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IMMUNOELECTROPHORETIC STUDY ON FIVE STRAINS OF *TRICHOMONAS VAGINALIS*

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Kua-Eyre Su (1980) Immunoelectrophoretic study on five strains of *Trichomonas vaginalis*. *Bull. Inst. Zool. Academia Sinica* **19**(2): 41-55. The antigenic composition of five stains of *Trichomonas vaginalis* were analyzed by immunoelectrophoresis methods to determine their antigenic relationships.

Strains JH30A, JH31A, JH34A, JH162A and JH384A were used. They were stored in liquid nitrogen since a few weeks after their isolation and were cloned before being used in the present study. Hyperimmune sera were prepared by seven weekly subcutaneous inoculations of disrupted antigens with complete Freund's adjuvant. Nonadsorbed and cross-adsorbed antisera were used in reactions with homologous and heterologous antigens.

Three major groups of antigens, I, II, and III were differentiated on the basis of precipitin line patterns formed in immunoelectrophoretic reactions. With the exception of strain JH384A which contained a unique group-III antigen, the qualitative differences were reflected mainly in a few group-I antigens; in two instances there were also differences in one group-II antigen. Quantitative differences in antigenic compositions and variation in the electrophoretic mobilities of the components among the five strains were also noticed.

Immunoelectrophoretic method has been employed in the study of antigenic relationships among strains of *Entamoeba histolytica* and *E. histolytica*-like organisms and among different species of *Entamoeba*. Ali Khan and Meerovitch[1] and Lunde and Diamond[12] using this technique were able to differentiate true *E. histolytica* strains from those of *E. histolytica*-like organisms. Krupp[8], on the basis of his experiments involving ten strains of *E. histolytica* and one strain of *E. invadens* with three anti-*E. histolytica* sera, reported the existence of immunologic differences among the various strains; the pathogenic strains had similarities of immunoelectrophoretic patterns not shared by the nonpathogenic strains and *E. invadens*.

The use of immunologic methods in the study of trichomonads has made it possible to demonstrate the existence of antigenic differences not only among various trichomonad species, but also among strains of each species[6,7,10,14]. Recently, Dwyer[5] employed immunoelectrophoresis techniques in studying the antigenic relationships among *Trichomonas*, *Histomonas*, *Dientamoeba*, and *Entamoeba*. The results were in agreement with those data he obtained previously for the fluorescent antibody and gel diffusion analysis of the same species[3,4], but the resolution of precipitin lines by immunoelectrophoresis was better than those observed observed by double diffusion methods.

In the present research, the antigenic composition of five strains of *Trichomonas vaginalis* was analyzed by immunoelectrophoretic methods to determine their antigenic relationships.

MATERIALS AND METHODS

Five strains of *T. vaginalis* were used in

this study. They were JH30A, JH31A, JH34A, JH162A, and JH384A. All strains were cloned by the method of Kulda and Serbus[9].

Preparation of antigens

Antigens for rabbit inoculation and for adsorption were prepared from a clone of each of the five strains. The trichomonads were recovered from liquid nitrogen storage and transfered twice in CTLM medium[13] with 5% (v/v) inactivated horse serum. To obtain large numbers of organisms, cultures were then transfered three times in CTLM medium modified by the omission of agar. Parasites in the culture obtained from the last transfer were centrifuged at 1,000 $\times g$ for 10 min. at 4°C, washed twice in Hanks' balanced salt solution (HBSS), then once in 0.01 M phosphate buffered saline (PBS), pH 7.0. Trichomonads were disrupted by repeated rapid freezing in a 95% ethanol-dry ice bath followed by slow thawing. Antigens for immunoelectrophoresis were prepared in similar way, except that the medium was supplemented with incativated newborn calf serum (7.5%, v/v) intesad of horse serum. Protein concentrations of the preparation were estimated by the Folin-phenol method[11]. All antigens were stored at -20°C until used.

Preparation of antisera

Antisera against each of the five trichomonad strains were prepared in two or three male New Zealand white rabbits weighing 2.5-3.0 kg. Each rabbit received seven weekly subcutaneous inoculations with antigens of 2.5, 3.5, 5.0, 7.5, 9.5, 12.0, and 15.0 mg proteins. In all instances, the antigens were homogenized with complete Freund's adjuvant (Difco) in a 1:1 ratio before being administered to rabbits. Anti-complete Freund's adjuvant serum was also produced in rabbits by seven weekly subcutaneous injections of the adjuvant in amounts comparable to those employed for the experimental groups. The adjuvant was homogenized with equal volume of PBS.

Pooled normal rabbit sera (NRS) collected before immunization served as experimental control for each group. The immunized rabbits were bled to death by heart puncture two weeks after the last injection. Sera prepared against a given antigen that produced similar precipitin reactions in gel diffusion tests were pooled. Pooled sera were preserved by the addition of merthiolate to the final concentration of 1: 10,000 (w/v), and stored at -20°C.

Immunoeletrophoresis

Veronal buffer, as described by Arquembourg[2] (diethylbarbituric acid, 1.4 gm; sodium diethylbarbiturate, 5.0 gm; sodium chloride, 1.0 gm; distilled water to 1 l; pH adjusted to 8.3), was used in all experiments. Gel plates were prepared by delivering 10 ml aliquots of hot 0.5% (w/v) agarose (Special Grade, Mann Res. Lab.) in buffer onto precoated 83 $\times$ 102 mm plates (projector slide cover glass, Eastman Kodak Co.). Sodium azide was added (final concentration 0.02%, w/v) to the agarose to prevent bacterial growth.

Two kind of templates were used, and the distance between antigen well (4 mm in diameter) and serum trough (2 $\times$ 76 mm) was always kept 4 mm, edge to edge. The adjacent-well method[15] was used for paired comparisons of homologous and heterologous antigens in reactions involving a given antiserum (e. g. Figs. 11-15 in "Results"). In one set of experiment this latter method was employed to compare reaction patterns of antigenic complex JH162A with its homologous and heterologous antisear (Figs. 26-29). Distance between the two adjacent wells was 2 or 4 mm, edge to edge.

Fifteen μl (300 μg protein) of antigen were added to each well, and the plates were left in the electrophoresis chamber for 10 min. to let the antigens equilibrate with the surrounding gel. Electrophoresis was then carried out at 4°C under constant current (10 mA/plate) until the bromophenol blue dye marker had moved 55 mm toward the anode end of the plate. The gel was then removed from the trough, the bottom of which was sealed with 0.2% agarose in buffer, and 200 μl undiluted antiserum were added. Photographs were taken after 72-hr incubation of the preparations at 37°C in humid chambers.

For adsorption of the antisera, each antiserum was adsorbed with each of the five

antigens. The minimum amount of antigen required to adsorb a given antiserum was determined experimentally in the following manners: during a 2-hr incubation at 37°C in a water bath, increasing quantities (in terms of total protein) of a given antigen were added in 5-10 μl aliquots every 5-20 min. to a series of test tubes, each containing 250 μl of antiserum. The antigen-antibody mixtures were left at 4°C overnight, then placed, without centrifugation, in the serum trough after electrophoresis of the antigens to be tested has been completed. The volume of mixture added to the serum trough exceeded 200 μl, which was the regular amount for nonadsorbed serum, the extra volume equalled the amount of antigen added for adsorbing the 200 μl antiserum. Photographs were taken after 72-hr incubation in moist chamber at 37°C. Adsorption was considered complete when all precipitin antibodies to the homologous antigen were removed from the antiserum.

RESULTS

No precipitin line was observed between either NRS of anti-complete Freund's adjuvant serum and the five antigens. Similarly, negative results were obtained when the five antisera were reacted with undiluted newborn calf serum or CTLM-NA medium containing 7.5% newborncalf serum. Positive results, therefore, could be attributed to specific antigen-antibody reactions.

Best resolution of the precipitin lines was usually observed in homologous reactions. Number of lines formed in these reactions ranged from 19-29 (Table 1); The lowest number

TABLE I

Number of precipitin lines resolved in reactions of a given antiserum with the five antigenic complexes in immunoelectrophoresis

Antiserum	Antigen	No. of precipitin lines			
		Group I	Group II	Group III	Total
Anti-JH30A	JH30A	11	3-4	9	23-24
	JH31A	11	3	9	23
	JH34A	10	3	8-9	21-22
	JH162A	10-11	3	8-9	21-23
	JH384A	11	3	8-9	22-23
Anti-JH31A	JH30A	11-12	1-2	8	20-22
	JH31A	13	2	8	23
	JH34A	10-11	1-2	8	19-21
	JH162A	11-12	1-2	7	19-21
	JH384A	12	1-2	7	20-21
Anti-JH34A	JH30A	10	2	5	17
	JH31A	8	2	5	15
	JH34A	10	3	6	19
	JH162A	9	3	5	17
	JH384A	9	2	5	16
Anti-JH162A	JH30A	9-10	3	7	19-20
	JH31A	10	3	7	20
	JH34A	9	3	8	20
	JH162A	13	3	9	25
	JH384A	11	3	8	22
Anti-JH384A	JH30A	11	3	10	24
	JH31A	9-10	2	9	20-22
	JH34A	9-10	3	9-10	21-23
	JH162A	11	3	10	24
	JH384A	13	5	11	29

of lines was formed in the reaction between antigenic complex JH34A and anti-JH34A serum, while the highest number was obtained in the reactions involving JH384A and its antiserum.

The precipitin lines were arbitrarily divided into three groups according to their relative position along the trough: group-I lines, between the antigen well and the trough; group-II lines, extending from or near the right side of the well toward the anode end of the plate; group-III lines, spreading over the right three fourths of the area between the antigen well and the anode end of the serum trough (e. g. Fig. 34: group-I, lines 1-13; group-II, lines 14-18; group-III, lines 19-29).

The precipitin lines were numbered sequentially, starting with those of group-I, in which line 1 was that closest to the antigen well, and the lines with the highest number, that closest to the antiserum trough; the latter line (e. g. line 13 in Fig. 34) was followed by the first group-II line located farthest away from the antiserum trough, the last line of this group (e. g. line 18 in Fig. 34) being located closest to the trough and to the anode end of the plate. As in the preceeding two groups, the first line in group-III was farthest away, the last line (e. g. line 29 in Fig. 34) closest to the antiserum trough and to the anode end of the plate.

Numbering the lines was first established in the homologous reactions (e. g. Figs. 34, 39). Identification of the major precipitin lines formed in the heterologous reactions with a given serum was obtained by the adjacent-well method (e. g. Figs. 35-39). In this method, one of the wells between two parallel troughs was filled with the homologous antigenic complex while the other with a heterologous complex; the troughs received the homologous antiserum. The line in the heterologous reaction which was continuous with a line in the homologous reaction (pattern of identity) was given the same number. If the lines of the homologous and heterologous reaction failed to meet, their curvature and relative position were employed for their identification.

The lines formed in reactions of each of the five antigenic complexes with its homologous antiserum were numbered independently, their designation being determined by their relative position alone. Thus, in reactions involving different antisera shown in the figures, lines designated by the same number do not necessarily denote identical antigen-antibody reactions. The number of lines resolved in reactions of five antigenic complexes with each antiserum are listed in Table 1; those obtained from reactions of adsorbed antisera are summarized in Table 2.

The results obtained with each antiserum will be discussed separately.

Anti-JH30A Serum

In the homologous reactions there were at least 23 precipitin lines: 11 in group-I, three to four in group-II, and nine group-III (Fig. 1).

It is evident from the results obtained by the standard immunoelectrophoretic method in reactions between the five antigenic complexes and anti-JH30A serum (Figs. 1-5) that the major group-I lines were best defined in reactions with complexes JH31A and JH162A. These lines appeared somewhat diffuse in reactions of this serum with strains JH30A and JH384A; they were diffuse in the reaction with strain JH34A. These results suggested that concentrations of group-I antigens were heighest in strains JH31A and JH162A, lower in strains JH30A and JH384A, and lowest in strain JH34A. Comparisons of the antigenic complexes by the adjacent-well method indicated that group-I lines 2, 3, 4, 7, and 8; group-II line 15; and group-II lines 18 and 19 were common to all five strains. The definition of the group-I lines in the reaction with strain JH34A could be improved by using the adjacent-well method, possibly because common antigens which diffused from the antigenic complex JH30A strengthened the reaction.

Four group-II lines were resolved in homologous reactions. Line 12, Which usually formed a composite band with line 13, could be resolved in the homologous reaction by the adjacent-well method. There were about three group-II lines

TABLE 2

The amount of protein required for homologous and heterologous adsorptions of the five antisera and the number of precipitin lines formed between adsorbed sera and their homologous antigenic complexes in immunoelectrophoresis

Antiserum	Adsorbing antigen	Ag. (mg)/ 100 μl As.	Ag. (μl)/ 250 μl As.	Amount Ag.-As. (μl)*	No. of lines formed with homologous Ag.		
					I	II	III
Anti-JH30A	JH30A	1.0	55	245	0	0	0
	JH31A	2.0	95	275	1	0	0
	JH34A	1.6	50	240	0	0	0
	JH162A	1.5	65	255	1	1	0
	JH384A	1.8	75	260	0	0	0
Anti-JH31A	JH30A	1.1	60	250	1	0	0
	JH31A	0.9	50	240	0	0	0
	JH34A	2.8	90	270	0	1	0
	JH162A	1.2	50	240	1	0	0
	JH384A	1.6	65	255	1	0	0
Anti-JH34A	JH30A	0.4	50	240	0	0	0
	JH31A	0.4	50	240	0	0	0
	JH34A	0.4	50	240	0	0	0
	JH162A	0.4	50	240	0	0	0
	JH384A	0.4	50	240	0	0	0
Anti-JH162A	JH30A	0.9	50	240	2	0	0
	JH31A	1.0	50	240	0	0	0
	JH34A	2.4	75	260	2	0	0
	JH162A	0.7	50	240	0	0	0
	JH384A	0.7	50	240	2	0	0
Anti-JH384A	JH30A	2.4	130	305	4	0	1
	JH31A	1.6	75	260	3	0	1
	JH34A	0.8	50	240	5	0	1
	JH162A	1.4	60	250	3	0	1
	JH384A	0.8	50	240	0	0	0

* Amount of antigen-antibody mixture added, without centrifugation, to the serum trough.

in the heterologous reactions. About nine group-III lines were resolved in the reaction involving all five antigenic complexes and the anti-JH30A serum (Figs. 1-5).

By the procedure used in this study, adsorption of anti-JH30A serum with strain JH34A or JH384A evidently removed all antibodies against the homologous strain. One group-I line, however, remained in the reaction involving JH30A complex and anti-JH30A serum adsorbed with JH31A trichomonads; one group-I line and possibly one group-II line were not removed when antigenic complex JH162A was employed for adsorption (Table 2). The foregoing results indicated a close relationship of strains JH384A and JH34A to strain JH30A. One or two antigens of the latter clearly were absent from JH31A and JH162A trichomonads, respectively.

Although the four heterologous strains shared most of the antigenic components with strain JH30A, the concentrations and/or electrophoretic mobilities of the components appeared to vary with strain; this caused differences in immunoelectrophoretic patterns among the strains. For example, in the reaction between anti-JH 30A

serum and JH31A trichomonads, the group-I lines were more elongate and better defined than the corresponding lines noted in reaction of this serum with other antigenic complexes (cf. Fig. 2 with Figs. 1, 3-5). Either strain JH31A had a higher concentration of group-I antigens or the electrophoretic mobilities of these antigens in strain JH31A were more heterologous. Since lines 5 and 6 were stronger in reactions with JH31A and JH162A (Figs. 2, 4) than those with the remaining strains, the concentration of antigens 5 and 6 might be higher in these two strains. Among the group-III antigens, mobility of antigen 19 was fastest in strains JH30A, JH34A, and JH384A (being similar in all three), slower in JH162A complex, and slowest in JH34A (cf. Figs. 1-5). Mobility of antigen 18 was almost identical in the former three strains, but slower in the latter two.

Anti-JH31A Serum

A total of about 23 precipitin lines: 13 in group-I; two in group-II; and eight in group-III, could be resolved in the homologous reactions (Figs. 7, 12). Line 22, not evident in the reaction shown in Fig. 7, was outlined faintly in the homologous adjacent-well reaction pattern (Fig. 12).

Antigenic complex JH31A stimulated a strong immunologic response to the group-I antigens in rabbits. Thus, the group-I lines were much better defined in the homologous reaction (cf. Fig. 7 with Figs. 6, 8-10). The group-I lines obtained with the heterologous antigenic complexes were rendered more distinct by the adjacent-well method (Figs. 11, 13-15). This might be due to common antigens diffusing from the adjacent JH31A antigenic complex and thus enhanced the reactions. The major group-I lines, 3-8, were shared by all five strains; however, concentrations of these group-I antigens might be lower in the heterologous strains than in the homologous JH31A trichomonads. One reason for such an explanation is that these lines developed slightly closer to the heterologous antigen wells (Figs. 11-15, cf. homologous vs. heterologous reactions). This phenomenon was more obvious in the reaction with strain JH34A that in those involving the remaining three heterologous strains.

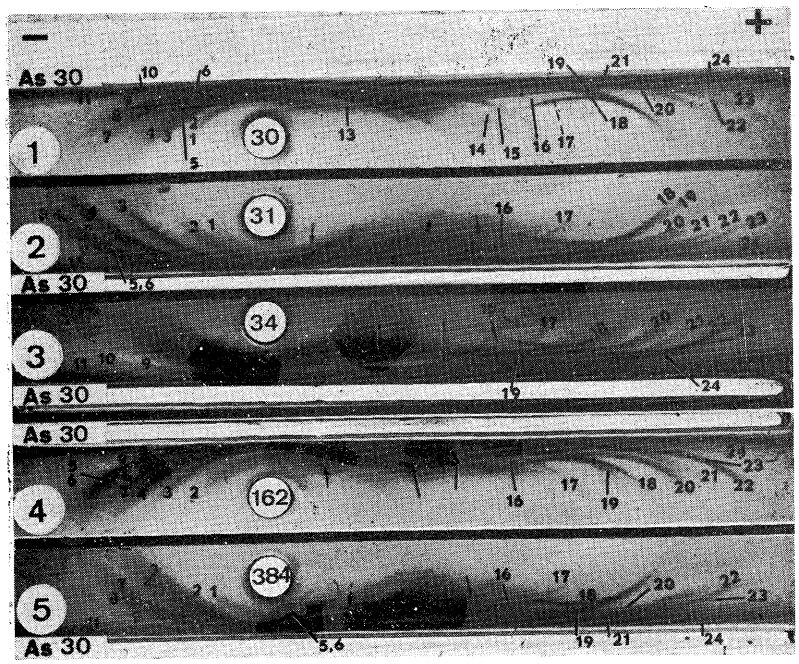
Among the group-III lines, No. 16, which appeared to continue into line 23, was observed only in reactions of anti-JH31A serum with strains JH30A and JH31A obtained by the standard immunoelectrophoretic method (Figs. 6, 7). The adjacent-well reactions revealed that the antigens represented by lines 19 and 20 were common to all five strains, however, the electrophoretic mobilities of these two antigens appeared to vary among the strains. Line 19 was vary similar in its relative position and intensity in reaction involving the homologous JH31A and the heterologous JH30A antigenic complex. It was stronger, however, in the reactions between anti-JH31A serum and the remaining three strains (cf. Figs. 6, 7 with Figs. 8-10). Although fairly strong in the reaction involving complex JH30A, line 20 appeared to be lagging behind its corresponding line in the homologous system. This line was much weaker and had lower mobility in the reaction between anti-JH31A serum and the remaining three strains (cf. Figs. 6-10). Mobility of antigen 23 seemed to be highest in reaction involving complex JH34A, lower in those of strains JH162A and JH384A, and lowest in those using JH30A and JH31A trichomonads.

Upon adsorption of anti-JH31A serum with strain JH30A, JH162A, or JH384A, one group-I line remanied in reactions with the homologous antigenic complex, and one group-II line was left in reactions between JH31A complex and its homologous antiserum adsorbed with JH34A trichomonads (Table 2).

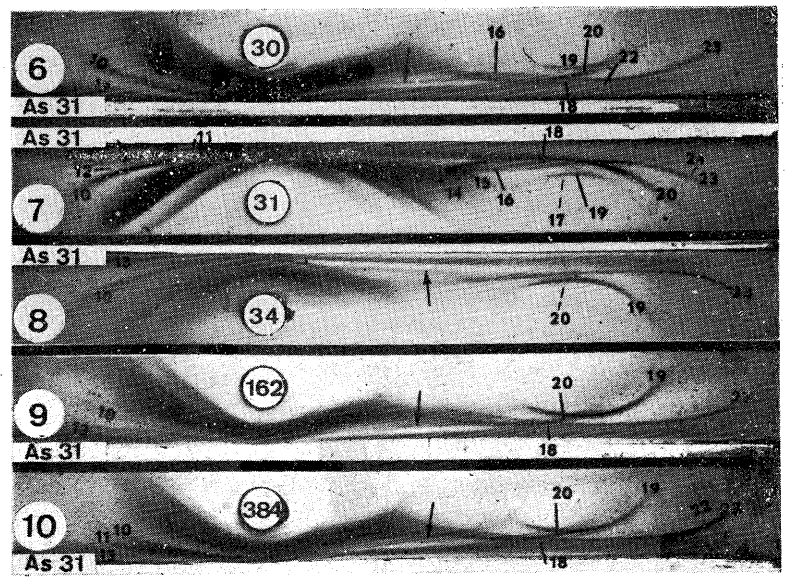
In general, the immunoelectrophoretic pattern in reaction of JH30A complex with anti-JH31A serum resembled most closely the homologous reaction pattern. Strain JH34A seemed to be most distant from JH31A, at least quantitatively, because the amount of protein required for adsorption was about three times that needed for homologous adsorption (Table 2).

Anti-JH34A Serum

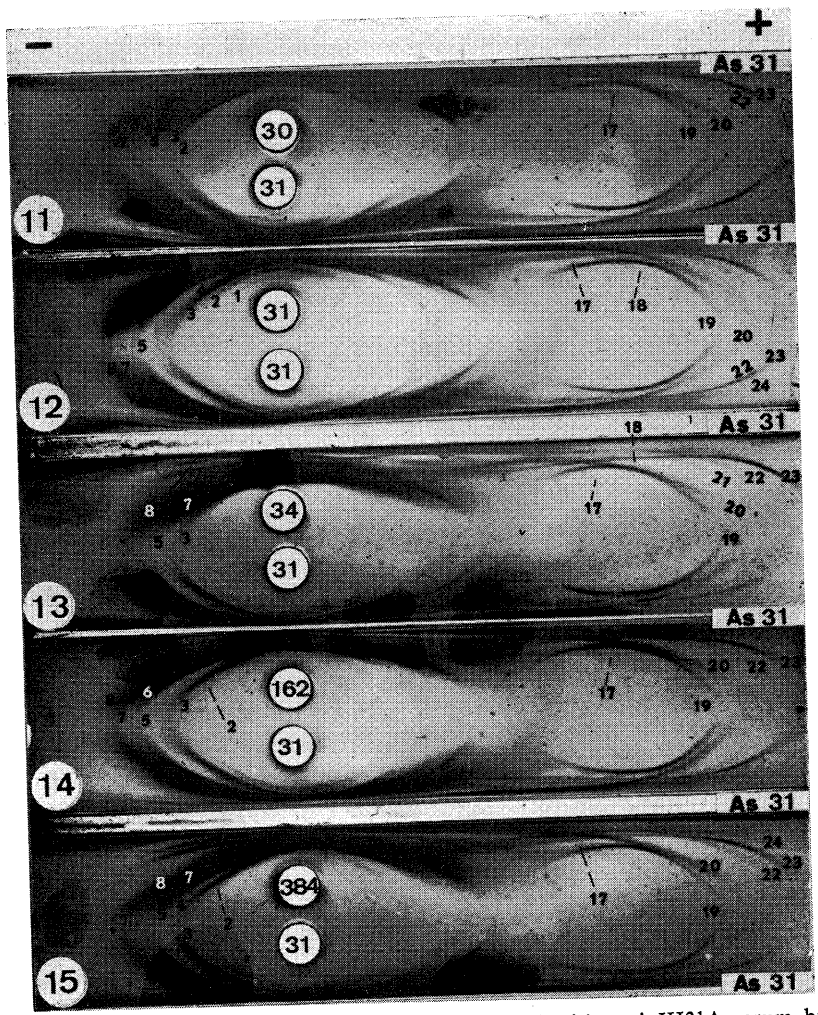
About 19 precipitin lines were resolved in reactions of the anti-JH34A serum with its



Figs. 1-5. Standard immunoelectrophoretic patterns in reactions of anti-JH30A serum with the five antigenic complexes.



Figs. 6-10. Standard immunoelectrophoretic patterns in reactions of anti-JH31A serum with the five antigenic complexes.

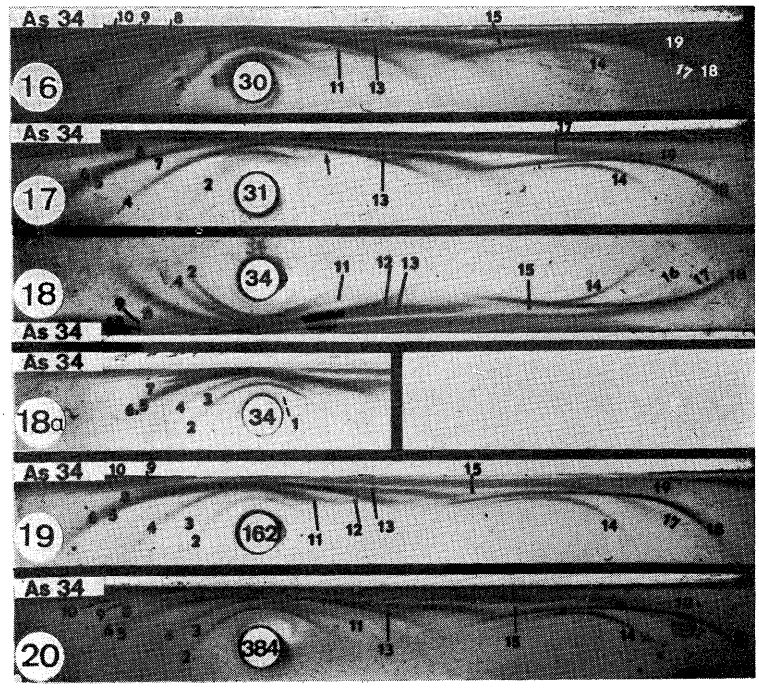


Figs. 11-15. Immunoelectrophoretic patterns obtained with anti-JH31A serum by the adjacent-well method. Distance between the two adjacent wells is 2 mm (edge to edge).

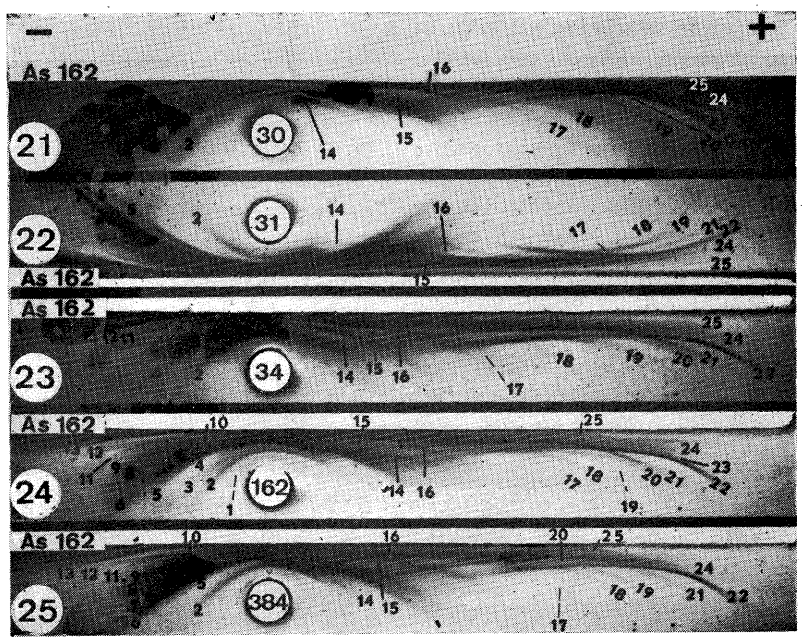
homologous JH34A antigenic complex (Figs. 18, 18a; Table 1). In reactions of this antiserum with all five antigenic complexes, the precipitin lines, although clearly defined, were generally weaker than those noted in reactions involving the other antisera. Relatively fewer group-III lines were formed.

Ten group-I lines were resolved in reactions of anti-JH34A serum with its homologous antigenic complex and with strain JH30A (Figs. 16, 18, 18a). Nine lines were observed in reactions with the antigenic complexes JH162A or JH384A (Figs. 19, 20, & the adjacent-well method), and only eight group-I lines in reactions of this antiserum with strain JH31A. Antigenic complex JH31A may have lower concentration of antigen 2. Most of the group-I lines in the heterologous reactions were more elongate than those formed in the homologous reaction. This suggested that either strain JH34A had low concentrations of these group-I antigens or that these antigens in complex JH34A had more homogenous electrophoretic mobilities.

Three group-II lines (11-13) were observed



Figs. 16-20. Standard immunoelectrophoretic patterns in reactions of anti-JH34A serum with the five antigenic complexes.



Figs. 21-25. Standard immunoelectrophoretic patterns in reactions of anti-JH162A serum with the five antigenic complexes.

rabbits; while reactions involving anti-JH34A serum with the five antigenic complexes resulted in the formation of both weaker and fewer precipitin lines than those obtained when other antisera were employed.

In addition to some qualitative differences, quantitative differences in the antigenic components also existed among these strains. Strain JH34A appeared to be most distant, at least quantitatively, from the JH31A and JH162A. This was reflected in the diffuse group-I lines formed in reactions of JH34A complex with anti-JH31A and anti-JH162A sera, and also by the excssive amount of protein of JH34A trichomonads required to adsorb either of these two antisera.

Most of the group-III antigens seemed to be shared by all five strains, except for JH384A, which had a unique group-III antigen in addition to the common ones. Although as many as ten common group-III antigenic components may exist in the five strains of *T. vaginalis*, a given component in different strains may vary in its ability to stimulate antibody formation in rabbits. For example, the group-III antigens of JH34A trichomonads appeared to have a lower capacity than those of the other strains to stimulate antibody formation in rabbits. While eight to ten group-III lines were formed in reactions of the JH34A antigenic complex with the heterologous antisera, only six such lines were observed in the homologous reactions. Therefore, JH34A had either very small quantities of, or incomplete antigens capable of combining with antibodies produced by the rabbits against the remaining four strains studied. In the case of JH162A trichomonads, one of its components (antigen 19) was able to elicit only weak antibody response but it formed a strong precipitin arc in reactions with antibodies produced in the rabbits against the four heterologous strains. Differences in the electrophoretic mobility of various group-III antigens among different strains were also noticed.

From these results, JH34A trichomonads appear to be least complex in its antigenicity, because every other strain also contain the antigenic components it has. JH384A organisms, on the other hand, possess the most complex antigenic composition as evidenced by at least four antigens unique to this strain. Excluding these specific antigens, JH384A, JH30A, and JH34A are fairly closely related antigenically. JH31A and JH162A seem to be more closely related in that cross-adsorption of anti-JH162A serum with antigenic complex JH31A also result in complete removal of the precipitin antibodies.

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陰道滴蟲之免疫電泳研究

蘇　霖　霧

本研究應用免疫電泳法，來分析五株陰道滴蟲之抗原成分，以探討各株滴蟲之關係。

實驗中所採用之五株滴蟲：JH30A, JH31A, JH34A, JH162A 及 JH384A，都是在由病人體內分離後數星期內，即予以冷凍儲存於液態氮中，使用前並曾分別單胞族羣化，爲獲得高度免疫血清，滴蟲抗原與佛羅恩特氏完全佐劑之均質液，分七次注入白兎皮下。然後以未吸附及經交叉吸附之免疫血清與各抗原進行免疫電泳試驗。

結果顯示，陰道滴蟲之抗原，依其免疫電泳沉澱線之形態，可分爲三類：Ⅰ、Ⅱ、Ⅲ。除 JH384A 具有其特異之第Ⅲ類抗原外，各株抗原成分之不同主要見於少數第Ⅰ類抗原，其中兩例亦有第Ⅱ類抗原之差異。此外各株滴蟲抗原成分間量的差異及電泳移動之速度，亦有不同。