SCHWYZER[2]; t-BOC Arg and Arg, if present (uncompleted reaction) were both precipitated with phosphomolybdic acid.

The mixture obtained by lyophilization was then fractionated on a cellulose chromatographic column using chloroform/methylalcohol/ammonia 17% (2:2:1) as eluent.

The Sakaguchi test was used to detect the presence of guanidinium group in the eluted fractions. The first Sakaguchi positive peak corresponds to the t-BOC Arg. After solvent evaporation of the pooled fractions, the residue was dissolved in water and finally freeze-dried. The white powder obtained has a m.p. 164 °C (dec.) (N%: calculated 20.45; found 19.75). Conversion into acetate by freeze-drying in dilute acetic acid gives a hygroscopic acetate which was also analyzed for its nitrogen content (N%: calculated 17.5; found 17.3). Acidolysis with trifluoroacetic acid gave only Arg when revealed with ninhydrin.

The last 2 steps of the synthesis proceeded without any difficulties. t-BOC His was added unprotected on the imidazole nucleus as it was the N terminal residue. The peptide was finally removed from the resin using C_2H_5ONa in ethanol and an aliquot was isolated by chromatography on carboxymethyl cellulose.

As expected, the tryptic digestion cleaved the peptide in two fragments: one of them was shown to be Trp-Gly. After total acid hydrolysis and paper electrophoresis in acetic acid/formic acid/water buffer (pH 1.9)[3], the 4 spots were identified as Phe, Gly, Arg and His. A very faint ninhydrin positive spot was also shown to be Ornithine (trace).

The barium hydroxide hydrolysate gave, under the same conditions, 5 spots which were identified as Orn, His, Gly, Trp and Phe.

The total yield of the synthesis was 60%[4].

Résumé. La synthèse du pentapeptide His-Phe-Arg-Trp-Gly a été réalisée par la méthode en phase solide de MERRIFIELD. L'Arginine a été introduite sans protection du groupe guanidinium; de même le noyau imidazole de l'Histidine n'a pas été protégé.

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Separation of Mast Cells from the Peritoneal Fluid of the Rat with a GE Nucleopore Filter

There are many methods available for the separation of mast cells from other cells in the peritoneal fluid of the rat. Most of these methods are based on centrifugation with a suitable gradient[1-3]. Trying to escape the great disadvantage of osmotic cell dehydration, UVNÄS and THON[4] used the sugar polymer Ficoll as a gradient. BLOOM and VALMET[5] constructed a special centrifuge which is suitable for mast cell separation on a large scale. The gradient consisted of saccharose and Na_3EDTA. Dicoumarol was added as an anticoagulant.

LAGUNOFF and BENDITT[6] separated mast cells by filtration through a millipor filter. This filter was tested in our laboratory. The results were unsatisfactory.

Material and methods. In 1964, FLEISCHER et al.[7] described a new filter, which they called the GE-nucleopore filter. The filter consists of a plastic membrane furnished with uniform holes occupying about 2% of the total filter surface. Filters of this type with hole diameters of 9 or 11 μ were used in this study.

With minor modifications, the filtrating apparatus described by SEAL[8] was used. It consists of 2 parts, a receiver and a draining part, which are tightly attached to each other by springs with the filter between them. The filtration rate is adjusted with a stop-cock and the filtration is carried out without variations in pressure.

The material consisted of 30 male rats weighing between 280 and 320 g. After decapitation, 10 ml, respectively, of the following out-washing fluids were injected into the peritoneal cavity: saline with and without sodium citrate, pH 4.8 and 6.5, respectively; phosphate-buffered saline with and without citrate, pH 7.1 and 6.9, respectively; Na_3EDTA-saline with and without dicoumarol, pH 7.1.

After light massage (for 1 min) a short section was made along the linea alba and the fluid was aspirated from the abdominal cavity with an injection syringe furnished with a large puncture needle. Of the cell suspension 5 ml were poured into the receiver. The filtration speed was adjusted to 6–8 drops/min. Then the filter was rinsed 4 times with 2.5 ml of out-washing fluid. With the filter in place in the receiver the preparation was fixed in absolute alcohol for 30 min. Finally the filter-cell preparation was clasped onto a slide and properly stained in a toluidine-blue solution. The cells in 5 visual fields, corresponding to 0.068 mm², were counted. Using a Buerker chamber the total number of mast cells and other cells were counted from the remaining cell suspension. The filtrate obtained was centrifuged, the volume was adjusted to 10 ml, and the mast cells and other cells in the filtrate were counted in a Buerker chamber.

Results and discussion. The results of filtration are shown in the Table. Apart from mast cells, an abundance

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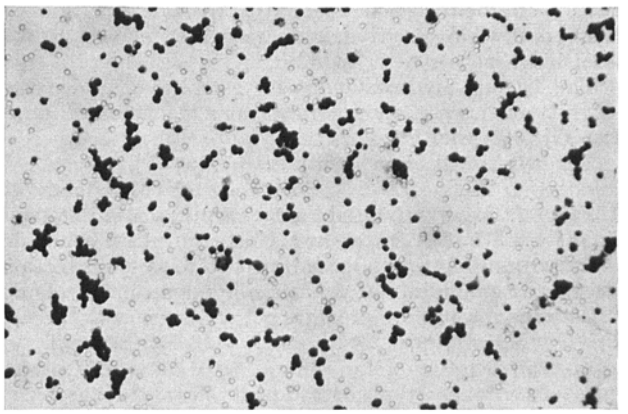

Fig. 1. A cell population from the peritoneal fluid of the rat on an 11 μ GE-nucleopore filter. The dark spots are mast cells and the light circles the holes of the filter. Staining: Toluidine blue. × 100.

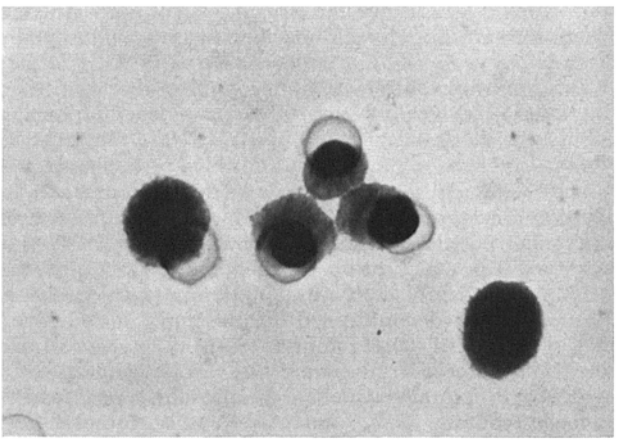

Fig. 2. 5 mast cells on an 11 μ GE-nucleopore filter, 4 of them passing in part into holes. Staining: Toluidine blue. × 800.

Filter	Medium	pH	No. of tests	% of mast cells in peritoneal fluid	% of mast cells on filter
11 μ	Physiological saline	4.8	3	2, 7, 2	33, 19, 17
11 μ	Phosphate-buffered physiological saline	7.1	3	4, 2, 2	75, 50, 36
11 μ	Physiological saline + 0.6% Na-citrate	6.5	3	3, 3, 3	68, 80, 61
11 μ	Phosphate-buffered physiological saline + 0.6% Na-citrate	6.9	4	3, 4, 3, 3	65, 84, 51, 40
11 μ	Na_3EDTA in physiological saline	7.1	3	2, 3, 4	89, 50, 56
11 μ	Na_3EDTA + 0.05% dicoumarol	7.1	5	2, 2, 3 3, 3	76, 97, 82, 72, 51
9 μ	Physiological saline + 0.6% Na-citrate	6.5	3	5, 5, 3	57, 70, 36
9 μ	Na_3EDTA-saline + 0.05% dicoumarol	7.1	3	4, 5, 3	49, 50, 34

of mononuclear cells, eosinophil leucocytes and epithelial cells settled on the filter. When physiological saline was used, good results could not be attained owing to agglutination of the cells. The difference between the results obtained with EDTA with and without dicoumarol was marked and puzzling. The results with the 9 μ filter were poorer than those obtained with the 11 μ filter.

In all groups, the results showed wide variations. In the group EDTA + dicoumarol and 11 μ filter, for instance, the range was 50–95%. As regards the 9 μ filter, it appeared that the larger the number of cells filtrated, the poorer the result. Large quantities of cells cannot be filtered with this sieve, which has a breadth of only 28 mm and a total hole surface of 2% of the total surface of the filter. However, using a medium consisting of EDTA + dicoumarol and an 11 μ filter, a cell population of 0.7 to $1.0 \cdot 10^8$ with a mast cell frequency of 3% can be successfully filtrated.

The yield was relatively low; it was evaluated at about 25% of the number of mast cells filtrated. No rise was obtained when the hole diameter was reduced from 11 to 9 μ, which gives an idea of the flexibility of the mast cells.

The mast cells were evenly distributed over the surface of the filter. The cells were morphologically in good condition, although they seemed to be somewhat distended. Cell ruptures were rare (Figures 1 and 2).

In order to investigate the condition of the cells, some preparations were fixed for 60 min in formalin vapour at 80 °C and examined in an UV-microscope. The mast cells exhibited an uneven fluorescence of lower intensity than the fluorescence of smear preparations of peritoneal fluid.

With the method of filtration described in the foregoing, viscous and dehydrating gradients as well as recentrifugations and decantations are avoided. Since the cells are retained on a transparent membrane, the preparation can be directly stained and studied in the microscope. The filter endures strong acids, weak bases and many fat solvents. With the apparatus and method described, filtration takes about 30 min.

Zusammenfassung. Filtrationsmethode der Trennung von Mastzellen aus der Abdominalhöhlenflüssigkeit der Ratte: Die separierten Zellen werden auf dem Filter fixiert, gefärbt und untersucht. Die Methode eignet sich für Untersuchungen von Mastzellreaktionen in vitro.

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