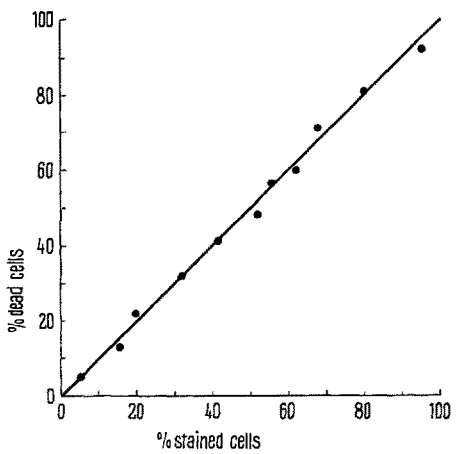
(3) The results with these dyes are always more or less variable, with high experimental errors, especially at high % of cytolysis[5]. The present authors found the same poor correlation between the number of dead and coloured cells.

Trying to find a better staining method for dead yeast cells, good results were obtained with a combination of the negative dye Ponceau Red and uranyl nitrate, commonly used for the tricomplex staining of phospholipids[8]. It is known that the phospholipids of the intact yeast cell membrane bind Ponceau Red and uranyl ions in a similar way[9]. This 'tricomplex staining' of the membrane phospholipids is not visible microscopically. Over a wide concentration range Ponceau Red and uranyl nitrate appear to have no cytolytic properties. Moreover, neither Ponceau Red[9] nor uranyl ions[10] permeate into the intact yeast cells. It appeared, however, that they do permeate into damaged cells, giving a bright staining of the cell contents. After preliminary experiments to find optimal staining conditions, the following procedure was adopted.

Reagents. (1) 0.04% Ponceau Red solution, (2) 5% $UO_2(NO_3)_2 \cdot 6H_2O$ solution, (3) 0.075 molar maleic acid, dissolved in $18 \cdot 10^{-3} N$ NaOH.



Relationship between % dead cells and % stained cells, after exposure to varying concentrations of alkyl dimethyl benzylammonium chloride. The number of dead cells was determined with the millipore-plate method; at each detergent concentration 12 plates, each containing about 100 cells, were prepared.

A suspension of yeast cells, exposed to a cytolytic agent, is centrifuged and the supernatant is discarded. The yeast cells are resuspended in ice cold water, to give a 0.2–0.5% suspension. This suspension is kept in melting ice for 10 min. 1 ml of this suspension is added to a mixture of 10 ml Ponceau Red, 2 ml uranyl nitrate and 2 ml maleic acid solution and kept at room temperature for 5 min, with continuous stirring. The final pH of this suspension should be 2.48–2.52. Subsequently proportional counts of stained and unstained cells are made by direct microscopic examination, utilizing a cell counting chamber. With this procedure accurate and reproducible results were obtained with various cytolytic agents (Figure).

Staining is positive, if the whole cytoplasm is coloured more or less uniformly. A local staining at a single spot should be considered negative; such local spots are presumably coloured cellular debris, attached to the surface of the cells. When 500 cells are counted, the experimental error is about 2%. In contrast to the methylene blue and Nile Blue method, the results are accurate over the whole range from 0–100% cytolysis. It seems probable that the same procedure can be used with respect to other microorganisms[11].

Zusammenfassung. Es wird eine neue Methode zur selektiven Färbung beschädigter (toter) Hefezellen mit Hilfe eines Gemischs von Uranylnitrat und Ponceau-Rot beschrieben, die grosse praktische Vorteile gegenüber der klassischen Methylenblau- und Nilblaufärbung besitzt.

MAGDALENA MAAS and J. VAN STEVENINCK

Laboratory for Medical Chemistry, Leiden (The Netherlands), 12th December 1966.

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Synthesis of a Pentapeptide by the MERRIFIELD Method

We wish to report the synthesis of the pentapeptide His-Phe-Arg-Trp-Gly by the solid phase method of MERRIFIELD[1], as an attempt to introduce arginine in a sequence without a protecting group on the guanidinium end of the molecule. ter-Butyloxycarbonyl (t-BOC) Gly was linked to the polymer according to the classical process and, after acidolysis of the t-BOC protecting group, was reacted with t-BOC Trp in methylene chloride using dicyclohexylcarbodiimide (DCCI) as coupling agent. A few drops of dimethylformamide (DMF) were added to ensure a better solubility of the t-BOC Trp in methylene chloride. No destruction of the indole nucleus seemed to occur during the acidolysis of the t-BOC group with HCl/HAc, as we were indeed able to isolate and identify (Ehrlich positive) the dipeptide Trp-Gly after refluxing an aliquot of the resin with sodium ethylate in absolute ethanol.

The next step of the synthesis was the addition of the Arg residue; α-t-BOC G.H.$^+$ Arg was used. This protected amino acid was prepared as follows: t-BOC Arg was synthesized according to the general method of

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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.

A. LOFFET

(UCB)-DIPHA, Direction Recherches et Développement, Département de Recherche Biochimique, Bruxelles (Belgium), 12th December 1966.

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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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