Arch. Inst. Razi, 1967, 19, 91 - 95

MYCOPLASMA AGALACTIAE

II – IMMUNO-ELECTROPHORESIS PATTERNS OF M. AGALACTIAE ANTIGEN (*)

by

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In a previous study (1) it was ascertained that 50% of the dry weight of M. agalactiae was protein. Whether this protein fraction could act as an antigen or not was unknown. To determine this Immuno-Electrophoresis was used to detect the Antigen components of M. agalactiae.

The present communication reports the results obtained with this test which indicate that in the all preparations precipitation are due to a special protein.

MATERIALS AND METHODS

Antigen Preparation: The M. agalactiae strain was cultivated in "Difco PPLO Broth w/o CV" medium supplemented by 20% horse serum, 1% Yeast Extract (BBL) and 500 units of penicillin per ml of medium. The culture was incubated for 36 hours on a shaker apparatus in the 37°C incubator. The organisms in the culture medium were sedimented by centrifugation in the Sorvall Continuous-Flow Centrifuge at 15,000 rpm.

Harvested material was washed several times in 0.15 M phosphate buffered saline (PBS), pH=7.2 The washed sediment was diluted in demineralized water and frozen at —20°C.

Disintegration: The X-Press (2) apparatus was used for disintegration of frozen materials. 25 ml of wet weighed bacteria were passed into the X-Press apparatus. The X-Press was operated at —20°C. The material was repeatedly forced

(*) Supported in Part by NIH Research Grant No. 1 RO5 TWOO238-01.

through a 2.5 mm hole. This required 5 to 10 Tone/Cm^2 pressure which is accomplished with the aid of a hydrolic jack.

Samples taken from the disintegrated material showed almost complete disintegration by the X-Press.

Disintegrated material was centrifuged at 20,000 rpm for one hour in an Ultra-Centrifuge (Phywe Germany, Type 1957). The supernatant thus obtained was carefully removed by means of a capillary pipette and was submitted to a second centrifugation to remove any remaining sedimentable materials. The clear supernatant was lyophilized in an Edwards Machine and labled (S).

Disintegrated sediment material after separation of lipids with Acetone reagent (3,4,5), was filtered through a Whatman No. 50 paper, then dissolved in 0.25 ml of N/NaOH and centrifuged. The residue obtained by centrifugation was labled (R).

Disintegrated material containing whole organisms in a soluble form was lyophilized and labled (W).

These three materials were used for Immuno-Electrophoresis.

Antiserum Preparation: Hyper-immune serum was prepared in donkeys. Two donkeys were inoculated intravenously with 5 ml of M. agalactiae antigen at 5 day intervals. Serum samples of these donkeys were examined separatly by serological tests, before and after different intervals inoculation. The pooled and concentrated sera was also tested (Table No. 1).

The concentrated and unconcentrated hyper-immune sera were used for Immuno-Electrophoresis.

Table No. I

Serum Samples	Pre-bleeding			After 3rd inoculation titers			After 5th inoculation titers		
	D	T	AG	D	T	AG	D	T	AG
Donkey No 1	-	-	-	10§	40	160	20	320	1280
Donkey No 2	-	-	-	10	40	160	20	320	640
Pooled	-	-	-	10	40	160	20	640	1280
Concentrated	-	-	-	40	160	320	80	1280	2560

D = Direct Agglutination Titers, T= Tube Agglutination Titers.

AG = Anti—Globulin Titers, § = 2+

Agar Gel Preparation: Difco Bacto Agar, prepared in 7.5% gel was cut into small cubes and washed by successive changes of distilled water for three days. The final concentration of Agar was established by dry weight determinations. This preparation was then diluted with distilled water and buffer solution in such proportions that the Agar concentration was 1% and the ionic strength of the buffer was 0.05. The solution was then filtered.

A rectangular plate of Photographic glass (13 X 18) was cleaned and fixed by allowing a thin layer of diluted Agar solution to dry on the surface. The Agar was melted and an experimental layer of 3 mm poured into level Pyrex dishes.

Electrical contact from the buffer reservoires to the Agar plates was made by placing strips of filter paper in the gel at each end of the plate when the solution was poured. Lateral canals, 4 cm apart were molded in the layers with glass rods. A well for the antigen mixture was cut large enough to contain 0.2 ml of the solution to be analyzed. The solution was included in the gel layer by mixing with buffered Agar before introducing it into the well.

Immuno-Electrophoresis: The method of Immuno-Electrophoresis as described by Grabar (6) was carried out. A veronal buffer solution (Sodium barbiturate N/10, 770 ml and ClH N/10, 230 ml), pH=8.2, at ionic strength 0.05 was found to maintain the pH of the gel during the course of the experiment and Electrophoresis was continued for a period of four hours with a voltage gradient on the plate of approximately 5 Volts/cm. (Total potential drop across the 13 X 18 cm plate was approximately 90 Volts). The total current per plate was 34-45 milliamperes. Antigen concentrations of 1 to 20% were used. 0.2 ml of concentrated antigen was mixed with 0.5 ml of warm buffered Agar and pipetted into slots which had a capacity of 0.2 ml. All experiments were done at room temperature. After electrophoresis of antigen, undiluted hyper-immune serum was pipetted into the trough each side of the antigen path, these had a capacity of 1.5 ml. The preparation was then put aside for diffusion at room temperature in a humidified chamber. Usually the first arcs of precipitation appeared after 18 hours and precipitation patterns were completed after 48 hours. The precipitation arcs were stained by Azocarmine and photographed.

RESULTS

Photograph No. I shows the Immuno-Electrophoresis by whole disintegrated material (W), Photograph No. II and No. III show the result with residue (R) and supernatant (S) respectively. Concentrated serum was used in all of these instances. The results by unconcentrated serum were similar.

According to these Photographs in all preparations from the Immuno-Electrophoretic patterns, it seems that two antigens with different mobilities but with common antigenic determinants are present. One antigen remains at point of inserssion. The two antigen-antibody precipitin bands which are formed, as seen on Photographs indicating partially indentical structure.

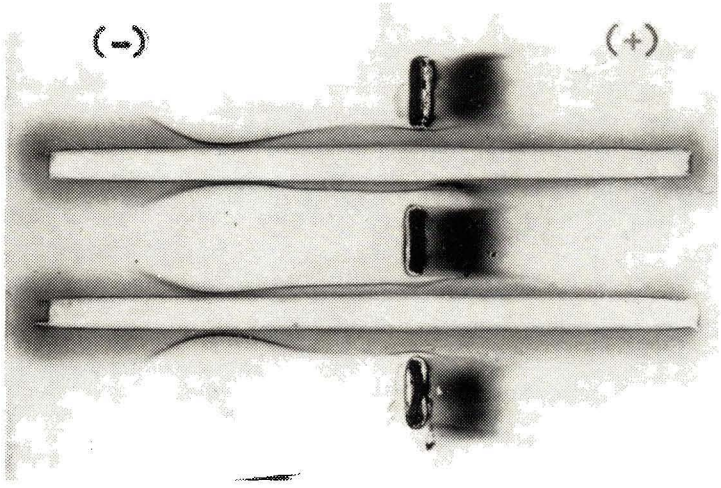

Fig. No I — Immuno-Electrophoresis by whole disintegrated material

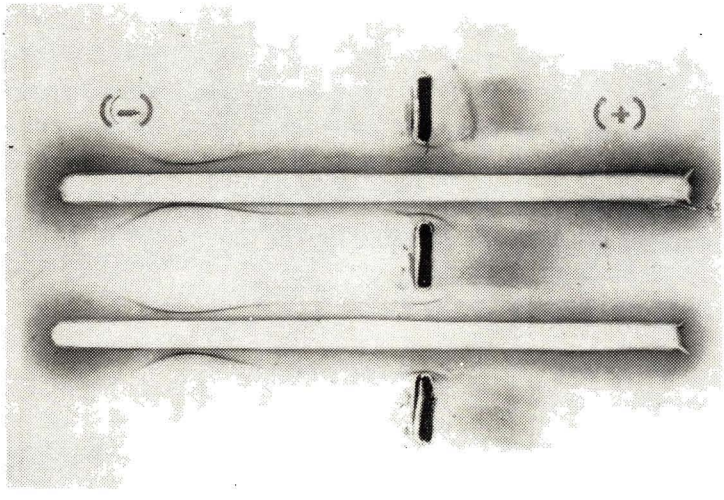

Fig. No II — Immuno-Electrophoresis by residue material

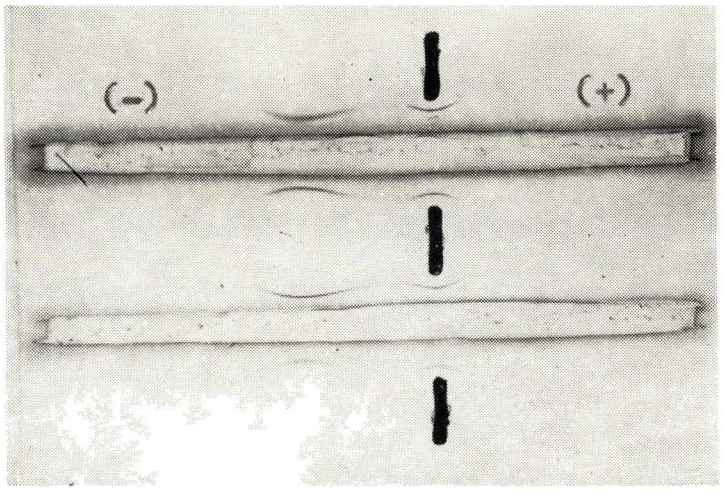

Fig. No III — Immuno-Electrophoresis by supernatant material

SUMMARY

Preparations obtained from M. agalactiae by the X-Press method and examined by Immuno-Electrophoresis showed that disintegrated materials regarded as antigen gave similar precipitation band with hyper-immune serum.

There were no significant differences in the precipitation patterns.

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