Immediate types of allergic hypersensitivity reactions are encountered in all fields of medicine. There is no simple, reliable and reproducible laboratory technique available for the detection of most of these reaction states, especially for those due to drugs. The clinical history of such a previous allergic reaction remains the only reliable guide for the clinician in the detection of these hypersensitivity states (3).

The circulating basophil/tissue mast cell systems are intimately involved in the release of active pharmacologic agents such as histamine and heparin during many immediate hypersensitivity reactions due to drugs (3, 7, 8). Morphologic, chemical, and bioassay methods have been used to monitor these cellular systems during immediate hypersensitivity reactions, but these have not met with universal acceptance (3). This paper describes a technique to monitor alterations occurring in mouse peritoneal mast cells by determination of the release of S\(^{35}\) labeled sulfated acid mucopolysaccharides from mast cells after their incubation with known mast cell degranulating substances [Compound 48/80 and distilled water (7)].

Technic
Forty \(\mu\)g of S\(^{35}\) sulfate (less than 0.1 mg total sulfate) in 0.2 ml of physiologic saline was given by intramuscular injection to each of 52 female albino mice weighing between 22-25 grams. There were four groups of mice, each containing the following number of animals: Group I—22, Group II—14, Group III—9, and Group IV—7. Forty-eight hours after the isotope injection, the animals were exsanguinated and sacrificed by cervical dislocation. Immediately thereafter, each animal of the respective groups was injected intraperitoneally with 2 ml of the following solutions: Group I—Ringer-Barron mast cell harvesting solution, Group II—distilled water, Group III—0.2 mg Compound 48/80 dissolved in Ringer-Barron solution, and Group IV—1.0 mg Compound 48/80 in Ringer-Barron solution. After 15 minutes the fluid was aspirated from the abdominal cavity with a prewarmed siliconized 18 gauge needle and plastic syringe and immediately filtered through a 3 micron plain white Millipore filter. The filter was then washed with 3 ml of Ringer-Barron solution. The filtered peritoneal fluid and wash solution from each animal were combined and reduced to dryness by a warm air stream. The acid mucopolysaccharide in the dry residue was isolated by a modification of the technique described by Schiller, Slover, and Dorfman (6). One ml of pH 5.5, 0.1 M acetate buffer containing 0.005 M versene, 0.005 M cysteine and 3 \(\mu\)g of crystalline...
Table 1. Experimental Results

<table>
<thead>
<tr>
<th>Solution used for Intrapitoneal Injection</th>
<th>Number of Animals</th>
<th>Average Value</th>
<th>Range of Values</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringer-Barron Solution alone (controls)</td>
<td>22</td>
<td>7.0</td>
<td>1.7-12.9</td>
<td>3.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Distilled Water alone</td>
<td>14</td>
<td>21.6</td>
<td>10.5-38.6</td>
<td>8.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Compound 48/80 0.2 mg in 2 ml Ringer-Barron Solution</td>
<td>9</td>
<td>23.4</td>
<td>10.8-36.2</td>
<td>9.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Compound 48/80 1.0 mg in 2 ml Ringer-Barron Solution</td>
<td>7</td>
<td>10.9</td>
<td>8.3-14.5</td>
<td>2.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Radioactive results expressed in cpm/ml of isolated acid mucopolysaccharide corrected to the reference date of the radioactive sulfur.

Results

Table 1 shows that the radioactivity of the acid mucopolysaccharide isolated from the peritoneal wash of the mice which received distilled water (21.6 cpm/ml) and 0.2 mg Compound 48/80 (23.4 cpm/ml) was significantly greater (p < 0.01 Student’s t-test) than that from the Ringer-Barron solution alone (7.0 cpm/ml). The amount of labeled sulfated acid mucopolysaccharide isolated from the peritoneal wash of animals given 1.0 mg Compound 48/80 (10.9 cpm/ml) also differed from the Ringer-Barron controls; however, the results were consider­ably lower than those obtained with the lesser amount of Compound 48/80.

Comments

This study demonstrates that the degranulation of mast cells can be monitored through the release of radioactive labeled sulfated acid mucopolysaccharide. Use of radioactive isotope technique to label the sulfated acid mucopolysaccharide of rodent mast cells is not new. Lagunoff, Calhoun, and Benditt (4) found that 48 hours after IM injection of radioactive sulfur the majority of the radioactivity in cells obtained from washing of the peritoneal cavity was in the mast cells. Other cellular elements in the washing had insignificant amounts of radioactivity when compared to the mast cells. The radioactivity was localized in the sulfated acid mucopolysaccharide portion of the mast cells.

In a preliminary report we stated that the technic described here can be used to monitor degranulating mast cells (2); however, in that preliminary report the effect of varying concentrations of Compound 48/80 (a condensation product of p-methoxyphenethylmethylamine and formaldehyde) was not evaluated. The paradoxical-

papain was added to each residue. The samples were incubated at 60°C for 16 hours and the dialyzed against distilled water for 24 hours. The papain digestion releases protein bound acid mucopolysaccharide. Particulate matter was removed from the digests by filtration with 0.45 micron plain white Millipore filters. The filtrates were reduced to dryness by a warm air stream and 1 ml of 0.04 M sodium chloride was added to each residue. Quantitative precipitation of the labeled acid mucopolysaccharide as their cetyl pyridinium chloride complexes was obtained by the addition to each sample of 100 μg carrier heparin and 50 mg of cetyl pyridinium chloride. The precipitated acid mucopolysaccharide—cetyl pyridinium chloride complexes were collected on 0.45 micron plain white Millipore filters. The radioactivity on the dried filters was determined by gas flow techniques and expressed as counts per minute (cpm) above background (approximately 15 cpm) per ml of aspirated peritoneal fluid corrected to the reference date of the radioactive sulfate. The samples were counted for 5000 total counts.
ly lower results (Table 1) with the higher concentration of Compound 48/80 results from its ability to combine with sulfated acid mucopolysaccharides to form insoluble complexes. In vitro, Compound 48/80 at 0.1 mg/cc does not precipitate sulfated acid mucopolysaccharides from solution, but at 0.5 mg/cc a visible precipitate is formed. When Compound 48/80 is present above a critical concentration, a portion of the sulfated acid mucopolysaccharide is probably lost in the preliminary isolation process of our technic. All components thus must be tested in concentrations used to insure against false negative results.

Others have used morphologic, chemical, or bioassay methods to examine alterations in circulating basophil/tissue mast cell systems undergoing degranulation due to specific degranulating agents or during antigen-antibody reactions (3). Microscopic techniques have proven inadequate in defining subtle changes in the morphology of mast cells. Rodent mast cells contain histamine, serotonin, and sulfated acid mucopolysaccharides, but chemical and bioassay techniques for these substances are technically difficult to perform at the microanalytical levels required for the study of small populations of tissue mast cells. It is anticipated that the technic described here can be used as an objective method to monitor mast cell degranulation during an antigen-antibody reaction. In Dermatologic laboratories at the University of Minnesota, Nelson et al. (5) have used the animal model which we developed to observe with the electron microscope mast cell degranulation in an antigen-antibody reaction. Their results show that the mast cell granules lose their normal morphologic characteristics in such a reaction. Their results suggest that the projected application of our radioactive technic to the same antigen-antibody reaction system will be useful in measuring these reactions in a quantitative manner.

**SUMMARY**

The method described in this paper presents an objective, microanalytical technique to monitor degranulation in the mast cells of an individual animal following incubation with known mast cell degranulating agents. It does not involve subjective evaluation which is the major drawback in the morphologic techniques.

The radioactivity of the isolated sulfated acid mucopolysaccharides from the peritoneal cavity of mice treated with known mast cell degranulating agents differed significantly from those of the control group.

**Acknowledgement**

Elizabeth Mindrup provided technical assistance in performing the tests described in this paper.

**REFERENCES**