which was still encountered in the peritoneal wash, was considerably reduced by the addition of 50 μg of heparin per ml salt solution. No effects from the heparin were noticed over a 1 hour period.

The high viscosity due to the gelatin in the medium of Padawer and Gordon made it difficult to use, and it was found that the Diodrast-sucrose medium(5,6) which avoids this difficulty renders the mast cells fragile and destroys their ability to stain metachromatically with toluidine blue. Accordingly, only sucrose was employed for the density gradient. The finding of Ottesen and Weber(6) that Diodrast-sucrose gradients require 24 hour standing at 2°C for the density to become continuous, as shown by schlieren curves recorded with an electrophoresis apparatus, was found to apply to the sucrose gradients used in the present work. It was noted that sucrose gradients could be stored in the cold room (4°C) for 2 days without noticeable diffusion. Phosphate buffer, as employed by Ottesen and Weber, was also used in the sucrose gradients. The addition of Versenate to the buffer reduced the tendency of clumping of the leucocytes and erythrocytes. Such clumping trapped mast cells and thus reduced their yield.

To observe whether convection was a disturbing influence in the centrifugation procedure carried out at room temperature (25°C), a comparison was made with the entire procedure conducted in the cold room (4°C). No advantage followed from the latter; in fact, the greater viscosity of the medium caused the mast cell layer to remain closer to the leucocyte layer and the latter retained more mast cells which resulted in poorer yields.

The pattern of centrifugation recommended by Anderson(7) was found to be advantageous, and the time and centrifugal force used by Padawer and Gordon(1) proved to be suitable for the present purpose.

The gradient tubes are set up as follows: Prepare and store in the cold (a) Versene-phosphate buffer, pH 7.4 (0.190 g disodium Versenate, Bersworth, 2.86 g $Na_2HPO_4 \cdot 12H_2O$, and 0.272 g KH_2PO_4 to 1 l. with distilled water), (b) Sucrose stock solution (53.7 g sucrose to 46.3 ml of Versene-phosphate buffer), (c) Sucrose working solutions of sp.gr. 1.200, 1.165, 1.130 (Dilute 7.95, 6.55 and 5.15 ml of stock sucrose solution respectively with Versene-phosphate buffer to final volumes of 10 ml). Carefully layer, with a minimum of mixing, 1 ml of each of the 3 sucrose working solutions in a Pyrex tube 13 x 100 mm, I.D. 11 mm. Place in the cold (2-4°C) for 24 hours.

The following is the final procedure adopted: 1. Decapitate the rat and drain the blood, or anesthetize with ether. 2. Wet the hair over the abdomen to keep stray hairs from getting into the peritoneal cavity later, cut away a skin flap from the abdomen and fold over the chest, and then open the peritoneal wall with a mid-ventral incision using an electric cautery knife. 3. Drop 3 ml of heparinized (50 μg/ml) Hanks' balanced salt solution over the intestinal surface and gently massage the outside of the abdominal cavity for about 1.5 minutes. 4. Hold the intestines aside and remove the liquid (about 2 ml) with a drawnout medicine dropper from the space on each side of the spine. 5. Remove the tube containing the sucrose density gradient from the cold room, where it had been set up 24 hours earlier, and allow it to reach room temperature. Layer the peritoneal cell suspension over the sucrose solution, and diffuse the interface by gentle stirring with a thin glass rod. 6. Spin in a horizontal centrifuge for a total of 5 minutes, gradually increasing the speed to a maximum of 110 x

g, and then gradually reducing to zero. Allow to come to rest without braking. (A Size 1, Type SB, International Centrifuge was used, and after the tubes were placed in the carrier the rheostat was set to division 9, the cover was closed, the tachometer was connected, and the rheostat was advanced 1 division every 5 seconds until 740 rpm, division 16, was attained. Then the rheostat was set back 1 division to maintain this speed for 3⅓ minutes, after which deceleration was begun at the rate of 1 division every 5 seconds to zero.) 7. Clamp the tube on a vertical stand. Four layers of cells will be apparent, a top red cell layer, under it a dense white cell layer, below this a diffuse white cell layer and farther down the mast cell layer. Remove the mast cell layer with a 4 inch, 20 gauge, stainless steel hypodermic needle attached to a 2 ml syringe. (Samples of the layers were also removed with a 10 μl constriction pipette. Removal is aided by clamping the syringe or pipette to a vertical rack and pinion stand for controlled lowering and raising of the needle or pipette.) 8. To each ml of mast cell suspension in sucrose solution, add about 3 ml of distilled water and centrifuge for 5 minutes at 1500 rpm (452 x g). Remove most of the supernatant fluid and replace with Hanks' balanced salt solution. 9. To observe the morphology, mix 10 μl of cell suspension with an equal volume of 0.05% toluidine blue in 0.85% NaCl and shake by tapping the tube. Cell counts may also be performed on this preparation, although the unstained mast cells can be distinguished for counting.

Results. An example of results that can be obtained is given in Table I. The mast cell layer is distinct and separated sufficiently from the nearest white cell layer to permit easy removal. The isolated cells are uncontaminated by other cell types.

The positions of the cell layers after 5 minutes of centrifugation at 110 x g and room temperature are not the equilibrium positions that would be attained ultimately by longer centrifugation. However, the relatively large size and density of the mast cells combine to give them a sufficiently greater sedimentation rate than the other cells and this results in an adequate separation for isolation purposes. No advantage was gained when the centrifugation was prolonged to 10 or 15 minutes, or the centrifugal force was increased to 330 x g. In the latter instances the red cell layer moves part way through the white layers as the equilibrium positions are approached, and the bottom of the white cell layer gets closer to the mast cell layer.

TABLE I. Separation and Purity of Mast Cell Fraction from Peritoneal Washings of the Rat Using a Sucrose Density Gradient. (5 min. centrifugation at 110 × *g* and 25°C.)

Sample	mm below interface	Mast cells per μl	% mast cells
Original suspension		840	11.2
Red layer	1– 2	0	0
Heavy white layer	2– 5		
Diffuse " "	5– 9	0	0
Mast cell layer	13–15	920	100
Bottom of tube	30		
Final suspension*		1340	100

* Mast cell layer withdrawn, diluted, centrifuged and resuspended in 0.5 ml balanced salt solution.

Summary. The procedure of Padawer and Gordon for the isolation of mast cells from peritoneal washings was modified to employ a sucrose density gradient, and along with other changes in certain details, a procedure was elaborated that regularly provides mast cells 100% pure with respect to cell type in yields up to 87% with respect to the original washings.

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