die Glasplatten mit den Ringen aufeinandergestapelt um Platz zu gewinnen. Dann wird ein steril verpackter Sartorius-Filtrationsvorsatz mit einem Bakterienfilter (SM 165 18, mittlere Porengrösse 0,45 µm) auf einen Dispenser aufgeschraubt. So lassen sich stets gleichbleibende Portionen von 0,3 ml steril filtrierter Nährlösung auf die UV-sterilisierten Membranen auftragen. Die Füllung der Sachets wird durch einen einfachen «Evakuationsapparat» (Figur 3) erleichtert: Ein bespannter A-Ring wird auf den Gummistopfen des Sockels aufgesetzt. Durch leichtes Ziehen am Sockel kann ein Unterdruck erzeugt werden, und die Membran wird nach unten eingedellt. Nach dem Auftragen der Nährlösung in diese Eindellung legt man einen B-Ring (mit der Membran nach unten) darauf, so dass der Tropfen sich ausbreitet. Sofern er nicht die ganze Fläche schön ausfüllt, schiebt man mit der linken Hand den Sockel soweit zurück, bis der Tropfen bis zum Rand ausgebreitet ist, und drückt dann mit der rechten Hand den B-Ring ganz nach unten auf den Sockel. Dabei reisst die Membran vom B-Ring los und verklebt von selbst über der Kante des A-Rings mit der unteren Membran. Der A-Ring mit dem fertigen Sachet und der leere B-Ring werden vom Gummistopfen abgenommen, und der nächste Ring wird aufgesetzt. Diese einfachen Handgriffe lassen sich auch mit relativ dicken Gummihandschuhen ohne Schwierigkeiten ausführen, und bei einiger Übung kann man auf diese Weise ohne weiteres 150 Sachets in 90 Minuten füllen.

Anschliessend an die Füllung der Sachets werden diese auf allfällige Beschädigungen hin kontrolliert und dann bei −25 °C bis zu ihrer Verwendung auf den A-Ringen eingefroren. In eigenen Versuchen wurden nach dieser Methode bisher über 6000 Parafilm-Sachets hergestellt, wobei die Ausfallquote bei der Herstellung (undichte Sachets, geplatzte Sachets) auf weniger als 5% gesenkt werden konnte.

Summary. A new technique is described which allows massproduction of parafilm-sachets, containing artificial diet for aphids, under sterile conditions.

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Visualization of Immunoadsorption by Immunofluorescence

Immunoadsorption by affinity chromatography has become a widely used tool in modern protein chemistry, enzymology and immunology. Proteins are covalently bound to the sorbent, i.e. microcrystalline cellulose (Merck) or Sephadex, or Sepharose (WIDE[1-3]), and thereby made insoluble. Enzymes can be bound to the sorbent and packed into a column, which enables the continuous breakdown of the respective substrate, and the reaction can be stopped without the necessity of destroying the enzyme.

By coupling antigen to the sorbent, its homologous antibody can be trapped in pure form from an antiserum by immunological specificity and thereafter desorbed by lowering the pH to 2.3–2.6 or by 3 *M* NaSCN (CARREL and BARANDUN[4]). This method has been used by MESSERLI and FEY[5] for measurement and classification of bovine colostral pure antibodies from cows immunized with different proteins. The same technique can be applied to the isolation of antigen by a column containing insolubly bound antibody, i.e. enzymes, haptens and ligands (KENYON et al.[6]). Recently KENYON et al. isolated the Aleutian Mink Disease Virus by affinity chromatography.

WIDE[2] introduced the immunosorbent technique into the routine determination of hormones and IgE in the so-called radio-immuno-sorbent-test (RIST). The RIST has since become a rapid test for mass screening and precise determination of several hormones.

In our attempts to adapt the RIST for application in the field of bacteriology, virology and serology, we started experiments with pneumococci of type 2 and the respective rabbit antibody, which we coupled to Sepharose 2B by means of the cyanobromide method (WIDE[2]). Being inexperienced in this field, we tried to prove the successful Sepharose activation and antibody coupling by using the immunofluorescence method as follows:

Methods. The rabbit *Pneumococcus* type 2 antibody globulins were precipitated by 50% saturation with ammonium sulfate, followed by 2 washings with 50% saturated ammonium sulfate and dialysis against 0.1 *M* $NaHCO_3$.

The coupling of the *Pneumococcus* antibody protein to CNBr activated Sepharose 2B (Pharmacia, Uppsala) was performed by the method of WIDE and PORATH[1] with some minor modifications. A raw polysaccharide fraction was extracted from a dense culture of *Pneumococcus* type 2 by ox-bile, acetic acid and absolute alcohol after ROESGAARD (1945) (cited by LUND[7]).

Antibody labelling with FITC was performed as described by THOMASON et al.[8] with our own modifications. For fluorescence microscopy we used a Wild equipment with a FITC interference filter and a quartz-iodine lamp illumination, as described by FEY and BRAUN[8]. For photography we used the film Ektachrome High Speed 23 DIN, developed at 32 DIN which increases ist sensitivity 4-fold (FEY and BRAUN[9]).

Results. First we filled a small column (5 × 40 mm) with the Sepharose-anti-*Pneumococcus* type 2 conjugate and passed 1 ml of a suspension of pneumococci type 2 with an optical density of 0.17 at 543 nm. The eluate appeared water-clear and had an OD of < 0.01. Even prolonged washing did not cause any turbidity of the

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[9] H. FEY and K. BRAUN, Festschrift Wild, Heerbrugg (1971), p. 24.

eluate. When we percolated the same *Pneumococcus* suspension through a column with anti-Ferritin-Sepharose as a control, the eluate showed the same turbidity as the original bacterial suspension. 1 drop of the contents from each of the 2 columns was then washed 3 times with PBS pH 8.0 and stained with the anti-*Pneumococcus*-FITC conjugate. Microscopic observation showed that the Sepharose beads containing spe-

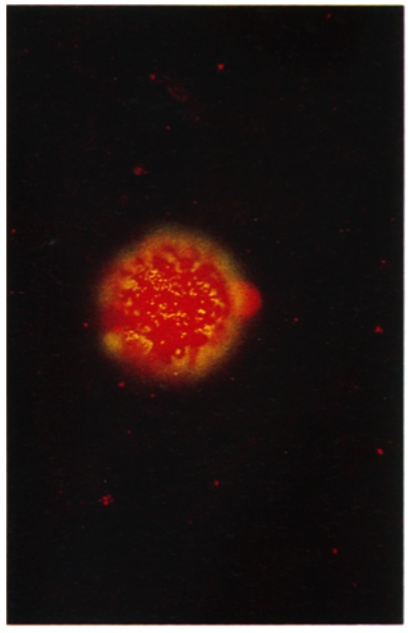

Fig. 1. Sepharose beads with covalently linked anti-*Pneumococcus* type 2 anti-body, then incubated with *Pneumococcus* type 2. After several washings staining with anti-Pneumococcus type 2 FITC-conjugate. Microscopical enlargement 6 × 10.

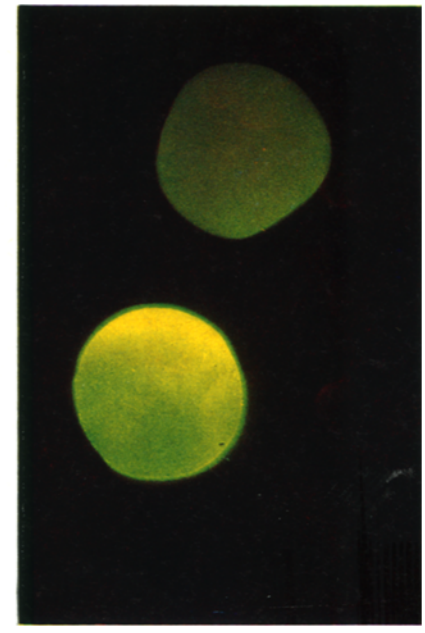

Fig. 3. Sepharose beads with covalently linked anti-*Pneumococcus* type 2 antibody, then incubated with *Pneumococcus* type 2 polysaccharide. After several washings staining with anti-*Pneumococcus* type 2 FITC-conjugate. Optical equipment: WILD Microcsope M 11, illumination with a quartz-iodine lamp 100 W. Interference FITC-filter, no blue filter BG 23. Film Ektachrome High Speed 23 DIN developed at 32 DIN.

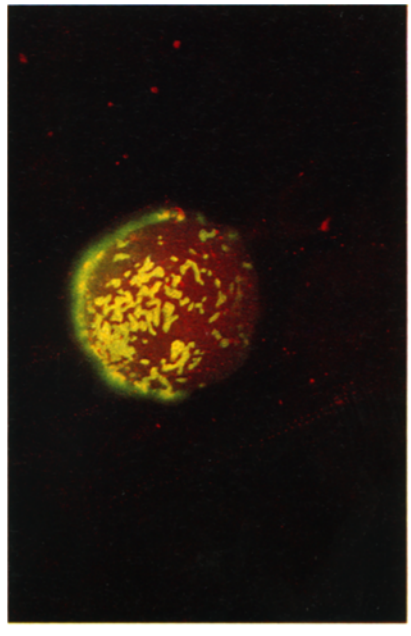

Fig. 2. The same as in Figure 1. Microscopical enlargement 6 × 40. Capsular swelling of the *Pneumococci* visible.

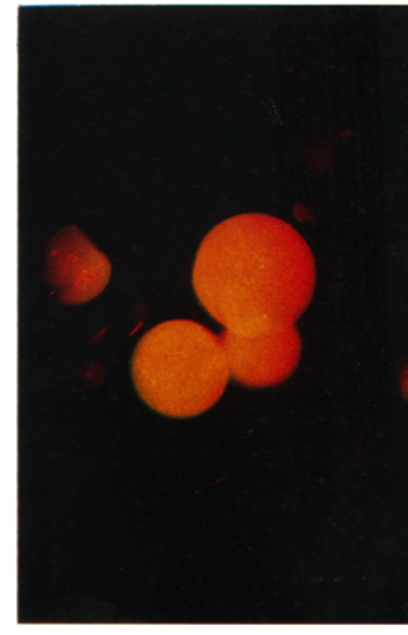

Fig. 4. Negative control. Sepharose beads with covalently linked anti-Ferritin antibody, then incubated with *Pneumococcus* type 2 polysaccharide, then stained with anti-*Pneumococcus* type 2 FITC-conjugate.

cific antibody were densely packed with *Pneumococcus* organisms at their surfaces (Figures 1 and 2). In Figure 2 a capsular swelling reaction of the bacteria is clearly recognizable. No bacteria were attached to the beads of anti-Ferritin-Sepharose.

The experiment proved that antibody coupling to the insoluble Sepharose beads was successful, and since we worked with particulate antigens, it was also possible to get a quantitative impression of the degree of antibody fixation. In a second experiment we used *Pneumococcus* polysaccharide as an antigen instead of bacteria, according to the following system:

$$\text{Sepharose-}\left(\begin{matrix}\text{anti-}\textit{Pneumococcus}\\ \text{type 2 globulin}\end{matrix}\right)\text{-}\left(\begin{matrix}\textit{Pneumococcus-}\\ \text{Polysaccharide}\\ \text{type 2}\end{matrix}\right)\text{-}\left(\begin{matrix}\text{anti-}\textit{Pneu-}\\ \textit{mococcus}\\ \text{2-FITC}\end{matrix}\right).$$

All the beads were now homogeneously and brilliantly stained whereas the negative control beads (anti-Ferritin-Sepharose + *Pneumococcus*-polysaccharide + anti-*Pneumococcus*-FITC) showed no specific fluorescence. They were pale greenish when using a blue filter BG 23, or red when the correction filter was omitted (Figures 3 and 4).

Apart from this very practical control of our coupling procedure we use this method for the following purposes: 1. The evaluation of fluorescent conjugates against soluble antigens (i.e. proteins, polysaccharides, etc.) to be used in immunohistology is difficult: The antigen can be incorporated into a thin layer of gelatine on a slide and then stained, but according to our experience this method is poor, because the homogeneous antigen layer allows no optical contrast.

On the other hand, the evaluation of the antigen directly with histological sections containing antigen is laborious. The direct (protein) or indirect (by means of specific antibody) fixation of the soluble antigen to Sepharose beads gives us an antigen carrier, which is easy to handle and can be washed. Furthermore it reveals an optimal optical contrast without any background, because of its particulate structure.

2. For the evaluation of new filter systems in the fluorescence microscopy, i.e. interference filters for green FITC and red Rhodamine compounds, which become more and more important for the simultaneous double staining of 2 different antigens, it is helpful to have a simple training system. *Ploem*[10] recommended the use of microdroplets of a watery solution of the fluorochrome in an artificial resin mixture. We coupled bovine IgG-FITC and bovine serum albumin labelled with Tetramethyl-Rhodamine-Isothiocyanate (BBL) to Sepharose 4 B and submitted mixtures of the brilliantly fluorescing beads (green = FITC, red = BSA-TRITC) to fluorescence microscopy. Such preparations can be stored at 4°C for many months.

After the completion of this work we learned that J. LASCH, M. IWIG and H. HANSON[11] made a similar experiment with leucine aminopeptidase labelled with FITC and coupled to Sephadex G 100 and Sepharose 6 B respectively.

Zusammenfassung. Durch Kombination von Immunoadsorption und Immunofluoreszenz wird die Adsorption von Proteinen an eine Trägersubstanz sichtbar gemacht.

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[10] J. S. PLOEM, *Standardization in Immunofluorescence* (Ed. E. J. HOLBOROW, Blackwell Scientific Publications, Oxford 1970).
[11] J. LASCH, M. IWIG and H. HANSON, Eur. J. Biochem. *27*, 431 (1972).

PRAEMIA

Prize Biochemical Analysis

The prize of DM 10,000.– is donated from Boehringer, Mannheim, and is awarded at the conference 'Biochemische Analytik' in Munich for outstanding work in the field of biochemical analysis. The donation will take place during the 1974 conference between 23 and 26 April. One paper or several papers concerning one theme, either published or accepted for publication between 1 October 1971 and 30 September 1973 may be sent in triplicate before 15 November 1973 to Prof. Dr. Ivar Trautschold, Secretary of the Prize Biochemical Analysis, Medizinische Hochschule Hannover, Karl-Wiechert-Allee 9, D-3 Hannover-Kleefeld (Germany).

CONGRESSUS

Switzerland

4th International Conference on Magnetic Resonance in Biological Systems

at Kandersteg, 16–21 September 1974.

The purpose of the conference is to bring together scientists of many disciplines who are concerned with the application of magnetic resonance in biochemistry, molecular biology, biophysics, pharmacology, and medicine. The program will include papers presented by invited lecturers, contributed communications, an discussion periods.

For further information write to: Professor Dr. K. Wüthrich, Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8049 Zürich (Switzerland).

Switzerland

9th EUCHEM Conference on Stereochemistry

at the Bürgenstock, near Luzern, 5–12 May 1974.

The number of participants will be limited. Inquiries and applications (no special forms are required) should be addressed before January 15, 1974 to the Chairman: Prof. J. M. Lehn, Institut de Chimie, Université de Strasbourg, 1, rue Blaise Pascal, F-6700 Strasbourg (France).