using the appropriate enzyme substrates. Figure 1 should be consulted to clarify the overall steps in the staining procedure.

In the following description, all concentrations are expressed in terms of the final concentration in the overlay.

The 1,3-DPG generating system consists of $0.00219M$ glyceraldehyde-3-phosphate (GAP), $0.00033M$ nicotinamide adenine dinucleotide (NAD), $0.01M$ K_2HPO_4, $0.06M$ pyruvate, 6.1 units lactate dehydrogenase (LDH) per ml, and 2.1 units GAPD per ml. These components are dissolved in a third of the *Tris*-HCl, $0.1M$ pH 7 buffer to be used and this is incubated at 37 °C for 30 min. Pyruvate and LDH are used to keep the levels of reduced NAD (NADH) low since high levels can produce too dark of a background in the overlay.

To the 1,3-DPG generating system, after 30 min incubation, are added the reagents of the ATP detection system, which are $0.0011M$ ADP, $0.01M$ glucose, $0.11M$ $MgCl_2$, $0.00043M$ nicotine adenine dinucleotide phosphate, $0.00039M$ phenazine methosulfate, $0.00029M$ MTT, 0.5 mg G6PD per ml, and 0.1 mg HK per ml. The resultant solution is mixed with a molten (40 °C) Ion-agar solution made from the remaining 2/3 buffer to give a final agar concentration of 0.75%. The final agar solution is then poured over the surface of the starch gel and incubated at 37 °C.

Bands of PGK activity will appear well within 30 min and should be photographed or scored. Because of the presence of components of the 1,3-DPG generating system, the overlay will darken with more time and bands will be difficult to see.

Some of the bands seen may be due to adenylate kinase (AK) activity and not PGK. Since AK can convert ADP to ATP without the need for any other cosubstrates, staining the other face of the gel with an overlay similar to the above but minus the 1,3-DPG generating system will indicate which are AK bands. AK bands usually take much longer to develop than PGK bands.

Starch gels were run for 8 h using the pH 7 histidine gel and citrate bridge buffer system and electrophoretic setup described by BREWER[6] for pyruvate kinase.

Results and discussion. In Figure 2 can be seen the starch gel PGK banding pattern of selected samples. The specificity of the stain is attested to by the fact that the PGK bands do not appear if either GAP or GAPD are omitted from the 1,3-DPG generating system. Further, a commercial PGK preparation (yeast) was found to produce a band only with the complete stain. Separate staining for LDH also removed the remote possibility that these bands could represent this activity.

The previous system for detecting PGK activity utilizes the reverse reaction of PGK. In this system 1,3-DPG is formed at the site of enzyme activity on gels and is detected by a GAPD/NADH system. The areas of activity can be visualized under UV-light as less fluorescent areas (NAD) against a fluorescent background (NADH)[5].

The commercial availability of 1,3-DPG would, of course, greatly simplify the positive staining technique described in this communication and add to its attractiveness. Nevertheless, our experience with the positive stain has been more satisfying than that with the fluorescent technique. In fact, our difficulties in visualizing banding patterns under UV-light (as required with the fluorescent technique) were the impetus for developing the positive stain.

In a study presently in progress involving over 15 species (both plant and animal) we have had no trouble obtaining PGK bands with the positive technique and distinguishing them from AK bands. Thus, the technique most likely has wide species applicability.

Zusammenfassung. Eine Methode zur positiven Anfärbung der Phosphoglycerat-Kinase (EC 2.7.2.3) nach Stärkegelelektrophorese wird beschrieben. Das in der Vorwärtsreaktion des Enzyms gebildete Adenosin-Triphosphat wird mit einem Indikatorsystem (Hexokinase, Glukose-6-phosphat-Dehydrogenase, Tetrazoliumsalz) erfasst.

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An Apparatus Suitable for Serial Determinations of Fibrinolytic Activity

Various methods have already been described for determining the influence of drugs on the fibrinolytic activity of plasma in vitro[1-9,11,13] or in vivo in pretreated animals[12]. Since the number of samples to be tested may be very large, the construction of a time-saving device to facilitate the estimation of fibrinolytic activity appears well worth the effort. Such an apparatus is particularly useful when, instead of a straight-forward measurement of lysis time, the clots have to be treated in a certain way or a sample of the incubation medium taken in order to define the mode of action of a preparation[1,12].

We have therefore designed an apparatus permitting the lysis of 20 clots to be studied in various respects. One point to which we attached particular importance was that the formation and treatment (e.g. rinsing) of the clots, as well as the removal of samples of the incubation medium, could be effected simply and as nearly as possible simultaneously in all specimens.

The apparatus consists essentially of the following parts (Figure 1): rectangular cabinet box (A); a support plate (B); exchangeable clot rods (C) and racks for test tubes (D) of different sizes.

Cabinet. The cabinet is a rigid box, on the base (a) of which special test-tube racks are placed (D). A vertical

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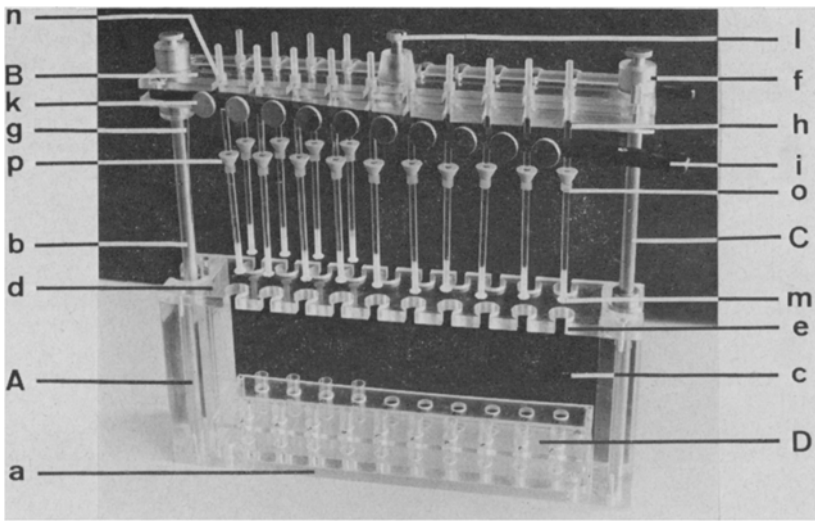

Fig. 1. Apparatus for studying clot lysis. Low rack with small tubes.

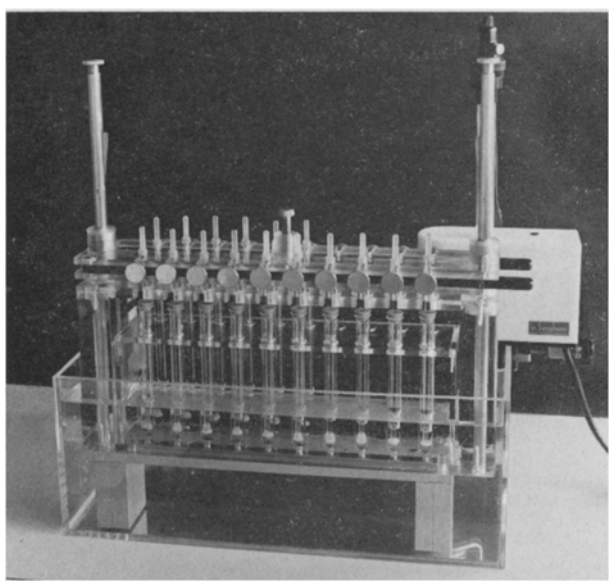

Fig. 2. Apparatus for studying clot lysis in water bath. Higher racks with test tubes containing buffer solution. Clots on the glass rods.

pillar (b) is set into each of the side walls; a matt-black plate (c) can be fitted to the back. The front is open. The top (d) has large slotted holes (e).

Support plate. The support plate slides up and down the two pillars and in axial ball-bearing guides (f) which prevent it from tilting and thus jamming. In its uppermost position, the plate is automatically held in place by catches (g) set into the pillars. Ten slots (h) cut into each of the longer sides accommodate the clot-rods, the latter are held in place by a pivoting bar (i) with spring-loaded stud bolts (k). The height of the support plate and clot-rods in the lower position can be regulated by means of an adjusting screw (l) in the centre of the plate.

Clot-rods. The clot-rods are roughened at the bottom and have flattened, indented ends (m). In our experience, even very loose clots adhere to these rods. The upper rubber collar (n) prevents the rod from slipping down when the bar is open; the lower collar (o) retains the conical stopper (p) for the test tubes.

Tube racks. The racks are placed on the base plate and sit firmly on pegs, so that there is no danger of the clots being wiped off on the edge of the test tube when the support plate is raised or lowered. Various racks may be used for different purposes: e.g. low racks with small tubes for the formation and washing of the clots, and tall racks for tubes containing incubation medium.

These compounds are made of Plexiglas (box, support plate and racks), stainless steel (pillars, axial ball-bearings and screws), anodized aluminium (retaining bar and a mattblack back-plate) and glass (clot rods).

We have used this apparatus to good effect in recent experiments described elsewhere[12,13]. Briefly, the procedure is as follows: First, the rods are hung in position in the slots of the raised support plate and kept in place with the check bar. Small tubes containing citrated plasma or euglobulin in buffer solution are inserted into a low rack. Clot formation can be induced with a $CaCl_2$ or thrombin solution, or with a combination of both. The rods are then lowered into the tubes and the apparatus is placed in a water-bath at 37 °C. After 20 min the rods with the adhering clots are either rinsed off or immersed for a short time in washing fluid. To determine lysis time, a higher rack with tubes containing buffer solution is placed on the base plate and the clot-bearing rods are

Euglobulin clot lysis time (ECLT) in plasma of rats with adjuvant arthritis (AdA)

Rats	Treatment	ECLT (min)	P
Normal rats	–	77 ± 8[a]	
AdA rats	water/tragacanth (0.5 ml/100 g)	329 ± 48	<0.001[b]
AdA rats	sodium salicylate in water/tragacanth		
	300 mg/kg	221 ± 29	<0.1 [c]
	600 mg/kg	131 ± 33	<0.01[c]

[a] $n = 5$, determinations duplicated in each case; SD according to LORD[10]. [b] Calculated according to LORD[10] *vs.* normal rats; [c] *vs.* sham-treated rats.

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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