again lowered into the tubes. In this position, the conical stoppers seal off the test tubes (Figure 2). The rods may now be left until complete lysis takes place, in which case the matt-black plate serves as a good background for estimating the degree of lysis. Alternatively, it is possible to withdraw all the rods at once at any wanted time interval by raising the support plate, or any individual rod by lifting it partially and then taking it sideways out of its slot. To determine the residual protein content, the rod and clot are introduced directly into Biuret reagent. A further method of estimating the degree of lysis is to examine an aliquot of the incubation medium at fixed intervals for lysis products[12].

The usefulness of the apparatus described above is exemplified in the following experiment. The euglobulin clot lysis time had to be determined in duplicate in a total of 20 rats (4 groups of 5). The plasma samples were obtained form normal rats and rats with adjuvant arthritis, treated from the 18th until the 21st day post adjuvant with either water/tragacanth or sodium salicylate, once daily by mouth in a dose of 300 or 600 mg/kg[12, 13]. As can be seen from the Table, fibrinolytic activity in the arthritic rats was already restored to an almost normal level by treatment with 300 mg/kg of sodium salicylate. A statistically significant increase in fibrinolysis was, however, only obtained with a dose of 600 mg/kg.

Zusammenfassung. Es wird eine Apparatur beschrieben, die es ermöglicht, die Lyse grösserer Serien von Plasma- oder Euglobulingerinseln nach verschiedenen Methoden zu untersuchen.

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A Rapid Fluorescent Solid-Phase Method for Detecting Antibodies Against Milk Proteins and Gliadin in Different Immunoglobulin Classes

Estimating the different immunoglobulin classes of a specific antibody is a new technique which furnishes valuable information about the course of a disease. Immunofluorescence allows the identification of the immunoglobulin class of an antigen-bound antibody by use of a fluorescent heavy chain specific antihuman antiserum. However, this technique is restricted to systems with a cellbound antigen-antibody complex which can be visualized in a fluorescence light microscope.

The identification of immunoglobulin classes of antibodies reacting with non-cellbound antigens is in general done by a two step procedure. First a fractionation of the serum into the different immunoglobulin classes is carried out. A second step involves the determination of antigen specific antibody in the different serum fractions. Although IgM and IgG, and after improving gel filtration by use of agarose[1] also IgA, are well separated from each other, this method cannot be used for minor immunoglobulin classes as IgE.

In the fluorescent solid-phase method presented here, these difficulties were overcome by covalent binding of soluble antigen to agarose beads and using the immunofluorescence method described for cellbound antigens.

As coeliac disease and milk sensitivity have been studied for years in our laboratory[2–4] antibodies against milk proteins and gliadin[5–7] were chosen for the development of the new technique.

Methods. 1. *Coupling of antigen to agarose* (according to Porath et al.[8]). 2 g hydrated (100 mg dry) agarose Biogel-A-5m, 200–400 mesh (Calbiochem) are suspended in 20 ml H_2O at 0 °C, the pH adjusted to 11 with 2 *N* NaOH and 0.2 g BrCN added. The suspension is stirred at 0 °C for 30 min and the pH kept at 11 by adding further NaOH. The agarose is washed with cold water and 0.1 m $NaHCO_3$ and suspended in 2 ml 0.1 *M* $NaHCO_3$. 20 mg of antigen are dissolved in 2 ml of 0.1 *M* $NaHCO_3$ (for gliadin in NaOH pH 10) at 0 °C, mixed with the agarose suspension and stirred slowly over night at 2°–4 °C. Then 100 mg of glycine are added, followed by stirring for 1 h at room temperature. The product is washed with 0.1 m $NaHCO_3$, 0.1 m acetate buffer pH 5, 0.1 *M tris* buffer pH 7.5 with 0.9% NaCl and water. The washing procedure of agarose-gliadin is modified using 0.1 *M* acetic acid and H_2O due to the insolubility of free gliadin in the buffers mentioned above. The final product is stored in the hydrated form with the addition of sodium azide or lyophilised. After lyophilisation the diameters of the agarose beads are diminished, but this has no effect on the reactivity of the antigen.

2. *Testing for serum antibodies.* 1 mg dry weight of antigen loaded agarose is suspended in 0.02 ml of veronal buffer pH 7.2 and 0.3 ml of diluted (usually 1:50 or 1:100) serum to be tested is added. After incubation for 1 h at room temperature the beads are washed with veronal buffer and 0.3 ml of fluorescent rabbit-anti-human-IgG or -IgM or -IgA (Behringwerke) are added. The appropriate dilution of the fluorescent antiserum must be determined beforehand. The mixture is again incubated for 1 h at room temperature and washed with veronal buffer. After the last centrifugation the sediment is viewed under a blue light fluorescence microscope. The presence of specific antibodies is shown by brilliantly green globules with sharp fluorescent borders. In samples without antibody the agarose is only faintly green.

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Results. The sera of 14 children hospitalized with the diagnosis of coeliac disease were tested for antibodies against gliadin and 5 different cow milk proteins (casein, α-lactalbumin, β-lactoglobulin, bovine γ-globulin, bovine serum-albumin).

The sensitivity of the new fluorescent solid-phase method was compared with the Ouchterlony precipitation technique (final reading after 5 days) and for gliadin to the complement fixation test in addition (see Table). The fluorescence technique is 10 to 100 times more sensitive for the detection of IgG-antibodies toward gliadin and milk proteins than the conventional Ouchterlony technique. In many sera, antibodies to single protein classes could only be revealed by fluorescence. All sera giving negative results with this new method gave no precipitation reaction or complement fixation.

Sensitivity of different methods for detecting antibodies against cow milk proteins and gliadin

Antigen	Test for antibody detection	Coeliac disease[a]
Gliadin	complement fixation	9/14
	Ouchterlony	7/14
	fluorescence[b]	14/14
Casein	Ouchterlony	9/14
	fluorescence[b]	14/14
β-Lactoglobulin	Ouchterlony	12/14
	fluorescence[b]	13/14
α-Lactalbumin	Ouchterlony	13/14
	fluorescence[b]	13/14
Bovine serumalbumin	Ouchterlony	1/14
	fluorescence[b]	8/14
Bovine γ-Globulin	Ouchterlony	3/14
	fluorescence[b]	9/14

[a] Number positives/number tested; [b] tested with rabbit anti-human-IgG

Further studies evaluating the diagnostic significance of antibodies against food-proteins in different immunglobulin-classes (IgG, IgA, IgM, IgE) are in progress.

Zusammenfassung. Mit Hilfe einer neu entwickelten Methode (Bindung von Antigen an Agarose-Partikel, AK-Nachweis mit fluoreszierenden Anti-human-Ig-Seren) gelingt es auf einfache Art, Serum-AK gegen Gliadin und Milchproteine bei Coeliakiepatienten in verschiedenen Immunglobulinklassen nachzuweisen.

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[9] We gratefully acknowledge the able technical assistance of Mrs. ZAIN and Mrs. CAHN.

CONGRESSUS

Yugoslavia

IAEA Symposium on Nuclear Activation Techniques in the Life Sciences

in Ljubljana, 10–14 April 1972.

The Symposium will be concerned with the applications of nuclear activation techniques in the life sciences and the significance of the results obtained in such applications. It follows the IAEA Symposium in Amsterdam 1967.

As regards techniques, topics to be discussed include sample preparation procedures particularly in multicomponent systems and biological analytical reference materials. As regards interpretation of data, results obtained in studies in both cellular and subcellular systems in plants and animals will be discussed. Contributions relating to agriculture, biochemistry, ecology, nutritional studies, pharmaceutics and pharmacology, as well as applications in medical diagnosis, research and therapy, will be included. Contributions relating to human ecology will deal especially with the problems of public health, environmental pollution and food additives and contamination.

Further information by the Scientific Secretaries G.B. Cook and R.M. Parr, IAEA Symposium on Nuclear Activation Techniques, Kärntnerring 11, P.O. Box 590, A–1011 Wien (Austria).

Great Britain

6th European Symposium on Bio-Organic Chemistry (ESBOC)

in Gregynog Hall, mid-Wales, 19–22 May 1972.

Chairmanship Professor A.R. Battersby, Cambridge. The symposium will include lectures by Professor D. Arigoni (Zurich) and Professor F. Lynen (Munich). Further information by Dr. J.S. Davis, Secretary of ESBOC Symposium, Department of Chemistry, University College, Swansea SA2 8PP (Wales), UK.