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# Demonstration of Unique Mycelial Components in *Candida Albicans*

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# DEMONSTRATION OF UNIQUE MYCELIAL COMPONENTS IN *CANDIDA ALBICANS*

by

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ABSTRACT

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IN CANDIDA ALBICANS

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Mycelial (M) and blastospore (B) cell homogenates of Candida albicans strain B385 were compared to detect and recover unique M components. Qualitative differences in the protein and lipid content of M and B homogenates were suggested by discontinuous polyacrylamide gel electrophoresis (DPAGE). In addition, a quantitative and possibly qualitative shift in the protein content during germ tube formation was implied by DPAGE. Unique M components were detected using both DPAGE and immunoelectrophoresis (IE) techniques. One unique M component by DPAGE, four by IE, and two by combined DPAGE and IE techniques were suggested. Of these three methods, only DPAGE and the combined DPAGE and IE techniques were suitable for the quantitative recovery of unique components. Of these two, the combined DPAGE and IE techniques seem to have greater potential for the detection of unique M components.

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# INTRODUCTION

Candida albicans is a common inhabitant of the normal human intestinal flora, occurring in 15 to 40% of the population (Dubos and Hirsh, 1965). In the compromised host, Candida albicans is responsible for more systemic infections than any other fungal species (Portnoy, et al, 1975). In patients subjected to prolonged or multiple surgical procedures, antibiotic, immunosuppressive, or cytotoxic therapies, treatment involving catheters or respiratory aids, or compromised by some underlying primary disorder such as leukemia or carcinoma, systemic candidiasis has become an increasingly serious complication and is frequently the major or actual cause of death of the patient (Preisler, et al, 1969; Toala, et al, 1970; Venezia and Robertson, 1974; Hurley and Fauci, 1975; and Portnoy, et al, 1971). Often the lack of pathognomonic signs may hamper diagnosis of candida infection. The organism may thus invade the host and cause a systemic infection which is followed by death without producing any overt clinical manifestations. In such cases the diagnosis is made at autopsy, frequently by demonstrating candida endocarditis. The diagnosis of candida infection is made in only about 50% of the patients (Hart, et al, 1969).

It is not safe merely to treat high risk patients for candidiasis. Treatment by amphotericin B and 5 fluorocytosine is a rather harsh and detrimental procedure for the patient (Ommen, 1974). Isolation of Candida albicans from the human host is not diagnostic since many healthy people are colonized with Candida albicans.

The currently available serological diagnostic tools are plagued with false positive reactions (Salvin, 1969; Taschjian, et al, 1967; Preisler, et al, 1969). As a further limitation, many compromised hosts, particularly those on corticosteroids, are anergic. It is therefore desirable to diagnose systemic candidiasis before proceeding with an antibiotic regimen.

There is evidence that Candida albicans almost always exhibits a mycelial phase in the infected host (Braun and Schwatz, 1974; Barlow, et al, 1974). Virulence of individual strains seems to be roughly correlated with the in vitro capacity to form hyphae (Louria, 1967). Thus, some investigators have suggested that a mycelial antigen could possibly serve as a good reagent in a diagnostic test for systemic candidiasis (Evans, 1973; Barlow, et al, 1974). Germ tube antigens, in addition to mycelial antigens, have potential as a source of a diagnostic reagent considering that the established laboratory procedure for the identification of Candida albicans is the growth of germ tubes in serum (Barlow, et al, 1974).

The development and standardization of purified microbial antigens is of great value for the serological testing of a variety of microorganisms involved in infectious diseases. At the present time, antigens for many serological tests are crude and impure. Procedures which are generally used for the chemical analysis of microbial cellular extracts are insensitive and of limited value in the comparative analyses of related organisms or of the different growth phases of specific organisms.

The polyacrylamide gel electrophoretic procedures of Wright (1971, 1972) and Houchens and Wright (1973) and the immunoelectrophoretic procedures described in A Manual of Quantitative Immunoelectrophoresis. Methods and Applications edited by Axelsen (1973) have great potential in this regard as methods for the improved separation and resolution of many microbial components. In this study, these methods were applied to the study of mycelial (M) and blastospore (B) phases of a strain of Candida albicans for the detection of components unique to the mycelial phase of growth.

## MATERIALS AND METHODS

### Antigen Preparation

*Candida albicans* strain B385 (C.D.C.) used in all procedures was maintained at room temperature by monthly subculture on Sabouraud's dextrose agar in 25 $cm^2$ tissue culture flasks (Corning). To prepare a standard inoculum for propagating M and B antigens the growth from a 24 hr culture which had been incubated at 37 C in a humidified atmosphere of 5% $CO_2$ was suspended in two ml of sterile 0.9% saline solution. For M or B phase growth, four Erylemyer flasks containing 100 ml liquid growth medium which had been autoclaved at 15 $lb/in^2$ for 15 min at 121 C were each inoculated with one ml of standard inoculum and incubated in a shaker (New Brunswick Scientific) for 48 hr. The medium for M growth described by Chattaway et al (1968) was modified to contain 3.75% Neopeptone (Difco) and incubated at 40 C and 100 rpm. For B growth the medium described by Sweet and Kaufman (1970) was used with incubation at 37 C and 130 rpm.

After incubation, the growth was examined for mycelia or blastospores and a Gram stain was performed to check for contaminants associated with the antigen. The pooled M or B growth was then washed in physiological saline three times, each for 10 min at 2200 rpm, and adjusted to 25 ml. Fifty gm of dry glass beads (0.45 to 0.50 mm) were placed in a 50 ml homogenization tube and moistened with physiological saline. The growth was then transferred to the tube and homogenized in

a Braun cell-homogenizer (Melsungen) for two min x three using liquid $CO_2$ as a coolant. An examination of the volume of the pellet recovered after centrifugation of the homogenate at 2200 rpm indicated that 95% cell breakage had occurred. The supernatant from the low speed centrifugation was subjected to ultracentrifugation at 100,000 x g for one hr in a Beckman analytical ultracentrifuge with a 41.2 constant angle rotor. The supernatant recovered after ultracentrifugation was dialysed overnight against distilled water. The protein content of the dialysate was determined by the Lowry method and samples were subsequently lyophilized, reconstituted to eight mg/ml, and stored at -20 C.

Preparation of Germ Tube Homogenate

Subcultures having confluent growth were prepared by using a sterile swab to spread a standard inoculum from a stock culture of B385 onto petri dishes (100 x 15 mm) containing Sabouraud's dextrose agar. The growth from 24 hr subcultures incubated at 37 C in a humidified atmosphere of 5% $CO_2$ was suspended in physiological saline. The concentration of the suspension was adjusted to a packed cell volume (PCV) of 5. Five ml of the PCV solution were transferred to 1000 ml flasks containing 250 ml of pooled human serum and incubated for eight hr at 37 C.

An homogenate from the germ tube suspension was prepared and stored in the same manner as described for the M and B phase antigens.

Preparation of Antisera

New Zealand white rabbits were used as a source of all

antisera. The schedule of B antigen injection was originally as described by Axelsen (1971). After eight months, no antibody response could be detected and a new schedule of weekly injections was begun. One ml of antigen containing three mg of protein was injected intradermally on both sides of the vertebral column in 0.2 ml aliquots with as much as 0.5 ml being injected at the base of the neck. This latter protocol was used for M antigen except that two ml of an emulsion containing equal volumes of Freund's incomplete adjuvant and antigen having a protein concentration of one mg/ml were used for the initial injection of M antigen.

After the fifth injection, rabbits were bled from a lateral ear vein using a Bellco glass vacuum bleeding apparatus. By this method 35 - 40 ml were collected from each rabbit at each bleeding. Blood was allowed to clot at room temperature for one h, rimmed, and then placed at 4 C overnight. The serum was centrifuged, filter sterilized, and stored in one ml aliquots at -20 C.

Polyacrylamide Electrophoresis

Three polyacrylamide gel techniques were used in an attempt to qualitatively and quantitatively resolve specific components from the M homogenates.

Analytical discontinuous polyacrylamide gel electrophoresis (ADPAGE). An ADPAGE technique developed by Wright (1971) was used in which homogenate components were separated according to electrophoretic mobility and molecular size on columns made up of layers of polyacrylamide gel having increasingly smaller

pore size. The banding patterns resulting from this separation were revealed by the use of several stains: the Coomasie brilliant blue R (CbbR) stain for protein, the Oil Red O (ORO) stain for lipoprotein, and the Periodic acid Schiff (PAS) stain for glycoprotein.

Two dimensional polyacrylamide gel electrophoresis (2DPAGE). The procedure of Wright (1972) was used in which the separated components on an ADPAGE column were further separated for greater resolution in a gel slab having a continuous gradient of acrylamide gel.

Preparative discontinuous polyacrylamide gel electrophoresis (PDPAGE). Quantitative recovery of M and B components was achieved with the PDPAGE technique of Houchens and Wright (1973). The banding patterns of M and B components on ADPAGE columns were used as a reference for slicing preparative columns into discs. Fractions eluted from discs were dialysed, lyophilized, and reconstituted to one ml. In the analyses of fractions for the presence of unique M components, 100 ug volumes were applied to ADPAGE columns. A unique M component was defined as a band which 1) did not have its counterpart in a B ADPAGE column and 2) had the same electrophoretic behavior when combined with B phase antigens.

Immunoelectrophoresis

M and B antigens were examined using six different immunoelectrophoretic techniques. These as described below were: crossed immunoelectrophoresis (Weeke, 1973), immunofiltration (Axelsen, 1973), the Osserman technique (Osserman and Lawlor, 1966),

rocket immunoelectrophoresis (Svendsen, 1973), column acrylamide immunodiffusion (Catsimpoolas, 1969), and the acrylamide Osserman technique. The procedures of the above authors were modified by using a different size glass slide, concentration of agarose (Marine colloids), electrophoretic current, and time interval for electrophoresis.

Crossed immunoelectrophoresis (CIE). In this technique, antigenic components were first separated according to their electrophoretic mobility in an agarose gel. The separated antigenic components were then electrophoretically driven perpendicular to the direction of the first electrophoresis into an antibody-containing agarose bed where they would interact with antibody to form a characteristic pattern of precipitin lines. Slides having the dimensions 8.2 by 10 cm were prepared according to the template presented in Figure 1.

A well was cut in slab A (3 by 8.2 cm) of agarose gel I (Table 1). The sample for electrophoresis was prepared by adding 0.037 ml of a 3% solution of agarose in water to a microtiter well containing 0.15 ml of antigen solution. This was immediately mixed and applied to the well in slab A. After gelation, 20 ul of additional antigen were injected into the well. A small amount of bromophenol blue tracking dye (BPB) was injected below the well as indicated in the template in Figure 1.

The agarose-coated plate was inverted in the electrophoresis chamber (Gelman) and supported at one end by an adjustable plexiglass strip. Bibulous paper wicks connected the agarose-

coated plate to the well buffer (Table 1) in the chamber. First dimension electrophoresis was carried out at 4 C using a 4 ma current supplied by a constant current power source (Buchler regulated power supply). Samples were electrophoresed for approximately 4 hr until the bpb marker had migrated to the 0.8 cm mark indicated on the plate. The first dimension agarose was trimmed back to the dotted line in the template (Figure 1) and space B was filled with agarose gel II (Table 1). The second dimension of electrophoresis was carried out at 4.5 ma for 21.5 hr.

After electrophoresis, the slide was washed for two days in 0.9% saline with two changes of saline a day and a final day in distilled water. After drying, the slide was stained with CbbR as described by Axelsen (1973).

Immunofiltration. In this method, CIE was performed with an intermediate gel containing heterologous antisera (space C in Figure 2) interposed between the first dimension and second dimension gels (spaces A and B in Figures 1 and 2). In this way the two antisera could be compared for antibodies against blastospore and mycelial components.

The antisera-containing gels in spaces B and C were prepared to have antibodies in the same dilution as for CIE. In the second dimension, antigen was first electrophoresed into space C containing 4.67 ml of agarose gel III (Table 1). Any unbound components were driven into space B containing 8.41 ml of agarose gel II (Table 1). Washing, drying, and staining were performed as for CIE.

Osserman technique. The template for the Osserman technique appears in Figure 3. The Osserman technique was essentially a modified CIE technique in which the location of the antigen well and width of the first dimension gel were altered to accomodate the addition of a trough 0.5 cm wide and 0.6 cm from the bottom of the slide containing agarose gel IV (space C in Figure 3). The first dimension gel in space A was trimmed back to the dotted line 2.7 cm from the bottom of the slide to allow space for both the well and the trough.

Rocket immunoelectrophoresis. Space A was filled with agarose gel I using the template (Figure 4). A series of wells were cut in the positions indicated by the template (Figure 4). Fractions collected from PDPAGE were applied to the well using four drops from a Pasteur pipet. Diffusion was allowed to occur for one hour at room temperature and eight ml of agarose gel V was applied to space B. After 18 hr of electrophoresis the slides were washed, dried, and stained as in the CIE technique.

Acrylamide immunodiffusion. A 0.1 ml volume of M homogenate was electrophoretically separated by ADPAGE and the column was placed on a slide in the position indicated by the template in Figure 5. Ten ml of 0.75% agarose dissolved in water were added to the slide. Troughs were cut on each side of the column as indicated in the template in Figure 5. The slide was placed in a humidified chamber at room temperature for seven days followed by washing, drying, and staining as described for CIE.

Acrylamide Osserman. One mg of M antigen was electrophoretically

separated by ADPAGE and the column placed on a slide in the position indicated by the template in Figure 6. The entire slide was covered with 16 ml of agarose solution I (Table 1) and allowed to incubate overnight in a humidified chamber at room temperature to permit antigen diffusion from the column into the surrounding gel. The gel column was gently cut from the surrounding gel and removed. The agarose was cut along the dotted line (Figure 6) and the rectangular slab A was moved to close the gap left by the column. The 0.5 cm wide trough thus formed between A and B in Figure 6 was filled with agarose gel IV (Table 1). The gel in space C was removed and replaced with agarose solution VI (Table 1). Electrophoresis was at 4.5 ma for 21.5 h. Washing, drying, and staining was as described for CIE.

## RESULTS

### ADPAGE and PDPAGE

The M and B CbbR-stained ADPAGE electropherograms (Figure 7) were very similar making the location of possible unique M components for recovery from PDPAGE columns uncertain. In one instance, however, the suggested differences in the M and B banding patterns led to the recovery of a PDPAGE fraction containing a unique M component. Because of suggested differences in the region 3.2 to 3.4 cm from the top of analytical columns, discs were cut 3.2 to 3.4 cm from the top of preparative columns to which M or B homogenate was applied. Subsequent ADPAGE analyses of the fractions eluted resulted in the banding patterns shown in Figure 8. The light staining of the ADPAGE columns did not allow clear visualization of bands in photographs of the tubes. Thus, a drawing of the ADPAGE columns is presented in Figure 8 in place of a photograph to show the positions of the CbbR staining bands. Seven B and M CbbR stainable bands were seen on the ADPAGE columns. However, an additional band was seen in the M banding pattern. This band also appeared on columns to which the M fraction was combined with B fractions eluted from discs cut 3.0 to 3.2 and 3.2 to 3.4 cm from the top of a PDPAGE column and thus it was concluded that this band indicated the presence of a unique M component.

Although an exhaustive analysis of the fractions recovered was not undertaken, the analysis of several other fractions

for unique components was negative. These fractions were recovered from the following regions of preparative columns (given in cm from the top of a column): 2.9-3.2, 3.1-3.2, 5.4-5.6, and 6.9-7.0. In addition to the similarity of M and B ADPAGE electropherograms, the low protein yield of fractions (0.13-0.55 mg/ml) made the PDPAGE technique alone impractical for the analysis of Candida albicans components.

ORO staining revealed two wide bands at the 3.5/4.75 and the 4.75/7% gel interfaces of M but not B ADPAGE columns. Ten bands in the 4.75, 7, and 12% gels appeared in the B electropherograms none of which were seen in the same locations as the two M bands. The molecular weight of the M components if they are pore limited (Wright, et al, 1971) in the 3.5% gel region may be greater than 500,000. Similar banding appeared at the 3.5/4.75 and the 4.75/7% gel interfaces in both M and B electropherograms stained with CbbR and PAS stains. The use of sample concentrations as large as 120 ug did not improve the intensity of banding for either ORO or PAS stained columns. The positions of the major ORO and PAS staining bands are represented schematically in Figure 9.

2DPAGE

There are apparently many more components in M and B homogenates than can be resolved with ADPAGE columns. Fifty-one M and 70 B spots could be distinguished using the 2DPAGE technique (Figures 10 and 11). By superimposing the M and B 2DPAGE electropherograms no unique M spots could be detected. One very prominent spot, however, was seen in the B electropheregram (Figure 11) which did not appear in the M electropherogram.

CIE

Differences in the M and B CIE precipitin patterns (Figures 12 and 13) suggested that there were unique M antigens. Thirty-five precipitin lines could be detected with M antigen-antibody interactions (Figure 12) while 25 precipitin lines could be detected in B antigen-antibody interactions (Figure 13). Most conspicuous in the M CIE electropherogram was a very large biphasic precipitin line of high electrophoretic mobility labelled number 27 in Figure 12. Subsequent analysis suggested that one unique M antigen was associated with this complex precipitin line.

Immunofiltration

In the immunofiltration technique, the intermediate gel acted as a filter for antigens unique to the B phase or common to both the M and B phases. However, it allowed those antigens to go through for which there were no antibodies. These included any unique M antigens which could form precipitates in the second dimension gel in space B (Figure 2).

Antibodies against seven M components identified as A-G in Figure 14 formed precipitates only in the M antisera. The 'feet' of these precipitins continued into the intermediate gel because of diffusion and electroendosmosis (Figure 14). The shapes of two more precipitin lines implied that antibodies against two more M antigenic components (H and I in Figure 14) were in very low titer in the B antisera. The 'feet' of these precipitins penetrated further into the intermediate gel and turned in slightly as they would if confronted with antibodies

in lower titer.

Osserman technique

In the Osserman technique, antigens common to both phases formed common precipitin lines. Unique M antigens had no lines of identity with B antigens. The large number of precipitin lines made positive identification of unique antigens difficult with the Osserman technique alone. A comparison of the precipitin patterns of the CIE, immunofiltration, and Osserman techniques, suggested that four antigens labelled W-Z in Figure 15 were unique to the M phase. These antigens appeared to correspond to precipitin lines 8, 14, 23, and 27 and A-D in the CIE and immunofiltration techniques respectively.

Rocket immunoelectrophoresis

The rocket immunoelectrophoresis technique allowed the analysis of several PDPAGE fractions at a time thus affording a means for the rapid detection of unique components in PDPAGE fractions. Rocket immunoelectrophoresis of PDPAGE fractions revealed two unique M components in the fraction cut in region 5.4 to 5.6 from a PDPAGE column (Figure 16). Precipitin lines q and r in Figure 16 show lines of identity indicating the existence of a common M and B component. Lines x and y, however, cross q and r indicating the presence of unique M components.

Acrylamide immunodiffusion

Only three well-defined precipitins could be detected against either M or B antisera using the acrylamide immunodiffusion technique. The pattern of precipitins was the same on either side of the column (Figure 17).

Acrylamide Osserman

The acrylamide Osserman technique, in contrast to the acrylamide immunodiffusion technique, allowed the detection of 20 precipitin lines. Although the large number of lines did not allow detection of lines of nonidentity, eight precipitin lines were reminiscent of lines of nonidentity, their precipitin lines originating at the interface between the gel slabs in spaces B and C (Figure 6). B antigen migrating from the trough would have the effect of increasing the concentrations of common M components thus raising the precipitins away from the interface between spaces A and B and forming lines of identity extending across the slide (line x in Figure 18). The eight precipitin lines were obviously different in this regard. The location of each of these components on an ADPAGE column was accomplished by extending a line perpendicular to the column which passed through the apex of the precipitin line (Figure 18). The values obtained may then by used for slicing PDPAGE columns for the recovery of unique M components. The eight M components had the following locations on the ADPAGE column: 2.9, 3.4, 3.6, 3.9, 4.1, 4.2, and 4.3 cm.

Germ tube homogenates

Although no attempt was made to recover germ tube components, the ADPAGE banding pattern of germ tube homogenate was strikingly different from that obtained for M and B homogenates (Figure 7). While PAS and ORO staining resulted in no detectable variation from the M pattern, CbbR staining of germ tube homogenate electrophoretically sepatated on ADPAGE columns revealed a pattern

of banding greatly suggestive of major quantitative differences between germ tube homogenate and either M or B homogenates.

The behavior, during electrophoresis, of germ tube homogenates reconstituted from a lyophilized state contrasted greatly with the behavior of M, B, and nonlyophilized germ tube homogenates. While the electrophoretic time interval for all markers was approximately 1.5 h, the germ tube marker lagged behind the others, remaining on top of the column for the greater part of the electrophoretic period before finally entering the columns.

# DISCUSSION

While other authors (San Blas, et al, 1974; Chattaway, et al, 1968, Marriot, 1975) have demonstrated quantitative differences in the protein, lipid, and carbohydrate content of M and B phases of Candida albicans, the analytical techniques used were not sensitive enough to detect qualitative differences. The use of immunological techniques, however, has suggested the existence of unique M components. Evans (1973) used the CIE and Ouchterlony techniques to compare the M and B phases of Candida albicans. In his study, CIE of M and B antigens resulted in precipitin patterns suggestive of qualitative differences between the two phases. Subsequently, with the Ouchterlony double diffusion technique, he was able to show a reaction of nonidentity between M and B phase antigens suggesting that antigenic components unique to the M phase of growth were present.

The IE techniques used in the present study provided a means for the comparative analyses of a large number of antigens. CIE resulted in 35 M and 21 B precipitin lines some of which appeared to be unique to the M antigen preparation when the precipitin patterns shown in Figures 12 and 13 were compared. This led to further analyses with the immunofiltration and Osserman techniques. These techniques, which are modifications of the CIE technique, allowed a direct comparison to be made of the antigenic similarity of M and B components detectable with the CIE techniques. A comparison of the results of the CIE, immunofiltration, and Osserman

techniques indeed suggested that four antigenic components were unique to the M phase. These three techniques were limited however in that the unique components detected could not be quantitatively recovered for further analyses.

In the current study, ADPAGE and PDPAGE techniques alone have limited value in the isolation and characterization of unique M components due to the similar banding patterns of M and B phase components. Thus only one unique component was readily detected by DPAGE techniques.

There is evidence that the number of components detected by ADPAGE represents a small proportion of the total number of components. Regions as narrow as 2 mm and presumably containing a single band on analytical columns gave rise to as many as 13 bands upon subsequent electrophoresis on analytical columns. A 2DPAGE of M and B homogenates suggested that atleast twice as many components were present than could be detected with analytical columns. Affronti, et al (1972) used 2DPAGE to detect more than 200 components in species of mycobacteria. Some of the banding on ADPAGE columns may represent protein fragments or complexes. Furthermore, components with only slight structural variations or similar molecular weight or charge could have antigenic differences which could not be detected by ADPAGE.

For the reasons mentioned above, a combination of the DPAGE and IE techniques may ultimately provide a means for overcoming the limitations of each, providing a more efficient method for the detection and isolation of components. The acrylamide Osserman

technique developed here may provide one such combination. This technique readily permitted the location of antigenic components on an ADPAGE column. Several refinements of this technique which may increase its value are: 1) the addition as a control, of a second format lacking the trough containing heterologous antigen, 2) the use of a higher concentration of heterologous antigen applied to the trough so that antigens common to both phases would be raised above the interface and more clearly separated from unique components, 3) finally, the use of a format in which only components diffusing toward the cathodic side of columns placed on slides are used as antigens so that a more definite starting zone would result and a more concentrated front of antigen would migrate into the agarose-containing antisera.

Rocket immunoelectrophoresis was successfully used to detect two unique M components and has allowed the analysis of several PDPAGE fractions at a time. Rocket immunoelectrophoresis can be used in conjunction with ADPAGE or the acrylamide Osserman technique to reinforce their value for the study of subcellular components of Candida albicans.

Results of ORO and PAS staining suggest that the use of other stains may provide greater versatility in the detection and characterization of subcellular components of Candida albicans. A complex high molecular weight glycoprotein unique to the M phase may be implied by the similar location of two bands in CbbR, ORO, and PAS-stained columns at the 3.5/4.75 and the 4.75/7% gel interfaces. Though the staining was very light, the banding

patterns of ORO-stained M or B ADPAGE columns suggested that qualitative differences exist in the lipoprotein content of M and B phases. The rather large differences in the locations of bands may allow particularly successful application of the ADPAGE technique.

Germ tube homogenate may provide a better source of material for the diagnosis of systemic candidiasis. It has already been pointed out that there is evidence to suggest that the growth of germ tube material in serum provides a very convenient medium for study. The different growth conditions used to develop M, B, or germ tube growth may be responsible for some of the quantitative and qualitative differences observed between these growth forms in this study (Hunter and Rose, 1971). Chattaway et al (1968) found some variations in the values for glucose and mannose units present in the cell wall of Candida albicans grown under different conditions. Otherwise the cell wall composition for the M and B phases appeared to be constant. Thus, the quantitative and possibly qualitative differences seen in this study may be due to the growth medium used. These differences may not necessarily reflect those occurring in individuals becoming systemically infected with Candida albicans. Thus, it is possible that the use of serum as a growth medium may more closely simulate what occurs in vivo.

ADPAGE analysis suggested quantitative and possibly qualitative differences between the germ tube and either the B or M homogenates. Germ tube formation seems to be a period of high metabolic activity and turnover of cellular components and for this reason dramatic

uantitative shifts might be expected. The observed differences
n banding on columns may thus be exploited for the isolation
f unique components or of components predominantly associated with
he germ tube phase of growth. Components unique or predominantly
ssociated with the germ tube phase of growth thus isolated may
e useful in the diagnosis of systemic candidiasis. The similarity
f M and B banding on discontinuous columns, however, suggests
hat a quantitative shift occurring during germ tube formation
pparently does not persist into the M phase. This finding is in
greement with the electron microscopy studies of Cassone, et al (1973).

The detection and isolation of unique M or germ tube
omponents is just the first step in developing a reagent for the
linical diagnosis of candidiasis. In this paper, several techniques
re presented which may be useful in the detection and recovery
f unique M components. Several components which may be unique
o the M phase of growth were detected by these techniques. The
irst step in the isolation of one of these components was, furthermore,
monstrated. It remains to be shown 1) that any of these components
e truly unique or predominantly associated with the M phase, 2)
at they can be recovered in purity from M organisms, 3) that
ese components are specifically found in Candida albicans
rains causing candidiasis and that there is no cross reactivity
tween these components and components of unrelated organisms,
d 4) that the presence of these components can be shown to correlate
th the onset or presence of candidiasis in individuals.

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Figure 1. Template for crossed immunoelectrophoresis (CIE). First dimension agarose (agarose I in Table 1) was applied three c wide in space A. A well was cut 0.6 cm from the side and one cm f the bottom with a no. 2 cork borer. A mixture of 0.15 ml sample a 0.037 ml solution of 3% agarose in water was added to the well. Twenty ul additional sample was injected into the well. Bromophe blue was injected below the well as indicated in the figure and electrophoresis was carried out in the first dimension. Space A agarose was cut back to the dotted line, 2.2 cm. Space B was fil with agarose gel II (Table 1) for electrophoresis in the second dimension.

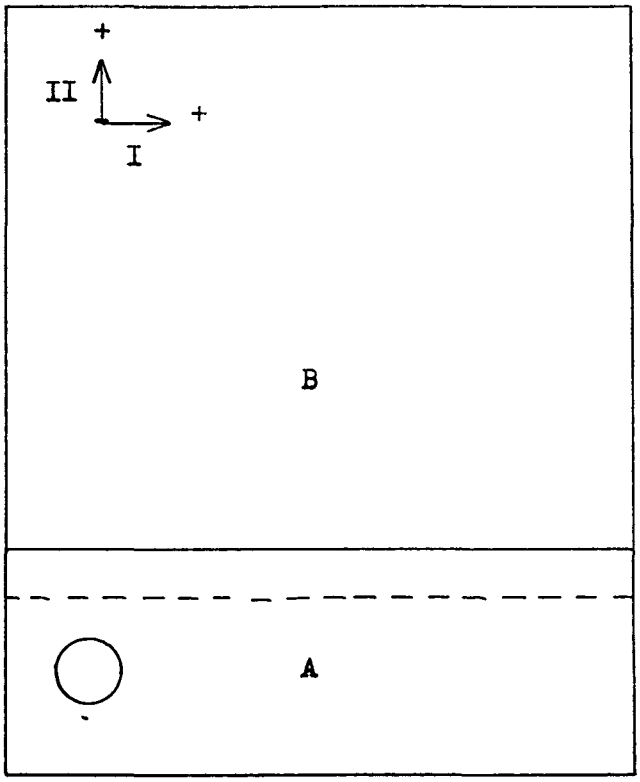
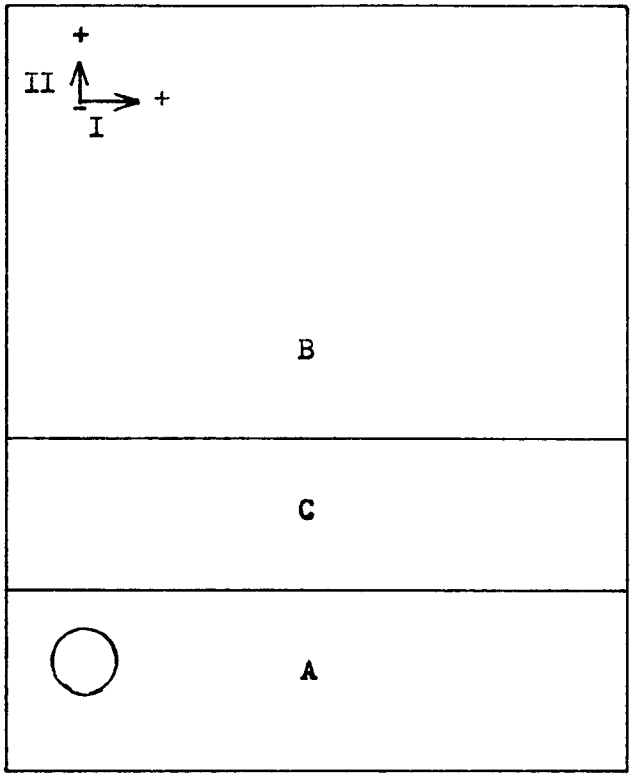

+
II
+
I
B
A

Figure 2. Template for immunofiltration. The slide was prepared the same as for CIE in Figure 1 except that after filling space B of the template in Figure 1, space C was cut 2 cm wide and filled with 4.67 ml of agarose solution III (Table 1).


+
II
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I
B
C
A

Figure 3. Template for the Osserman procedure. The slide
prepared the same as for CIE in Figure 1 except that after the fi
dimension electrophoresis, slab A was cut back to the 2.7 cm dott
line and agarose gel IV (Table 1) was added to trough C, 0.5 cm w
and 0.6 cm from the bottom of the slide.

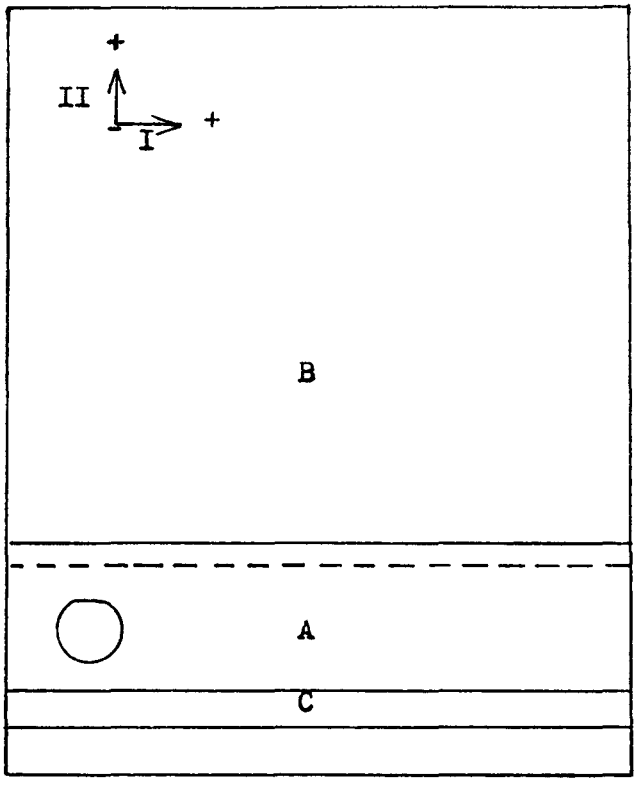

+
II
+
I
B
A
C

Figure 4. Template for Rocket immunoelectrophoresis. Space A, 3.5 cm wide contained agarose gel I (Table 1). Preparative column fractions recovered from the region 3.4-5.4 cm from the top of a PDPAGE column were applied to wells b-f with blastospore antigen (B) in the anodic row and mycelial antigen (M) in the cathodic row. The PDPAGE fractions were identified by numbers indicating the position from which they were recovered on a PDPAGE column. From left to right the following fractions were added to wells b-f: 3.4-4.0, 4.0-4.2, 4.2-5.0, 5.0-5.4, and 5.4-5.6. Bromophenol blue was added to well a in B and M rows. After a one h diffusion period at room temperature, eight ml of agarose gel V was added to space B and electrophoresis was carried out as indicated in the text.

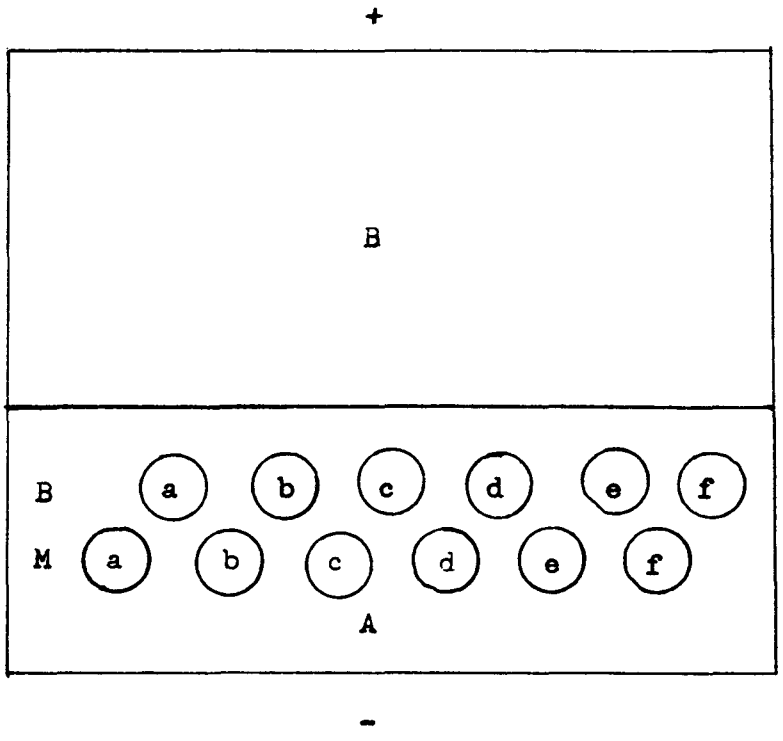

+
B
B
a
b
c
d
e
f
M
a
b
c
d
e
f
A
-

Figure 5. Template for the immunodiffusion technique. Ten of 0.75% agarose dissolved in water were added to the slide on whi an ADPAGE column (A) had been placed. B and M troughs were cut ou and filled with B and M antigen respectively. Precipitin bands formed on each side of the column as antigen and antibody diffused into one another in the agarose.

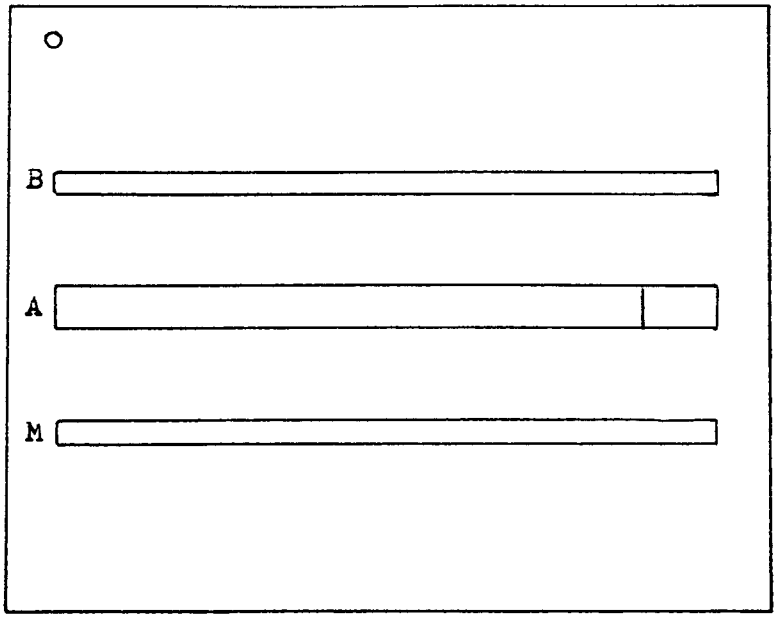

B
A
M

Figure 6. Template for the acrylamide Osserman technique. Sixteen ml agarose solution I (Table 1) were added to the slide c which an ADPAGE column was placed (indicated by the striped secti the template). After incubation overnight, the column was cut fi removed. The agarose was cut along the dotted line and the recta slab A was moved to close the gap left by the column. The 0.5 cr trough between A and B was filled with agarose gel IV (Table 1). Gel C was removed and replaced with agarose solution VI (Table 1 Electrophoresis was carried out as indicated in the text.

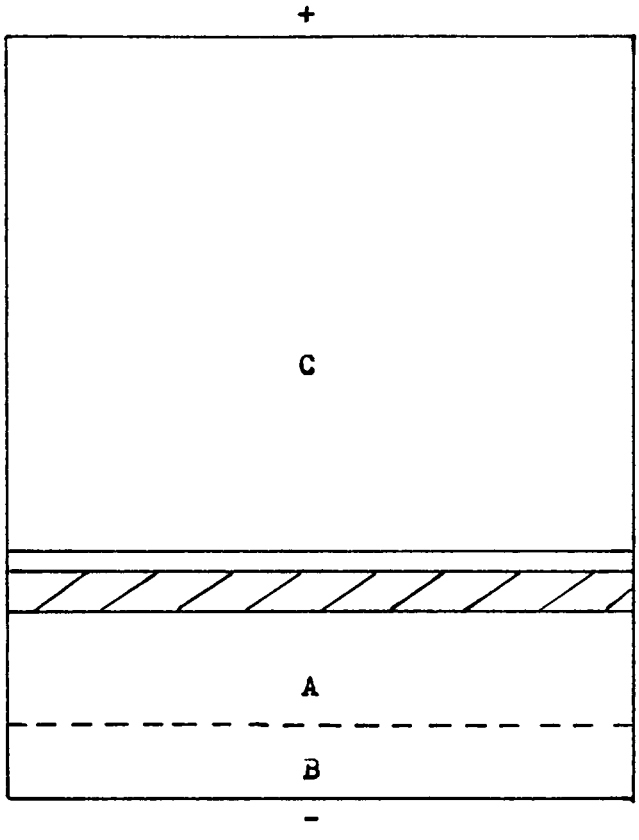
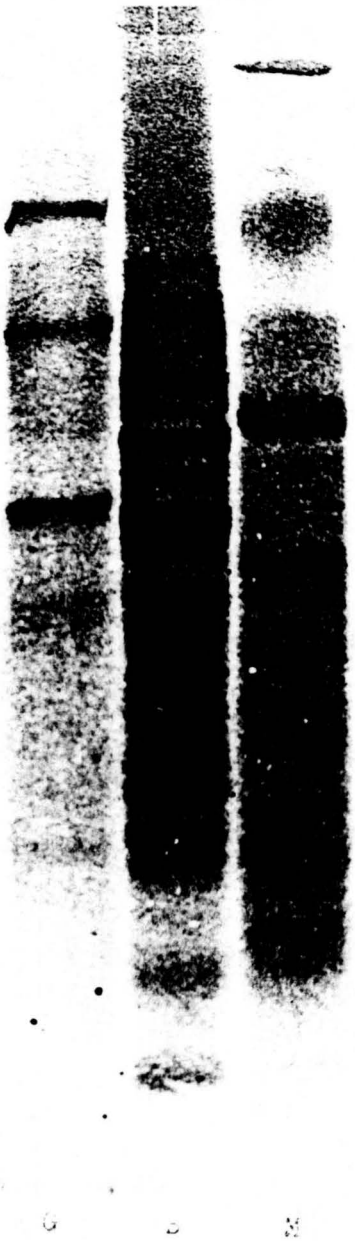

+
C
A
B
-

Figure 7. CbbR stained ADPAGE columns of germ tube, B, and M homogenates from left to right. Though the banding is indistin in the photograph, there is much greater similarity between the M B patterns than between either M or B patterns and the germ tube banding pattern. The germ tube banding pattern indicates that a quantitative and possibly qualitative shift in CbbR staining comp occurs during germ tube formation. While differences were seen between M and B patterns, ADPAGE was not sufficient by itself for positive identification of unique M components due to the large number of bands appearing on the columns. Comparison of M and B banding patterns does, however, suggest that there are both quantitative and qualitative differences between M and B componen



Xca. 2.87

Figure 8. ADPAGE of PDPAGE fractions demonstrating a uniqu
M component at region 3.2-3.4. Columns were stained with CbbR.
Shown is a diagrammatic representation of the ADPAGE columns on
which M, B, or combined M and B PDPAGE fractions were electrophor
From left to right these electrophoresed fractions were: B fracti
3.0-3.2, B fraction 3.2-3.4, M fraction 3.2-3.4, B fraction 3.0-3
plus M fraction 3.2-3.4, and B fraction 3.2-3.4 plus M fraction 3
In spite of weak staining, a narrow M band (arrow) not appearing
the B patterns was seen in the last three columns to the right.
This band indicated the presence of a unique M component.

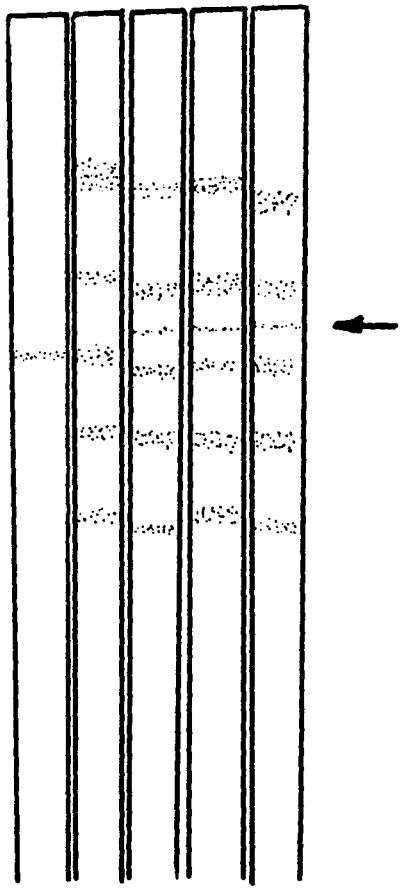


Figure 9. Diagrammatic representation of ORO and PAS stain ADPAGE columns on which M or B homogenate was electrophoretically separated. At the left are shown two columns on which B and M homogenates were electrophoresed and stained with ORO. The M and B staining patterns differed greatly suggesting the presence two unique M and atleast three unique B lipoprotein components. At the right are M and B PAS stained ADPAGE columns. No differe in staining patterns could be detected.

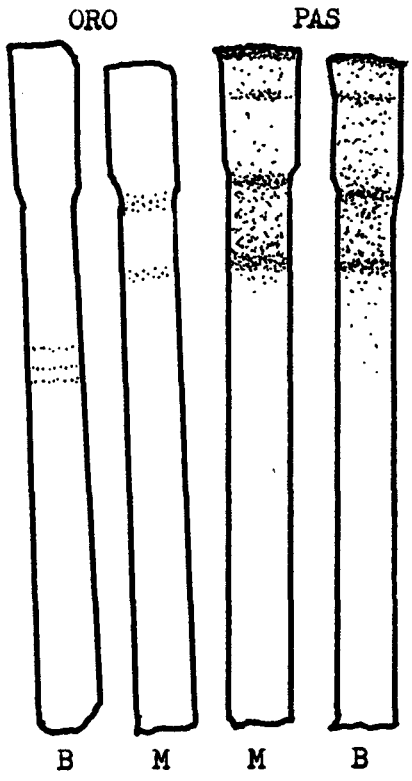

ORO
PAS
B
M
M
B

Figure 10. CbbR stained 2DPAGE of M homogenate. Fifty-one spots could be seen on the CbbR stained slab. A comparison of M and B 2DPAGE patterns did not reveal any obvious differences indicative of the presence of unique M components.

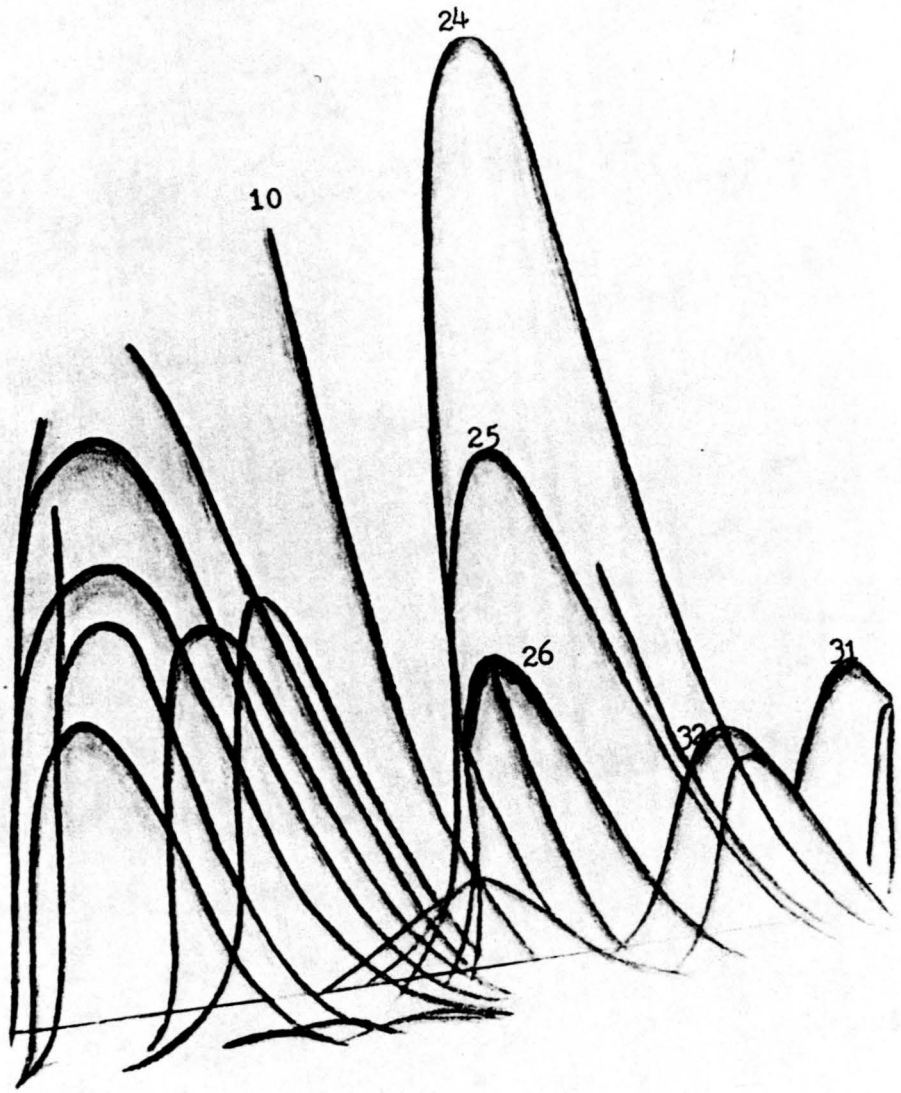
Figure 11. CbbR stained 2DPAGE of B homogenate. Seventy C staining spots could be counted on the slab. One prominent spot indicated by the arrow in the photograph did not appear in the M 2DPAGE pattern. The presence of this spot suggested the presence of a unique B component.

Figure 12. Crossed immunoelectrophoresis of M antigen vs M antisera. The retouched photograph in the figure shows 35 precipitin lines that could be detected.


14
10
27
23
30
4
24
11
5
6
15
1
25
26
28
16
17
31
12
2
18
32
29
3
13
19
20
9
7
33
8
21
22
35
34

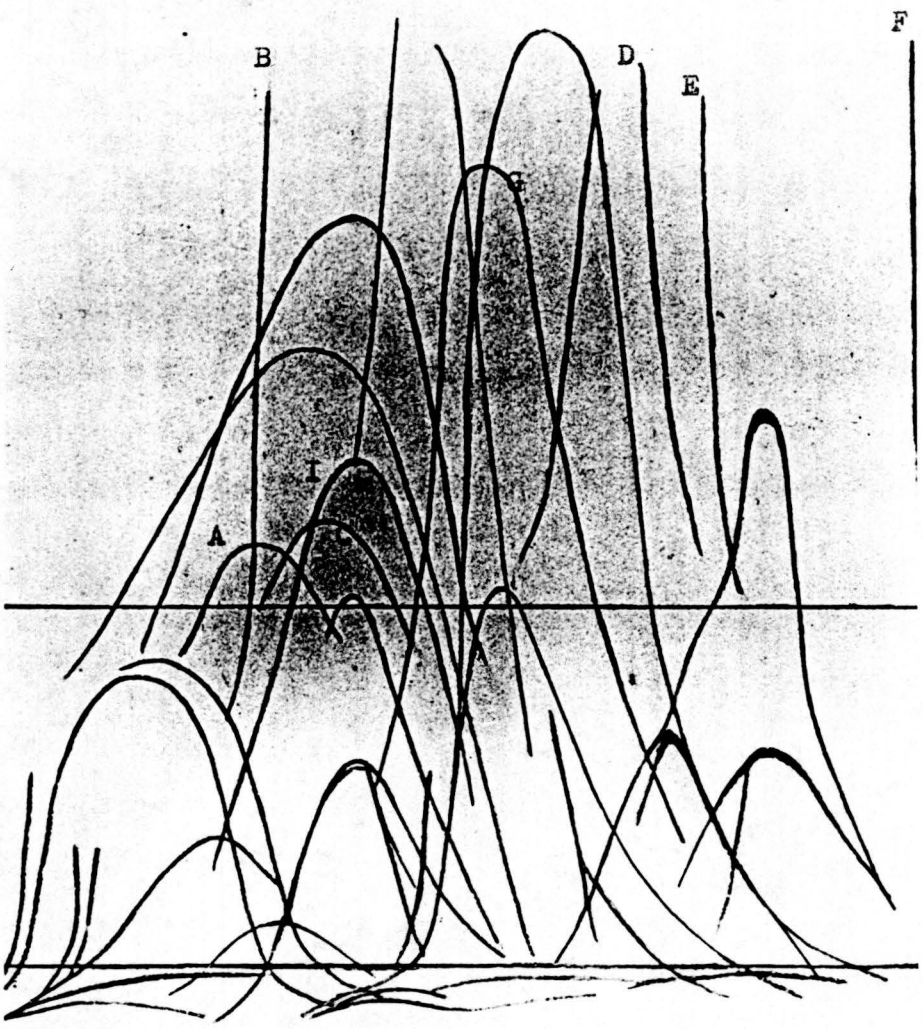
Хса. 2.86

Figure 13. Crossed immunoelectrophoresis of B antigen vs B antisera. The retouched photograph in the figure shows 25 precipitin lines which could be detected. The M and B precipiti patterns (Figures 12 and 13) looked different suggesting that there were differences in the antigenic composition of M and B homogenates. M and B bands which may correspond (indicating identical components) were given the same numbers in Figures 12 and 13.




Xca. 2.86

Figure 14. Immunofiltration of M antigen through a B antis intermediate barrier and into M antisera. Eight antigens (A-G) showed no precipitin reaction in the B antisera intermediate barrier. Thus no B antibody was present against these components which may therefore be unique to the M phase homogenate. Antibod against two more M components (H and I) were predominantly in the M antisera.




Хса. 2.4

Figure 15. No precipitin lines of identity could be detect for four M antigens (W-Z) using the Osserman procedure. These antigens may correspond respectively to antigens A-D in the immur filtration technique (Figure 14) and antigens 8, 14, 23, and 27 i the CIE technique (Figure 12) and may thus be unique to the M phase.

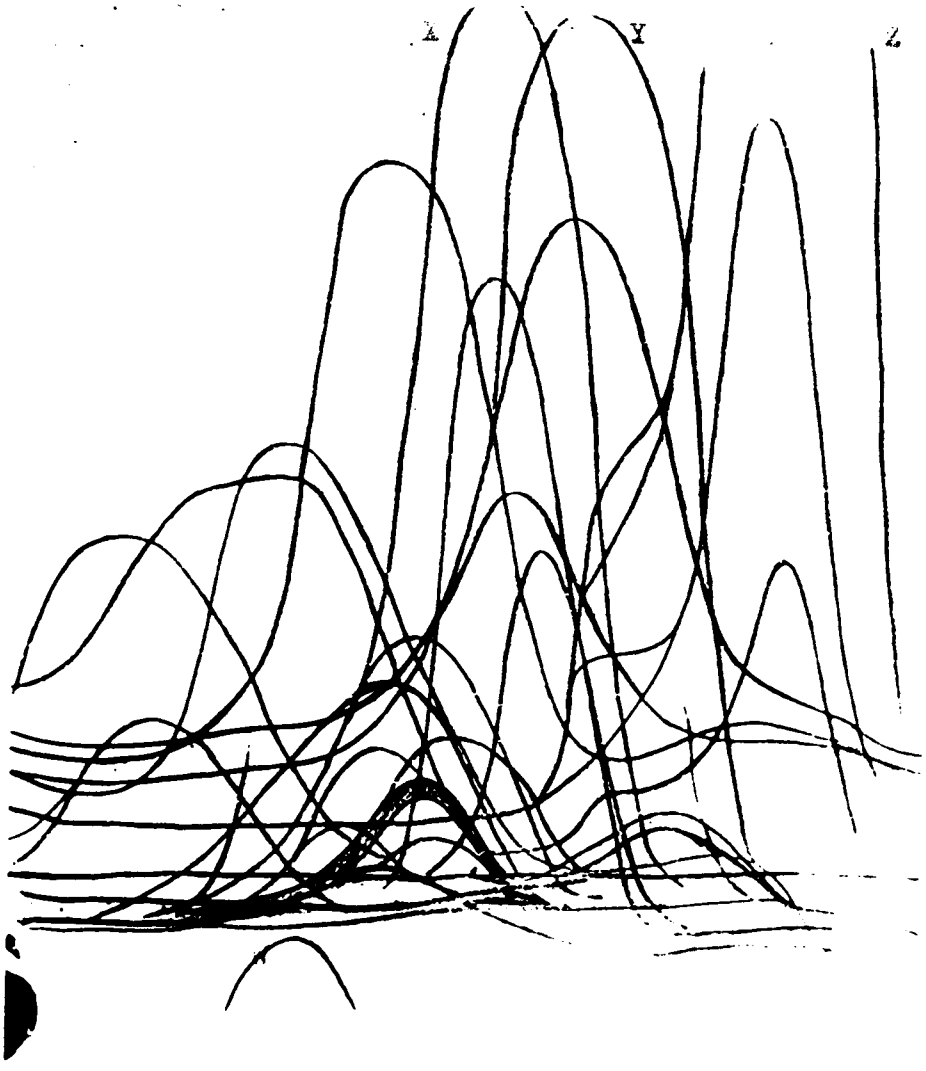

X
Y
Z

Figure 16. Rocket immunoelectrophoresis. Two components, x and y formed no common precipitin bands with B antigens reacti in M antisera and thus appeared to be unique to the M phase homogenate. Antigens in q and r, in contrast, formed common precipitin bands and were thus common to both M and B phases.

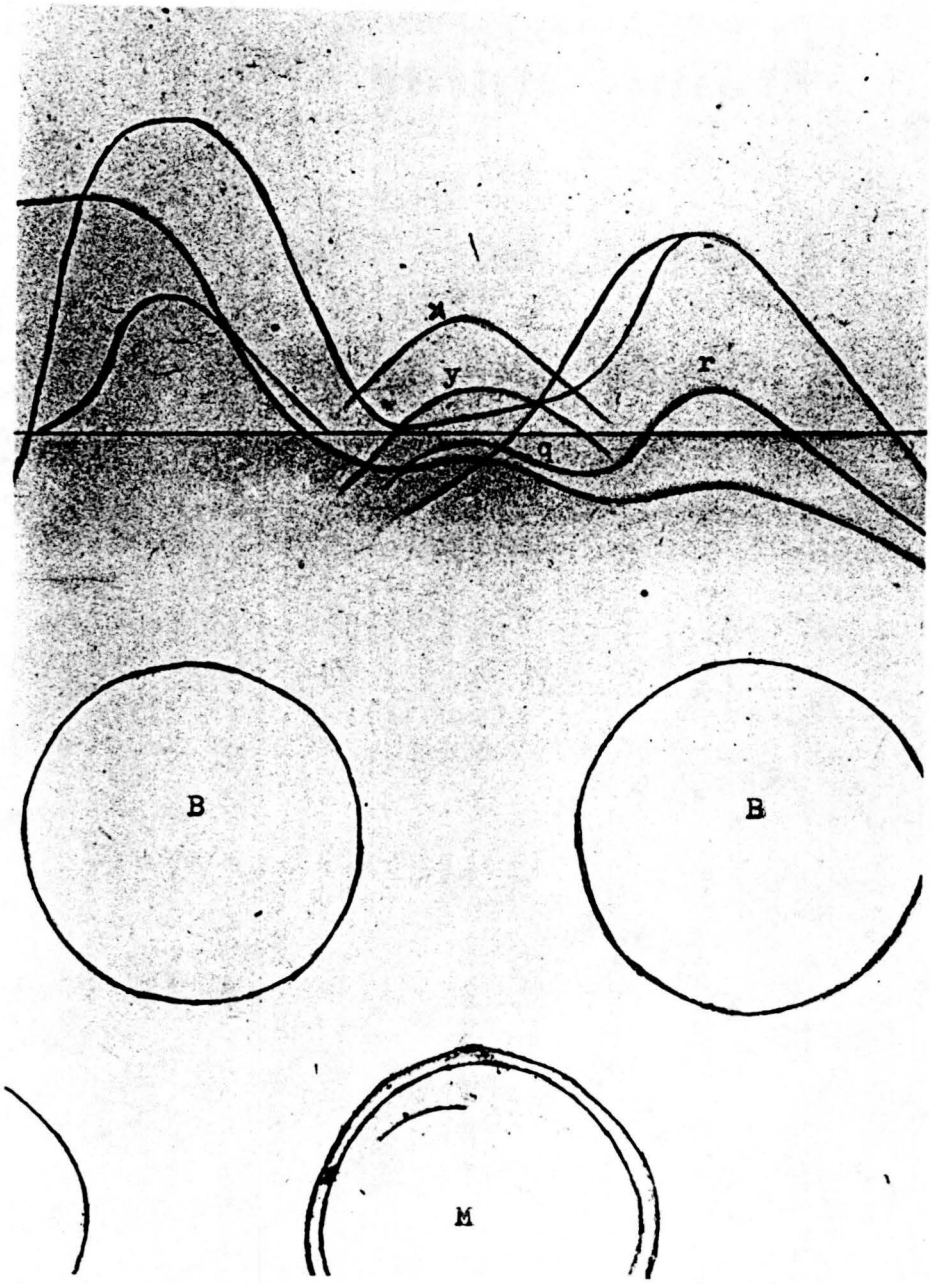

x
y
r
q
B
B
M

Figure 17. Acrylamide immunodiffusion. M antigen-M antibod and M antigen-B antibody reactions each gave rise to three precipi which appeared as mirror images on each side of the acrylamide col Thus no unique M antigens could be detected by this method.

Figure 18. Twenty bands were detected by the acrylamide Osserman technique as shown in this retouched photograph. Eight precipitin bands appeared to be either unique to the M phase or predominantly M antigens since all eight precipitins originated below the interface between antigen and antibody containing agaros Antigens in line x, in contrast, appeared to be common to both M and B homogenates. The large number of precipitin lines did not permit definitive identification of unique M antigens which may be present.

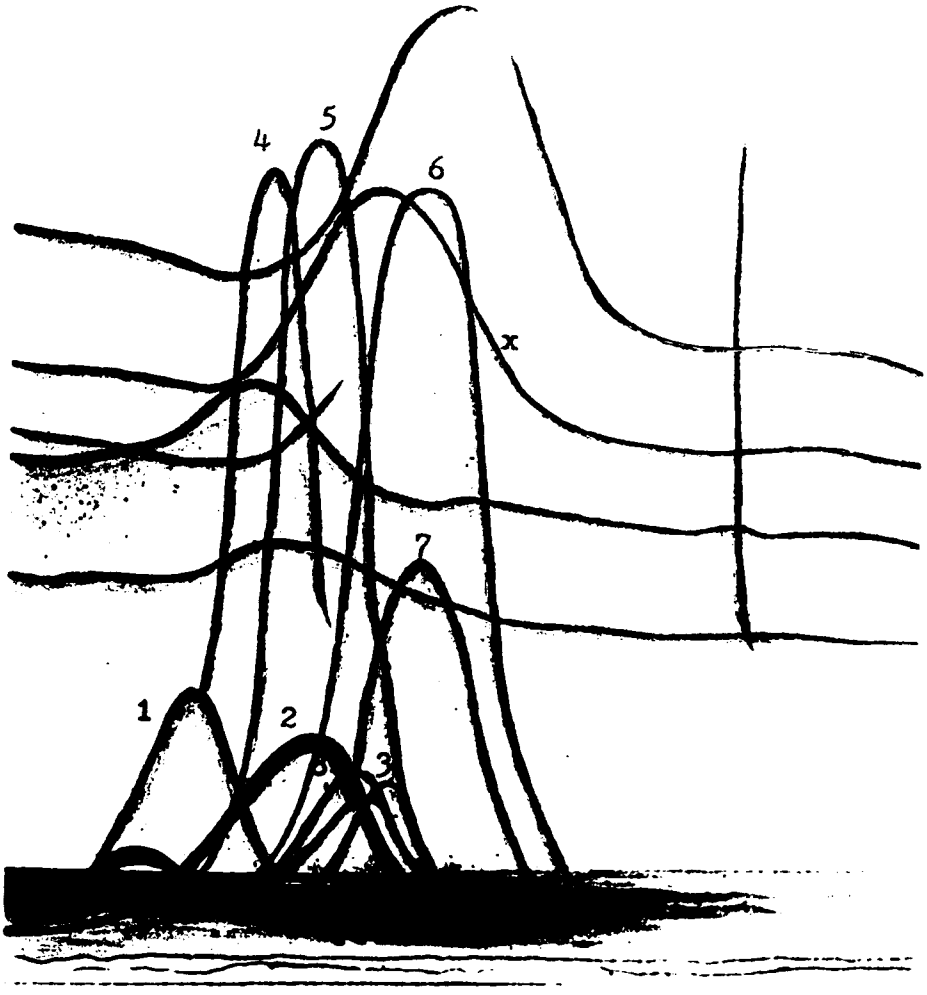

4
5
6
x
7
1
2
3

Table 1. Composition of agarose gels used in immunoelectrophoresis procedures.

TABLE 1

| | Agarose gels (ml) | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| A. Molten agarose solutions (55 C) | | | | | | |
| 1.5% agarose in water solution | 3 | 6 | 3 | 0.5 | 4 | 6 |
| distilled water | 1.5 | 2 | 1 | -- | 1 | 2.4 |
| well buffer (pH 8.3)* | 1.5 | 3 | 1.5 | 0.25 | 2 | 3 |
| B. Sample solutions (25 C) | | | | | | |
| mycelial antisera | -- | 1 | -- | -- | 1 | 0.6 |
| blastospore antisera | -- | -- | 0.5 | -- | -- | -- |
| blastospore antigen | -- | -- | -- | 0.25 | -- | -- |

B solution was mixed with A solution and immediately added to the electrophoresis plate.

* Barbitol well buffer was made up with 1000 ml of 0.023 M HCl and 15.85 gm sodium barbitol (Mallinckrodt).