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Light Chain Typing of Immunoglobulins in Small Samples of Biological Material

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Summary. A method is described for the typing of the light chains of immunoglobulins in small samples of sera or external secretions and without their previous isolation. It consists of immunoelectrophoresis in agar plates which contain specific antisera against one of the light chain types. All immunoglobulins of this type are thus selected by precipitation in the central area during the electrophoretic phase. Immunoglobulins of the opposite light chain type diffuse through the agar and react with the class specific antisera from the troughs. This results in the precipitin lines as in conventional immunoelectrophoresis. This technique has proved most useful for typing heterogenous or homogeneous immunoglobulins in normal and low concentration. The antisera used for incorporation in the agar should fulfil special requirements. They should contain a high level of antibodies against common surface determinants of the immunoglobulin light chains. The further possibilities of this immunoselection technique for typing different protein mixtures is discussed.

INTRODUCTION

The determination, by means of immunoelectrophoresis, of the light chain type of the immunoglobulins present in a mixture like serum offers no problem provided the relevant immunoglobulin is present in relatively high concentration. This is usually the case in myeloma or Waldenström's macroglobulinaemia. Nor are there any difficulties in immunoelectrophoretic typing of the slowly migrating part of IgG, where there is no interference by the other immunoglobulins. Antisera which are directed against the determinants specific for the various light-heavy chain combinations can also be used successfully (Prendergast, Grey and Kunkel, 1966; Korngold and Madalinski, 1967; Mul and Ballieux, 1968). These antisera are, however, difficult to prepare and are not commercially available. Finally one can resort to the isolation of the immunoglobulin to be examined. However, the methods used for purification or isolation are laborious and relatively large amounts of material are needed. Therefore a method will be presented which allows the typing of immunoglobulins in very small quantities of material, usually without the need of prior isolation of the component under investigation.

In principle this method consists of the standard immunoelectrophoretic procedure, but carried out in agar containing specific antibodies against one of the light chain types. All immunoglobulins of this type will then be precipitated during the electrophoretic phase by the antiserum incorporated in the agar and only the immunoglobulins of the other type will be able to diffuse through the agar. An antiserum against class (or subclass) specific determinants, is placed in the trough, and produces a precipitin line with immunoglobulin of the freely diffusible light chain type.

MATERIALS AND METHODS

The methods which have been used in our investigations will be described in detail. It is to be expected that other, comparable, reagents and modifications of these techniques can also be applied with success.

Materials

Sera, tears and saliva were obtained from patients and healthy persons in the University Hospital in Leiden and from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. Four sera from patients with alpha heavy chain disease were provided by Dr M. Seligmann and Dr J. W. Stoop. Serum and external secretions were obtained from a patient J. M. suffering from lymphopenic immunological deficiency (de Koning, Dooren, van Bekkum, van Rood, Dicke and Rádl, 1969).

Buffer

(a) Stock solution—Diethylbarbituric acid 7·36 g
Sodium barbiturate 41·20 g
Dissolve in 2000 ml distilled water (pH = 8·6; ionic strength $\mu = 0{\cdot}12$).

(b) Working solution—Dilute stock solution with equal amount of distilled water (pH = 8·6; $\mu = 0{\cdot}06$).

Agar

A 3 per cent Difco Bacto agar solution is prepared in buffer by boiling in a water bath until clear. The temperature is then brought down and kept at 50°.

Antiserum incorporation in the agar

An appropriate amount of antiserum (usually about 0·6–2 ml anti-κ or anti-λ light chain antiserum, depending on the titre of antibodies) is made up to 5 ml with the working solution. The temperature of this solution is brought to 50° in a water bath. Five millilitres of the warm 3 per cent agar solution is added to the preheated antiserum. Merthiolate at a final concentration 1 : 10000 is used to prevent bacterial growth.

Staining solution

Amido black 10B	0·5 g
Glacial acetic acid	50 ml
Methanol	450 ml

Washing solution

Methanol	500 ml
Glacial acetic acid	50 ml
Distilled water	400 ml

Antisera for testing

Monospecific rabbit antisera against κ and λ type of Bence Jones proteins were prepared in this Institute as described previously (Hijmans, Schuit and Klein, 1969); monospecific swine antisera against human IgG, IgA, IgM and IgD and rabbit antisera

against κ and λ type Bence Jones proteins were obtained from Sevac (Prague); monospecific rabbit antisera against κ and λ type Bence Jones proteins were purchased from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.

Methods

A volume of 9·5 ml of the antibody containing agar solution is applied to a methanol cleaned and preheated 8·5×8·5 cm glass plate. Holes with a diameter of 1·6 mm (for serum) or 2·0–2·5 mm (for fluids with a low concentration of immunoglobulins) are cut in the agar 3·5 cm from the anodic end of the plate. Longitudinal troughs are cut at the same time, but the agar is not removed. The samples to be tested are applied into the holes. The first one is used for the reference serum.

Electrophoresis is performed during 3 hours at 4–6 V/cm (10 mA/plate) in a chamber supplied by Sevac Prague. After electrophoresis the plates are kept in a moist chamber for one hour, whereafter the agar pieces from the troughs are removed and the troughs filled with specific antiserum against class specific determinants of the immunoglobulins under study. The troughs can also be filled alternately with a different class specific antiserum,

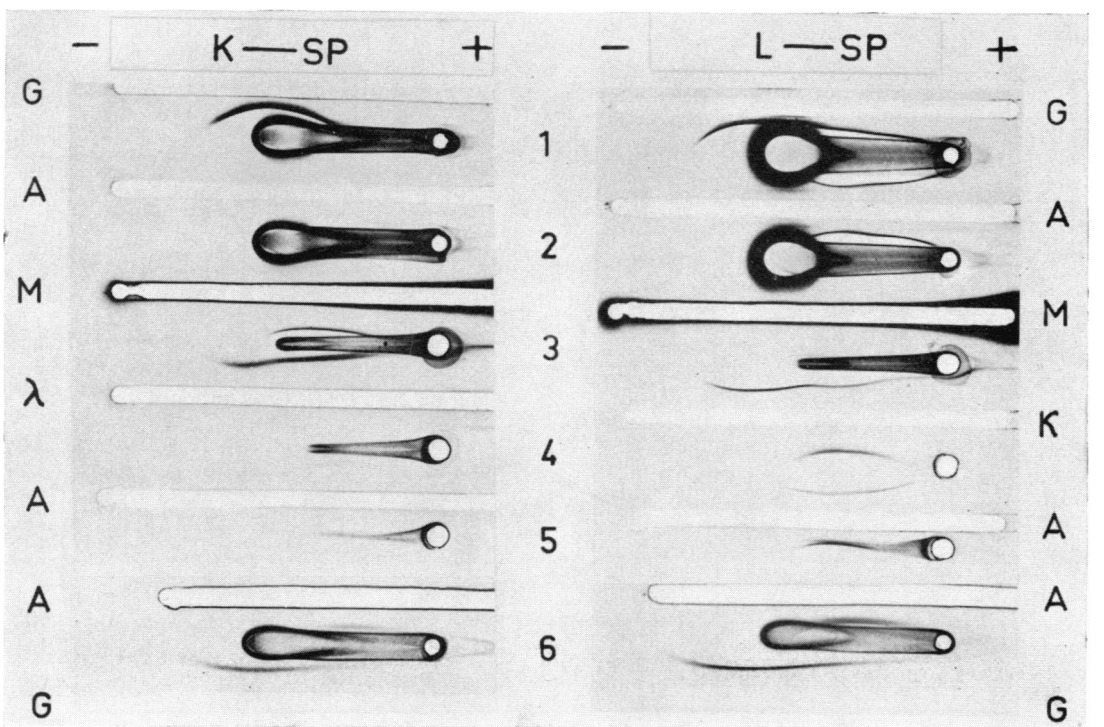

FIG. 1. Typing of immunoglobulin light chains in normal human serum (6), and in the serum (1, 2), the serum euglobulin fraction (3), and the tears (4) of a boy suffering from Swiss type agammaglobulinaemia, 3 months after transplantation of bone-marrow cells and foetal thymus. At that time there were IgG-L and IgA-K paraproteins, normal IgG-K, no detectable IgA-L, high IgM-L and low IgM-K in the serum. The tears of normal children (5) contain IgA of both types whereas in the boy's tears only IgA of κ type was detectable. The troughs contained specific anti-IgG (G), anti-IgA (A), anti-IgM (M) and anti-κ (κ) or anti-λ (λ) light chain antisera.

e.g. to test another immunoglobulin, or with antiserum against the light chains of the type, not incorporated in the agar, as a control (Fig. 1).

After 24 hours the diffusion and precipitation in the moist chamber is finished and the plates are intensively washed in buffered saline for 24 hours. The plates are then covered with a filter paper moistened in distilled water and kept at room temperature until dry. After removing the dry paper, the plate is carefully washed under tap water and stained with amido black 10B. The excess dye is removed by repeated washing in the washing solution. This solution can be recovered by filtration through activated carbon. The plates are dried at room temperature.

Nomenclature and function of the plates

The plates are indicated in an abbreviated form according to the antiserum which is incorporated in the agar.

Kappa selection plate (K-SP)

K-SP is the plate which contains anti-κ type light chain antiserum incorporated in the agar. All κ-type immunoglobulins are selected from the biological fluids under investigation during the electrophoretic phase by precipitation in the central area. This area is localized on the electrophoretic axis and extends from the starting hole to the cathodic part of the plate. Immunoglobulins of λ type diffuse through the agar and react with the class or λ type specific antisera from the troughs giving rise to the formation of precipitin lines as in conventional immunoelectrophoresis.

Lambda selection plate (L-SP)

L-SP with incorporated anti-λ type light chain antiserum represents the opposite situation where immunoglobulins of λ type are selected in the central precipitate and immunoglobulins of κ type induce precipitin lines with class specific or κ type specific antisera from the troughs.

Kappa–lambda selection plate (KL-SP)

KL-SP contains both anti-κ and anti-λ light chain antisera incorporated together in the agar. The immunoglobulins of both types are precipitated by these antisera during electrophoresis and give rise to a double contoured central precipitate. With the exception of free heavy chains immunoglobulin molecules do not then diffuse through the agar and therefore do not react with any of the anti-immunoglobulin antisera from the troughs. This plate is used as a control for the entire system.

RESULTS

THE TYPING OF HETEROGENEOUS IMMUNOGLOBULINS—NON PARAPROTEINAEMIC SERA

IgG and IgA were tested in the sera using holes of a diameter of 1·6 or 2·0 mm. In sixteen sera without paraproteins both IgG and IgA precipitin lines appeared in K-SP or L-SP and their amount could be roughly estimated by taking into consideration the length of the precipitin line and its distance from the antiserum trough. In addition no lines were observed by using a KL-SP. As previously mentioned, this type of selection plate was used to confirm that the quantity and quality of the incorporated antiserum were adequate for the antigens in the sera under test.

The typing of IgM when present at normal or low concentrations was more difficult. The precipitin line was often overlapped by the heavy precipitate in the central region. The filling of the trough with antiserum 6 hours after electrophoresis was sometimes beneficial and produced a precipitin line between the central precipitate and the trough. Better results were achieved using an enriched sample such as a euglobulin fraction obtained by precipitation with 1 per cent boric acid (Badin and Levesque, 1961). The redissolved precipitate from 0·1 ml serum yielded enough material to fill two or three holes (Fig. 1).

For typing IgD or other immunoglobulins in very low concentration a different arrangement was used. After electrophoresis, holes were cut instead of troughs and alternately filled with antiserum and standards (Fig. 2). Purified myeloma proteins of the same light chain type as the immunoglobulins under investigation served as standards. With this system immunoglobulins are detectable at concentrations of less than 10 mg/100 ml.

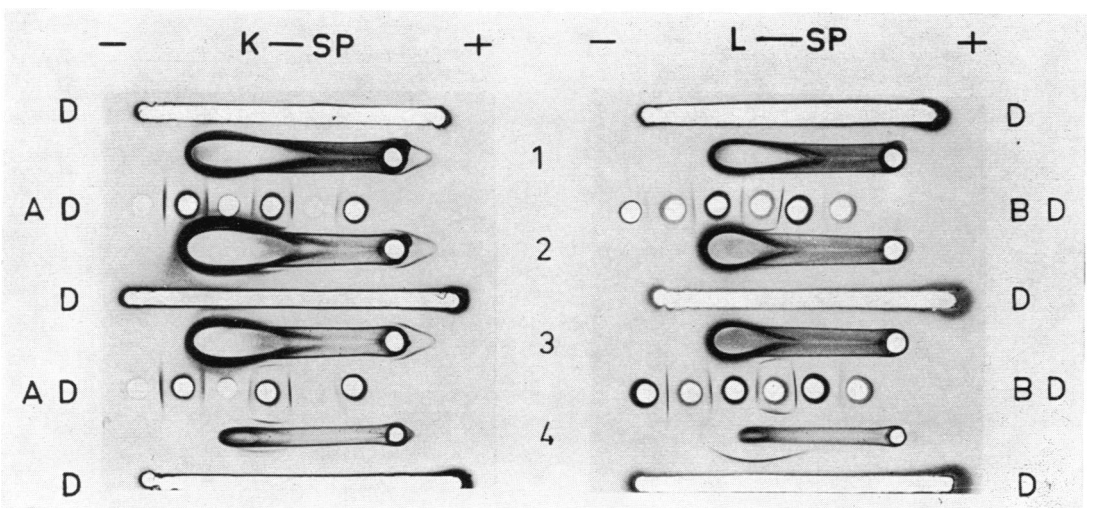

FIG. 2. Arrangement of selection plates for typing immunoglobulins in very low concentration. This shows an example of IgD typing in normal human serum (1) and in the sera of different patients (2, 3, 4) in which the total IgD concentrations were 3, 20, 13 and 60 mg/100 ml, respectively. The troughs contained specific anti-IgD antiserum (D) and the longitudinally punctured holes contained alternately standards A or B and anti-IgD serum (D). In the K-SP IgD paraprotein of lambda type (A) and in the L-SP IgD paraprotein of κ type (B) served as standards.

THE TYPING OF HOMOGENEOUS IMMUNOGLOBULINS—PARAPROTEINAEMIC SERA

A total of thirty-two paraproteinaemic sera of different class, type and concentration of M-component were examined with this technique: seven IgG, thirteen IgA, five IgM, three IgD and four double paraproteinaemic sera. In all these cases it was easy to type the light chains of the paraproteins either in undiluted sera or after proper dilution (Figs. 1 and 3). In principle, paraproteins in low concentration (usually up to 100 mg/100 ml) were completely precipitated by the incorporated antiserum of the corresponding type whereas distinct and abnormal precipitin lines appeared in the plates using antiserum of the other type (Fig. 3). If normal immunoglobulin of the same class but opposite light chain type was present in the serum, the corresponding line with a normal shape appeared in the

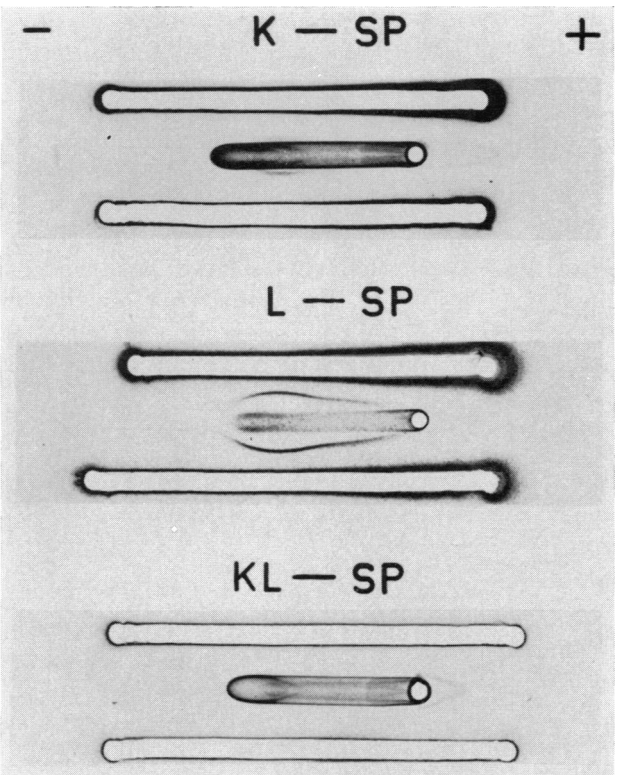

FIG. 3. Typing of IgA by immunoselection in the serum of a patient with IgA paraproteinaemia. The type of the paraprotein is IgA-K but some normal IgA-L molecules are still present. The absence of any line in the KL-SP shows the adequacy of the antigen–antibody system. The holes contain the patient's serum and the troughs contain specific anti-IgA antiserum.

opposite plate as well (Fig. 3). No line was detected when the KL-selection plate was used (Fig. 3).

In contrast, sera with higher concentrations of paraproteins gave variable results. The incorporated antisera were able to precipitate completely some of the paraproteins even when the latter were present at concentrations of about 2000 mg/100 ml. However, in some instances even high dilutions of the test sera could not prevent the appearance of a 'false' precipitin line with class specific antiserum. This could be due either to a local antigenic excess or an inadequacy of the entire system. In such cases the KL-SP clearly showed the inadequacy because precipitin lines were obtained with the class specific antiserum in the region of paraprotein mobility (Fig. 4).

In order to study possible differences in reaction of different paraproteins with the same specific anti-light chain antisera, the following experiment was performed. Ten sera with high levels of paraprotein (about 4–6 g/100 ml) and low levels of other immunoglobulins were diluted in order to give a comparable level of paraprotein. Serial dilutions of each paraprotein (1600, 800, 400, 200, 100, 50, and 25 mg/100 ml) were prepared and placed in holes in a plate with incorporated anti-light chain antiserum corresponding to the type

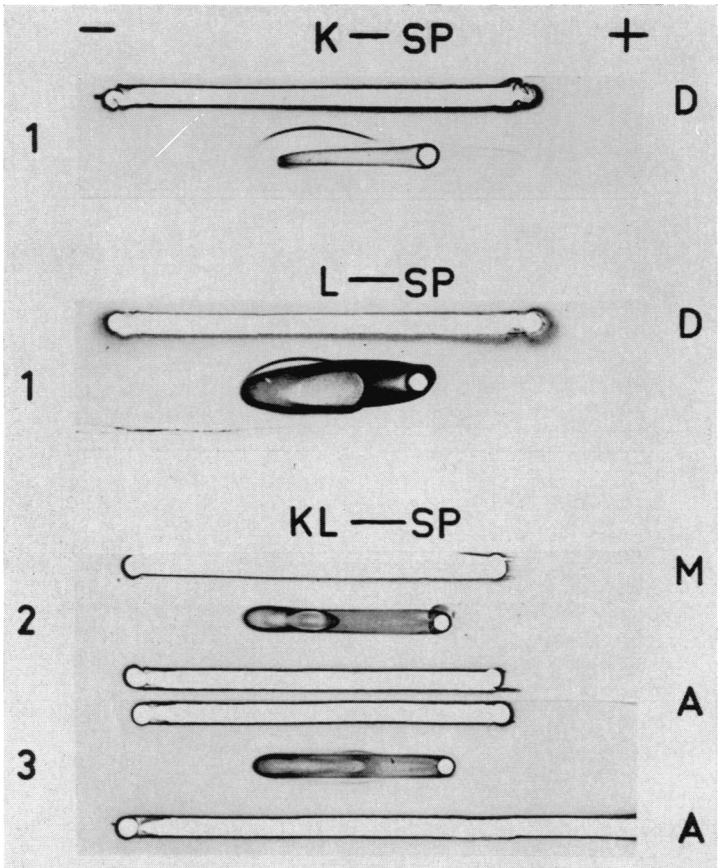

FIG. 4. Demonstration of striking differences of the central precipitin areas between K-SP and L-SP and of local irregularities of the precipititate in the KL-SP in paraproteinaemic sera. Hole (1) contained serum from a patient with a high level of paraprotein IgD and free Bence Jones, both of lambda type. The troughs contained specific anti-IgD antiserum (D). The L-SP shows a 'false' positive line due to antigen excess. In the holes of KL-SP there are IgM (2) and IgA (3) paraproteinaemic sera. Immunoglobulins in the former are completely precipitated, and in the latter incompletely precipitated, by the incorporated antisera. The troughs contained specific anti-IgM (M) and anti-IgA (A) antisera.

of paraprotein (Fig. 5). After diffusion for 4 hours the longitudinal troughs were cut and filled alternately with either the corresponding class specific antiserum or the same type of specific antiserum as had been incorporated in the agar. After a period of 24 hours diffusion and precipitation the plates were washed and stained. In eight plates four different anti κ and four different anti λ light chain antisera were incorporated and the same dilution range of antigens was used. The lowest concentration of antigen producing a precipitin line against class specific antisera was determined in each case. The results are given in Table 1.

Large differences were found in the ability of the antisera to completely precipitate different paraproteins in this arrangement. The system was more satisfactory for IgM and IgG paraproteins. However, even among paraproteins of the same class (IgA or IgG) there were conspicuous differences.

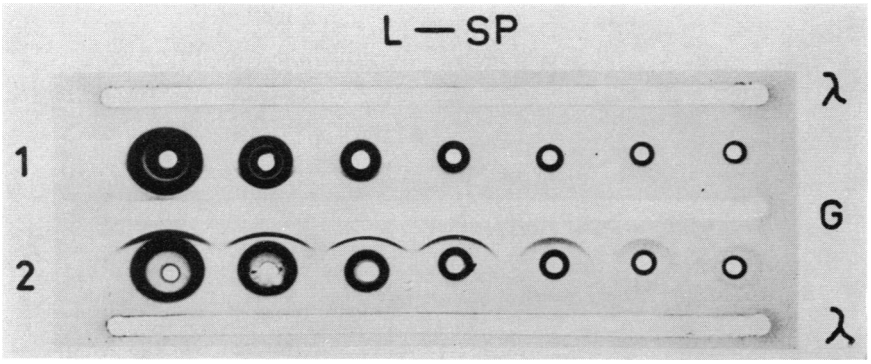

FIG. 5. The use of L-SP to test the complete precipitability of two different IgG-L paraproteins (1, 2) in serial dilutions. The troughs contained specific anti-lambda type light chain antiserum (λ) and anti-IgG antiserum (G). The first paraprotein is completely precipitated by the incorporated antiserum from a concentration of 400 mg/100 ml downwards, while the second one is not completely precipitated even at a concentration of 25 mg/100 ml.

TABLE 1

THE COMPLETE PRECIPITABILITY OF PARAPROTEINS BY TYPE SPECIFIC ANTI-LIGHT CHAIN ANTISERA*

Patient, class and type of paraprotein		Anti-κ type light chain antisera			
		K—I.IV	K—VII	K—VIII.A	K—IX.M
BI	IgD-K	50	50	25	25
BA	IgA-K	50	25	25	25
MI	IgA-K	200	400	50	50
BR	IgG-K	800	200	400	> 1600
NA	IgM-K	> 1600	> 1600	> 1600	> 1600
Patient, class and type of paraprotein		Anti-λ type light chain antisera			
		L—II	L—V	L—VI.A	L—VII.M
GR	IgD-L	25	25	25	25
RO	IgA-L	100	50	50	100
CR	IgG-L	25	25	25	100
KAL	IgG-L	400	800	200	> 1600
KAS	IgM-L	400	400	50	100

* The numbers indicate the lowest concentration of the paraprotein (in mg/100 ml) at which it was still not completely precipitated by a given antiserum. Serial dilutions containing 1600, 800, 400, 200, 100, 50 and 25 mg/100 ml of the paraprotein were studied.

THE TYPING OF IMMUNOGLOBULINS IN LOW CONCENTRATION—EXTERNAL SECRETIONS

By using an antigen well with a diameter of 2·5 mm (filled twice), IgA typing was performed on unconcentrated normal human tears and saliva of ten different persons and on similar material from a patient with selective IgA-L deficiency. In the normal tears and saliva, both IgA-K and IgA-L precipitin lines appeared in corresponding selection plates. In the patient's tears and saliva the line against specific anti-IgA serum was visible only in the L-SP. In the K-SP there was only a central precipitate and no precipitin line towards the trough with specific anti-IgA antiserum (Fig. 1).

For the typing of other immunoglobulin classes which are present in these fluids in very low concentration, a different arrangement was used. After electrophoresis of concentrated material an arrangement using holes instead of troughs was employed, as described

for the typing of IgD. Similar results can be obtained by a simple modification of the double diffusion technique without previous electrophoresis in a plate with anti-light chain antiserum incorporated in the agar.

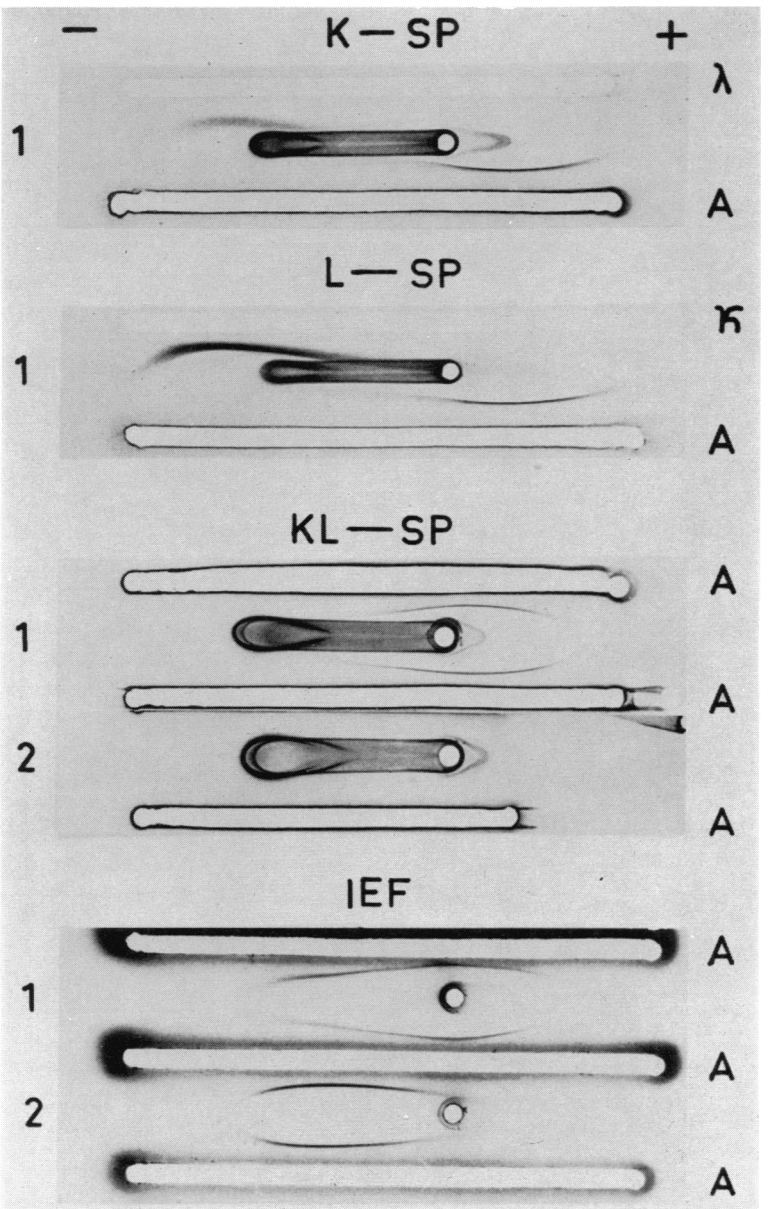

FIG. 6. Immunoselection and immunoelectrophoretic (IEF) analysis of free heavy chains in the serum of a patient with α-chain disease (1) compared with a normal human serum (2). The troughs contained specific anti-λ type (λ) and κ type (κ) light chain antiserum and specific anti-IgA antiserum (A). The precipitin lines of free α chains in the serum retain the same shape and anodic electrophoretic mobility in all plates.

THE ANALYSIS OF FRAGMENTS OF IMMUNOGLOBULINS

Fragments of heavy chains, such as are present in the serum of patients with heavy chain disease or in enzymatically split immunoglobulins, can also be detected by this method. In the presence of free heavy chains or parts of it, a precipitin line of the same shape and mobility appeared in the K-SP as well as in the L-SP against antiserum specific for the appropriate class. Also in the KL-SP this line appeared with the same intensity (Fig. 6).

Free Bence Jones protein in the serum can be detected by the use of type specific antisera, preferably by the use of antiserum against type specific hidden determinants of Bence Jones protein. In the K-SP the antiserum against λ Bence Jones determinants shows the presence of free λ Bence Jones protein in the serum and conversely anti-κ antiserum is used in the L-SP for detecting free κ Bence Jones protein in the serum. In the KL-SP no line will appear against any antiserum from the trough. Only a central precipitate in the anodic region (fast migrating Bence Jones protein) or a local deformation of any cathodic part of the central precipitate (slow migrating Bence Jones protein) can draw attention to the presence of free Bence Jones protein in the serum.

THE EVALUATION OF THE CENTRAL PRECIPITATE IN SELECTION PLATES

The evaluation of the central precipitate was performed from the qualitative and semiquantitative point of view. The areas of the central precipitates, as measured by weighing the enlarged photographs, were proportional to the concentration in a serial dilution of pooled normal human serum (Fig. 7). The evaluation of the central precipitates from different normal and pathological sera was performed only by comparison of these areas to the known standard area from pooled normal human serum. Such measurements gave only crude information about the total amount of immunoglobulins of a particular light chain type. This sort of information is probably inferior to the assessment of the precipitin lines in the opposite selection plate, but it can serve as useful supplement to the latter.

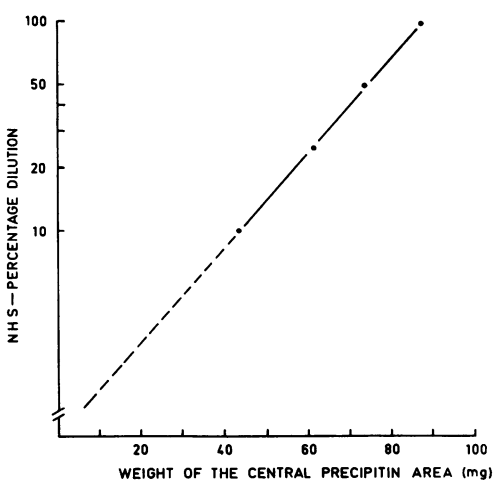

FIG. 7. Quantitation of kappa type immunoglobulins in a K-SP. There is a linear correlation between the size of the area of the central precipitate, as measured by weighing the enlarged photographs and the log concentration of pooled normal human serum (NHS). The percentage dilution is indicated on the ordinate.

Striking differences in the central precipitin areas between κ and λ selection plates and/or local irregularities (Fig. 4) of the precipitate have so far only been found in the paraproteinaemic sera.

DISCUSSION

The typing of light chains of the individual immunogloblin classes or subclasses in the serum and in other biological fluids can contribute substantially to the diagnosis of paraproteinaemia (M-components, monoclonal gammopathies) and this information can also be of interest in other conditions with disturbed immunoglobulin synthesis. For research purposes the determination of the light chain type of a given immunoglobulin or antibody is essential to define homogeneity or heterogeneity of the protein. The method presented in this paper is based on the principle of selection of immunoglobulins of one type during immunoelectrophoresis by a type specific antiserum incorporated in the agar. It improves the possibilities for typing immunoglobulins in a mixture without their previous isolation. Furthermore only small quantities of material (about 0·02 ml of serum) are needed for this analysis. It has proved most useful for typing both heterogeneous and homogeneous immunoglobulins present in low concentration, i.e. in conditions, where other methods usually fail.

From the technical point of view there are some crucial points which need special attention. Firstly the antiserum incorporated in the agar should meet special requirements. It should not only have a high titre of antibodies but most of these antibodies should be directed against common surface determinants of the light chains. Only antibodies against surface and not against hidden determinants of the immunoglobulin light chains can select complete immunoglobulin molecules of the same type by precipitation. A large number of antisera have been tested and only a few of them were suitable. The scheme for preparing antisera with alum precipitated antigens in addition to the use of Freund's adjuvant seemed most successful (Hijmans *et al.*, 1969).

The use of antisera which have been absorbed with excess amounts of antigens should be avoided. Otherwise unwanted effects, such as precipitin rims around the troughs or longitudinal precipitin lines between troughs, will occur. Absorption in the equivalence zone or preferably with insoluble immunoabsorbents is therefore indicated.

Difco Bacto agar was chosen for these examinations because due to high electroendosmosis all immunoglobulins migrate to the cathode. This results in long precipitin lines without any interference by the starting hole.

There is a definite limitation in the application of this technique because in the presence of a homogeneous immunoglobulin (paraprotein) in high concentration in the serum the relevant antiserum incorporated in the plate will often not give a complete precipitation of this local protein excess. Some of the nonprecipitated molecules will diffuse and then react with the class specific antiserum in the trough. This antigen excess will always be clearly visible in the KL-selection plate which is used as a control to test the adequacy of the entire system. In these cases one can, of course, apply the conventional method of immunoelectrophoresis or use diluted samples in our modification. In the latter case, however, it will often be very difficult to type the other immunoglobulins with the same electrophoretic mobility which are of the type opposite to the paraprotein. In some instances it is possible to complete the precipitation of the paraprotein by incorporating appropriate class specific antiserum. Then immunoglobulins of other classes can be typed.

The question why different paraproteins in the same concentration react differently with the same antiserum (see Table 1) needs further study. The large differences in precipitability of the paraproteins, even of the same class, and the fact that in some instances excess amounts of the antiserum cannot increase or complete the precipitation show that it is not only a simple question of the titre of antibodies against common surface determinants of the light chains. It seems that in some cases of monoclonal gammopathy not all of the paraproteins possess completely developed common surface determinants. Another possibility might be that those common surface determinants are not available due to a special steric configuration. Similar observations were described by Osterland and Chaplin (1966) and by Mul (1968) in a number of A-myeloma globulins. This phenomenon is indicated by the latter author as 'inhibition of precipitation'. Analogous difficulties in typing light chains and also gamma chain subclass specific determinants of some paraproteins have been observed by Škvařil (personal communication 1969).

The study of the size and the form of the central precipitate can yield limited but useful additional information. This immune precipitate results from the interaction of the anti-light chain antiserum in the agar with the mixture of immunoglobulins which carry the relevant antigenic determinants. As this mixture contains immunoglobulin molecules of different classes with different electrophoretic mobilities and diffusion coefficients it is unlikely that the measurement of the areas of precipitate will lead to an accurate quantification of immunoglobulins of one type of light chain. In this respect the conditions in our plates differ from the techniques of Laurell (1966) and Merill, Hartley and Claman (1967). These authors use a more homogeneous antigen–antibody system and more accurate results are, therfore, obtained. The evaluation of the central precipitate in the selection plates gives only semiquantitative information on the level of light chain types. Similar possibilities are offered by Becker's (1968) modification of the immunoelectrodiffusion technique.

The most useful information is given by striking differences in the areas of the central precipitate between K-SP and L-SP and especially by local irregularities of the precipitate in one plate. This can indicate the presence of a paraprotein. Therefore the use of both K-SP and L-SP in the same arrangement is recommended. By using antisera of similar quality against both types a good comparison of the shapes of the precipitin lines can be obtained by superimposing the two plates. This is especially helpful in detecting paraproteins in very low concentration in sera where the normal immunoglobulins of the same class but opposite light chain type are still present.

It should be possible to test other protein mixtures such as subclasses or subtypes by application of this method of immunoselection. Finally, antibodies could be classified and typed by incorporating the proper amount of antigen in the agar. The appropriate antibody would form the central precipitate and the weakening or, exceptionally, the absence of a particular precipitin line would then indicate to which class the antibody belonged. The incorporation of both the antigen and an antiserum against one type of light chain could show of which type and class or subclass the antibodies are.

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REFERENCES

BADIN, J. and LEVESQUE, H. (1961). 'Précipitation rapide du facteur rheumatoide dans une solution d'acide borique et titrage par l'agglutination des hématies humaines sensibilisées.' *Rev. Rheum.*, **28**, 101.

BECKER, W. (1968). 'Immunochemische Differenzierung γG-monoclonaler und polyclonaler Gammopathien durch Elektroimmundiffusen.' *Z. analyt. Chem.*, **243**, 442.

DE KONING, J., DOOREN, L. J., VAN BEKKUM, D. W., VAN ROOD, J. J., DICKE, K. A. and RÁDL, J. (1969). 'Transplantation of bonemarrow cells and fetal thymus in an infant with lymphopenic immunological deficiency.' *Lancet*, **i**, 1223.

HIJMANS, W., SCHUIT, H. R. E. and KLEIN, F. (1969). 'An immunofluorescence procedure for the detection of intracellular immunoglobulins.' *Clin. exp. Immunol.*, **4**, 457.

KORNGOLD, L. and MADALINSKI, K. (1967). 'Subgroups of G-myeloma globulins of type K and type L.' *Immunochemistry*, **4**, 353.

LAURELL, C. B. (1966). 'Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies.' *Analyt. Biochem.*, **15**, 45.

MERILL, D., HARTLEY, T. F. and CLAMAN, H. N. (1967). 'Electroimmunodiffusion (EID).' *J. Lab. clin. Med.*, **69**, 151.

MUL, N. A. J. (1968). 'L-Keten en Fd-Fragment van Immunoglobulinen. (Een immunologische studie van paraproteinen).' Doctoral Thesis, Utrecht.

MUL, N. A. J. and BALLIEUX, R. E. (1968). 'Immunologic specificity of the antibody fragments (Fab) of G- and A-immunoglobulins.' *Immunochemistry*, **5**, 399.

OSTERLAND, C. K. and CHAPLIN, H., JR. (1966). 'Atypical antigenic properties of a γA myeloma protein.' *J. Immunol.*, **96**, 812.

PRENDERGAST, R. A., GREY, H. M. and KUNKEL, H. G. (1966). 'Recombination of heavy and light chains of human γA-myeloma proteins: formation of hybrid molecules and configurational specificity.' *J. exp. Med.*, **124**, 185.