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ANALYSIS OF ANTIGENS IN MYCOBACTERIUM PARATUBERCULOSIS*

By

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GUNNARSSON, EGGERT and FINN H. FODSTAD: *Analysis of antigens in Mycobacterium paratuberculosis.* Acta vet. scand. 1979, *20,* 200—215. — Using crossed immunoelectrophoresis (CIE) and crossed line immunoelectrophoresis (CLIE), antigens from different strains and variants of Mycobacterium paratuberculosis were compared, and cross-reactions between 1 of these strains and Mycobacterium avium and BCG studied. In each of 4 bovine laboratory strains of M. paratuberculosis examined, altogether 44 different antigens were demonstrated. This is the largest number of antigens in M. paratuberculosis which has been described so far. No important difference in the antigenic structure of the strains was found. The 4 laboratory strains are being used routinely in the production of vaccine against Johne's disease in Norway and Iceland. One of the aims of the present work was to investigate the antigenic relationship between these strains and the goat-pathogenic Norwegian and the Icelandic variant of M. paratuberculosis. Out of 44 different antigens demonstrated in the laboratory strains, 39 and 31 gave cross-reactions against the Norwegian and the Icelandic variant, respectively. This is in accordance with practical experience, as the results of vaccination against Johne's disease, performed in Norway for many years, are very good.

Twenty-seven and 24 cross-reacting antigens between M. paratuberculosis and strains of M. avium and BCG, respectively, were observed. This finding agrees with clinical observations.

Another aim of the investigation was to identify species-specific antigens as regards M. paratuberculosis. One antigen showed a marked cross-reaction between the strains of M. paratuberculosis examined, but did not react with antisera against M. avium and BCG. Some other antigens showed partial specificity.

The results obtained stress the complicated antigenic situation in mycobacteria which is of decisive significance as regards the diagnosis and classification of mycobacterial infections.

Mycobacterium paratuberculosis; antigens; immunoelectrophoresis; cross reaction.

Antigenic relationships between different species of mycobacteria have been demonstrated by immunoelectrophoresis and double gel diffusion techniques (*Tuboly* 1965, *Roberts et al.* 1972, *Stanford* 1973, *Navalkar* 1977). This situation is responsible

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for, among other things, interfering cross-reactions when using immunodiagnostic methods. Thus animals vaccinated against Johne's disease react positively to tuberculin. Vaccination against Johne's disease has for this reason, been restricted in many countries (*Doyle* 1964, *Stuart* 1965, *Huitema* 1968).

In recent years, several modifications of the classical immunoelectrophoresis technique have been developed. These include crossed immunoelectrophoresis (*Laurell* 1965, *Clarke & Freeman* 1966). This method has proved to be well suited for the characterization and comparison of antigens from different mycobacteria, both cellular antigens and antigens in culture filtrates (*Roberts et al.*, *Wright & Roberts* 1974). Crossed immunoelectrophoresis with intermediate gel is a further modification of this method and seems to be especially suitable for the study of cross-reactions between different strains and species of mycobacteria (*Closs et al.* 1975).

The purpose of the present investigation was to compare antigens from different strains and variants of M. paratuberculosis, and to study cross-reactions between these and antigenically related bacteria. Differences in antigen structure in various strains and variants of M. paratuberculosis are of practical significance in connection with the production of vaccine, a production which must be based on laboratory strains adapted to grow on fluid synthetic media.

Furthermore a better knowledge of the antigenic structure of different strains and variants of Mycobacterium paratuberculosis, and of immunologic cross-reactions between these and other species of mycobacteria, is necessary if antigen fractions are to be produced which would allow an improvement in the sensitivity and specificity of immunodiagnostic methods.

MATERIALS AND METHODS

Antigens

A. Antigens from laboratory strains of M. paratuberculosis. Antigens were produced from each of the strains 2E, 316F, Teps and Str. 18, all of bovine origin*. Cultures performed on Reid's

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* 2E and 316F originate from the Central Veterinary Laboratory, Weybridge, England; Teps from the National Institute for Medical Research, Farm Laboratories, Mill Hill, London, England, and Str. 18 from the Animal Disease Research Laboratory of the Bureau of Animal Industry, Auburn, Alabama, USA.

medium were incubated at 37°C for 4—6 weeks. Approx. 1.2 g w/w of the growth were washed once in saline, centrifuged at 3000 × g for 30 min and resuspended in saline to a concentration of 60 mg/ml. The suspension was treated ultrasonically for 15 min at 80—100 w using a Branson Sonifier model B 12. The sonicates from the strains 316F, Teps and Str. 18 and half of the sonicate from strain 2E were centrifuged at 20,000 × g for 20 min. NaN_3 was added to the supernatants to a final concentration of 0.02 % and these were stored at 4°C. They contained the soluble antigens which were used in crossed immunoelectrophoresis. The other half of the sonicate from strain 2E was not centrifuged but divided into smaller doses and kept frozen to be subsequently used for the immunization of rabbits.

B. Antigens from strains of M. paratuberculosis isolated from Norwegian goats (M.ptb.CN). Isolations of the strains were made on Dubos' medium from intestine and lymph nodes from naturally infected goats (*Fodstad & Gunnarsson* 1979). An approx. 8-week old secondary culture was used for the production of antigens. Bacterial colonies were loosened from the surface of the medium using a wire loop, suspended in saline and transferred, using a sterile pipette, to 50 ml centrifuge tubes. The bacterial mass was washed twice in saline to a concentration of 60 mg/ml. The further procedure was as for the laboratory strains.

C. Antigens from strains of M. paratuberculosis isolated from Icelandic sheep (M.ptb. OI). A modification of a method described by *Closs et al.* (1975) for the production of antigens from mice infected with Mycobacterium lepraemurium was used. Scrapings from the intestinal mucous membrane of infected sheep were mixed with saline and homogenized in a Sorvall® "omni-mixer" for 2 min. The homogenisate was centrifuged for 20 min at 200 × g. Most of the tissue then sedimented. The supernatant was collected and centrifuged at 6000 × g for 10 min. Acid-fast staining of the sediment showed this to consist largely of acid-fast bacilli. The sediment was re-suspended in saline, mixed with equal amounts of mineral-oil in the homogenisator and centrifuged for 20 min at 2000 × g. The remainder of the tissue material was thus precipitated in the aqueous phase, while the bacteria remained in the intermediate layer between the aqueous phase and the oily phase. The centrifuge

tubes were then frozen and bacteria were collected the following day from the intermediate phase. The thick suspension was centrifuged at 3000 $\times$ g for 30 min. Excess oil was removed and the sediment was resuspended in saline to a concentration of 100 mg/ml and treated ultrasonically as described above for 15 min. After centrifuging at 20,000 $\times$ g for 20 min, the bottom phase contained the soluble antigens. After the addition of NaN_3 this was stored at 4°C. The top layer contained the cellular fragments in the oily phase. These were mixed with the soluble antigens in a ratio of 1:10 and frozen in small doses to be subsequently used for the immunization of rabbits.

Antisera

Four rabbits were immunized with the strain 2E and 2 rabbits with each of the strains M.ptb.CN and M.ptb.OI. The antigens were mixed with equal amounts of Freund's incomplete adjuvant to form a stable emulsion. Each rabbit was inoculated subcutaneously with 0.2—0.4 ml of this mixture distributed over approx. 10 different sites in the neck and shoulder region. The animals were immunized 7—8 times at intervals of 3—4 weeks and blood was tapped 10 days after the last inoculation.

The serum proteins were precipitated with ammonium-sulphate and IgA and IgG isolated and concentrated as described by *Harboe & Ingild* (1973). The immunoglobulin preparations from the different blood tappings from rabbits immunized with the same antigens were pooled.

Antiserum against Mycobacterium avium was obtained from the Institute for Experimental Medical Research, Ullevål Hospital, Oslo, and antiserum against BCG from Dakopatts, Copenhagen, Denmark.

Immunoelectrophoresis

Crossed immunoelectrophoresis (CIE) was carried out as described by *Weeke* (1973) and *Closs et al.* on 10 $\times$ 10 cm glass plates in the first dimension and on 5 $\times$ 5 cm glass plates in the second dimension. Electrophoresis chambers with cooling arrangements (LKB 2117 Multiphor) and power supply from Buchler Instruments, model No. 3-101417, were used. Electrophoresis was run in 1 % agarose (Agarose Litex, type No. HSB, batch No. 0322) in barbitural-tris buffer, pH 8.6 and ionic strength 0.02 (*Weeke*). The thickness of the gel in the first dimension

was approx. 1.5 mm. Ten μl antigen-solution was put into circular wells. Four antigen-complexes were run simultaneously in the first dimension. Parallel runs were made with bovine serum albumin stained with brom-phenol blue. Electrophoresis was run for 10 min at 5 V/cm and subsequently at 10 V/cm until the indicator protein had migrated 2.8 cm from the nearest edge of the well. The thickness of the gel in the intermediate and the reference gels was 1 mm and covered 7.5 cm^2 and 12.5 cm^2, respectively. Electrophoresis in the second dimension was run for 18—22 h at 2—2.5 V/cm. After electrophoresis, the plates were pressed, washed in 0.1 M saline and distilled water, pressed again and dried at 60°C. Finally they were stained with Coomassie brilliant blue R (Lot 44 C-1631, Sigma) and decoloured (*Weeke*). The plates were photographically enlarged and a copy was made. For identification purposes, the precipitation peaks were numbered from right to left and the relative electrophoretic mobility for each precipitation peak calculated in relation to the distance of a characteristic precipitation peak from the centre of the application well.

For the comparison of antigens from different variants of M. paratuberculosis and for the investigations of cross-reactions with M. avium and BCG the following immunoelectrophoretic analysis was carried out:

1. Ten μl antigens from strain 2E were run in the first dimension. In the second dimension the intermediate gel contained 20 μl/cm^2 of saline and the reference gel 20 μl/cm^2 of the homologous antiserum. This system was used as reference system in the investigations.

2. Ten μl antigens from each of the other 3 laboratory strains were run in CIE against the reference serum with saline in the intermediate gel. The reference system was run with antigens from the 3 last-mentioned laboratory strains replacing saline in the intermediate gel (Crossed-line immunoelectrophoresis, CLIE). Concentrations of 20, 40 and 80 μl/cm^2 were used.

3. CIE was run with antisera against M.ptb.CN, M.ptb.OI, M. avium and BCG in the intermediate gel in the reference system. Different concentrations of antisera were used (10, 20 or 40 μl/cm^2). The concentration of the reference serum was as before).

Each run was repeated several times. Four plates were run simultaneously in the second dimension. The reference system with saline in the intermediate gel was always run for direct comparison of the precipitation peaks.

RESULTS

Reference system

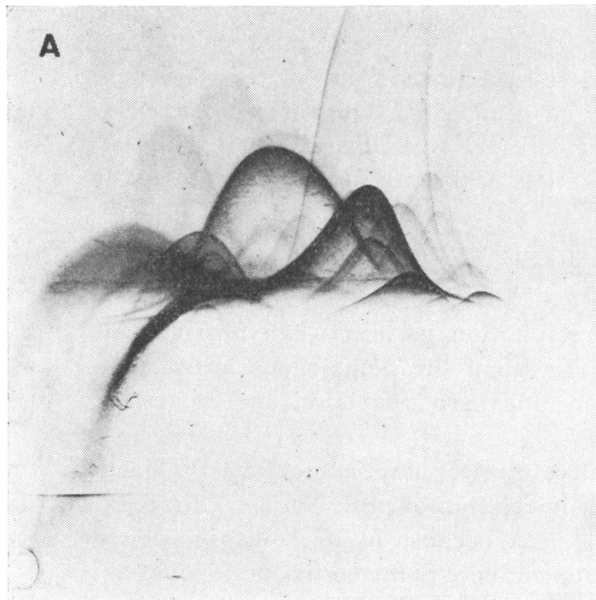
Figure 1 shows the precipitation pattern when antigens of strain 2E were run in CIE against the homologous antiserum. Altogether 44 precipitation lines are visible in the reference system.

Table 1 gives the relative electrophoretic mobility for each separate precipitation peak in relation to peak No. 20. This peak was chosen as a reference peak because of its constant occurrence and characteristic appearance. Some antigens gave weak precipitates and could be observed in only a few cases. When these in addition gave diffuse precipitation lines, it was difficult to draw them accurately. Relative electrophoresis mobility was therefore not calculated for such antigens (e.g. Nos. 1, 3, 6b, 28a and 38).

The relative mobility for each individual antigen was calculated from measurements made on plates from different days. The standard deviation shows relatively good reproducibility (Table 1). Variations in the relative mobility of some antigens are due to flat precipitation lines and consequent difficulty in finding distinct peaks as a basis for measurements (e.g. Nos. 2, 4, 8, 37, 40, 41 and 42). Antigen No. 34 did not show the characteristic bell-shaped precipitation pattern as did the other antigens. It was therefore impossible to calculate the electrophoretic mobility of this fraction.

The laboratory strains

The 4 laboratory strains all gave the same number of precipitation lines against the reference serum. Although quantitative measurements of the individual antigens were not carried out, a certain quantitative difference in concentration of the various antigens from the different strains seemed to exist. Especially obvious was the low concentration of antigen No. 20 in Teps and Str. 18. Strain 316F seemed to be identical to the reference strain 2E. In CLIE with antigen solutions from either strain



F i g u r e 1. Reference system for Mycobacterium paratuberculosis.

A Photograph of the precipitation pattern for antigens from strain 2E run in CIE against homologous rabbit antiserum. Saline in the intermediate gel.

B Drawing of the precipitation pattern in Fig. IA.

- I First dimension gel with strain 2E antigens.
- II Intermediate gel with saline.
- III Reference gel with antiserum against strain 2E.

Table 1. Relative electrophoretic mobility of antigens in the reference system.

Antigen No.	Relative mobility	s	Number of runs	Antigen No.	Relative mobility	s	Number of runs
1	N.P.	—		23	0.933	0.010	6
2	1.255	0.053	7	24	0.842	0.012	5
3	N.P.	—		25	0.835	0.011	6
4	1.245	0.039	7	26	0.834	0.020	6
5	1.225	0.034	6	27	0.818	0.011	7
6 a	1.224	0.038	6	28 a	N.P.	—	
6 b	N.P.	—		28 b	0.767	0.016	5
7	1.202	0.018	5	29	0.721	0.033	7
8	1.190	0.037	7	30	0.711	0.016	5
9	1.179	0.013	7	31	0.623	0.033	7
10	1.166	0.014	7	32	0.607	0.021	5
11	1.102	0.020	7	33	0.582	0.029	5
12	1.101	0.017	7	34	N.P.	—	
13	1.085	0.007	7	35	0.576	0.032	6
14	1.079	0.001	2	36	0.572	0.034	6
15	1.018	0.010	7	37	0.546	0.034	7
16	1.014	0.007	7	38	N.P.	—	
17	1.007	0.012	7	39	0.443	0.023	5
18	1.004	0.006	7	40	0.390	0.028	7
19	1.001	0.016	4	41	0.302	0.052	7
20	1.000	—		42	0.299	0.052	7
21	0.995	0.014	6				
22	0.960	0.008	7				

s: Standard deviation.
N.P.: Measurement for the calculation of relative mobility not carried out because of flat or diffuse precipitation lines.

316 F, Teps or Str. 18 in the intermediate gel in the reference system, all lines were either removed or raised to varying degrees (absorption of antibody in situ). This indicates identity of all antigens in the 4 strains. Example of this is given in Fig. 2 A that shows CLIE with antigens from strain 316F in the intermediate gel in the reference system.

Cross-reacting antigens

Figure 2 B, C and D shows the precipitation patterns when rabbit antiserum against M.ptb.CN, M.ptb.OI and M. avium, respectively, were added to the intermediate gel in the reference system. The results from these trials are summed up in Table 2.

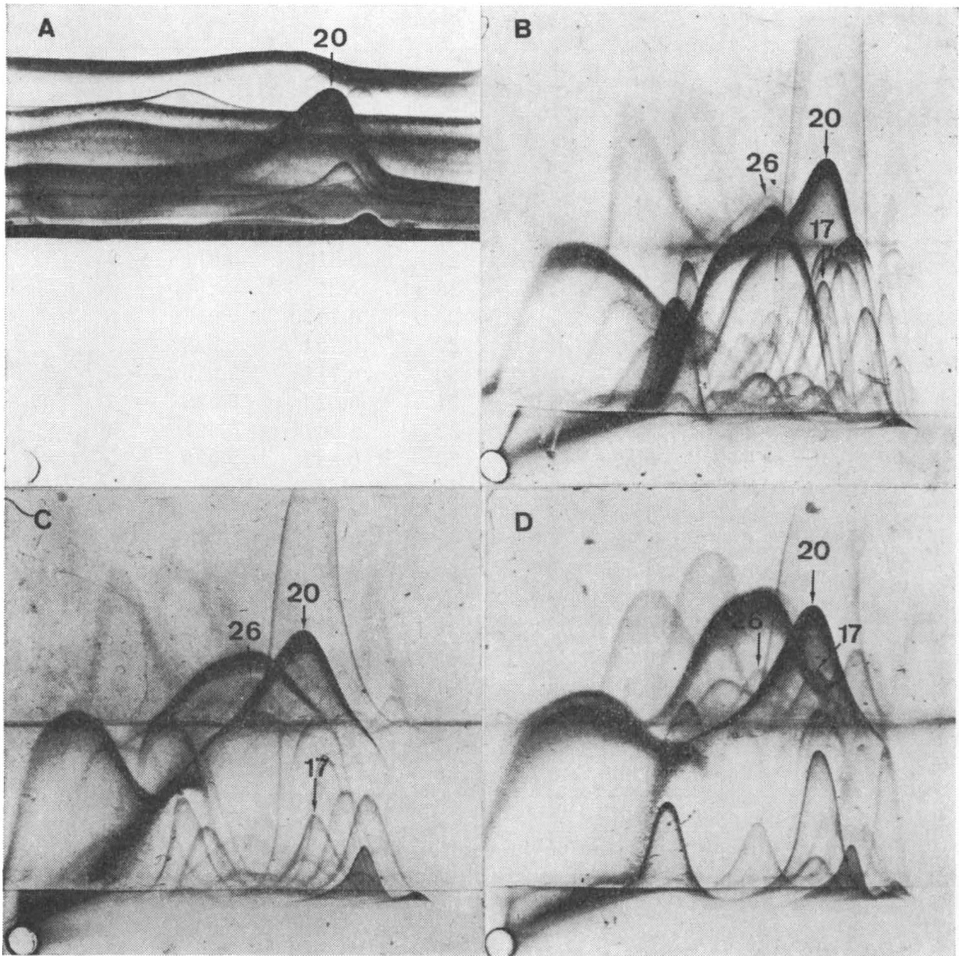


Figure 2. Crossed immunoelectrophoresis with intermediate gel of antigens from the reference strain 2E of Mycobacterium paratuberculosis against homologous rabbit antiserum in the reference gel.

A: Antigens from strain 316F in the intermediate gel (crossed-line immunoelectrophoresis).
B: Antiserum against strain M. ptb. CN in the intermediate gel.
C: Antiserum against strain M. ptb. OI in the intermediate gel.
D: Antiserum against Mycobacterium avium in the intermediate gel.

In A, 1 precipitate, the reference peak No. 20, is indicated. In this trial all of the precipitates of the reference pattern have been removed or elevated. Corresponding straight precipitate lines showing reaction of identity with the elevated reference precipitates are seen.

In B, C and D, the 3 precipitates, Nos. 17, 20 and 26, are indicated. Antigen No. 17 reacted with antisera against both the pathogenic strains of M. paratuberculosis examined, but not with antisera against M. avium. Antigen No. 26 did not react with any of these antisera (Cf. the reference pattern in Fig. 1).

Table 2. Cross-reaction between Mycobacterium paratuberculosis, strain 2E and M. paratuberculosis, strains CN and OI, M. avium and BCG employing crossed immunoelectrophoresis with intermediate gel.

M. ptb. 2E reference antigen No.	Antiserum in the intermediate gel				M. ptb. 2E reference antigen No.	Antiserum in the intermediate gel			
	anti-M. ptb. CN	anti-M. ptb. OI	anti-M. avium	anti-BCG		anti-M. ptb. CN	anti-M. ptb. OI	anti-M. avium	anti-BCG
1	?	?	=	?	22	=	=	=	?
2	=	=	=	=	23	=	=	=	= ?
3	=	?	=	?	24	=	=	÷	?
4	=	=	=	=	25	=	=	÷	=
5	=	=	÷	?	26	÷	÷	÷	÷
6a	=	=	?	=	27	=	÷	=	=
6b	=	?	?	?	28a	=	÷	?	?
7	=	=	=	=	28b	=	=	÷	?
8	=	=	=	?	29	=	?	?	=
9	=	=	=	?	30	=	=	?	=
10	=	=	=	= ?	31	=	÷ ?	÷	÷ ?
11	=	=	=	=	32	?	?	?	?
12	=	=	=	=	33	=	=	=	=
13	=	=	=	=	34	?	?	?	?
14	=	=	=	=	35	=	=	=	?
15	=	=	=	÷	36	=	=	=	=
16	=	?	÷	÷	37	=	=	=	=
17	=	=	÷	÷	38	=	=	=	=
18	÷ ?	÷ ?	÷	÷	39	=	÷	÷	=
19	=	=	=	?	40	=	=	=	=
20	=	=	=	=	41	=	=	=	=
21	=	=	=	=	42	=	=	=	=

? : Uncertain reaction. Precipitates either weak or completely lacking.
÷ : Cross-reaction not observed. = : Cross-reaction observed.

Of 44 different antigens in the reference system, 39 reacted with antiserum against M.ptb. CN, 31 with antiserum against M.ptb. OI, 27 with antiserum against M. avium and 24 with antiserum against BCG. In the last-mentioned trial reactions were somewhat uncertain as regards 2 antigens because of weak precipitation lines. Number of antigens in the reference system not reacting with antisera against M.ptb. CN, M.ptb. OI, M. avium and BCG were 2, 6, 10 and 6, respectively. However, as regards 1, 2 and 1 of the not reacting antigens in the trials with antiserum against, respectively, M.ptb. CN, M.ptb. OI and BCG, the results were somewhat uncertain because of weak precipitation lines. For a number of antigens precipitation lines were either

completely absent or so weak and diffuse that no conclusion could be drawn. This was the case for 3 antigens in the system with anti-M.ptb. CN in the intermediate gel, 7 in the system with anti-M.ptb. OI, 7 in the system with anti-M. avium and 14 in the system with anti-BCG in the intermediate gel. In the runs with anti-M.ptb. CN and anti-M.ptb. OI in the intermediate gel, 3 new precipitation lines appeared which could not be identified in the reference system.

Antigen No. 26 in the reference system was not found to react with any of the heterologous antisera used. It seemed therefore to be specific for the reference strain 2E. This is also probably true for antigen No. 18. For this antigen, however, it was difficult to find an exact base line in the reference system. Antigen No. 16 probably consists of 2 different fractions. Neither of them reacted with antiserum against M. avium or BCG. One of them reacted with antiserum against M.ptb. OI, while both reacted with antiserum against M.ptb. CN.

Antigen No. 20 showed marked cross-reaction with antiserum against the 2 pathogenic strains of M. paratuberculosis examined, but only very weak cross-reaction with antisera against M. avium and BCG in the form of the so-called "inward feet reaction".

Antigen No. 17 did not react with either anti-M. avium or anti-BCG serum, but did react with antisera against both the pathogenic strains of M. paratuberculosis.

DISCUSSION

Crossed immunoelectrophoresis seems to be a useful tool in the work for the characterization of the antigenic structure in different strains and species of mycobacteria. *Roberts et al.* (1972) recommend 1 fixed standard antiserum in the reference gel for this characterization. Based on the antigenic profile of each individual strain against the same antiserum, these authors found it possible to elaborate a simple taxonomy for all mycobacteria. However, detailed analysis of antigen profiles requires the use of homologous reference systems (*Closs et al.* 1975).

In the present study, it was decided to use antigens from sonicated bacterial cells, because antigens of this type have given good results in similar studies both on mycobacteria and other microbes (*Roberts et al., Closs et al., Høiby* 1975). While the

same antigen preparation was used for the immunization of rabbits, several antigen preparations were produced during the course of the investigation for use in CIE. Prepared under carefully standardized and controlled conditions, antigens giving very similar precipitation patterns will be obtained. Because of its poor growth characteristics, antigens from the Icelandic variant of M. paratuberculosis were prepared directly from infected intestine.

Certain individual differences in the ability of the various rabbits to produce precipitating antibodies were observed. This is in accordance with the experience of other investigators (*Axelsen* 1973, *Harboe & Ingild* 1973). These authors recommend the use of a somewhat larger number of rabbits than was the case during the present investigations. However, this is not considered to have had any great significance for the results obtained.

Due to a powerful resolution, CIE has proved to be the superior method used in antigen studies of mycobacteria. In the present reference system 44 different antigens are described. This is the largest number of antigens observed so far in M. paratuberculosis.

No important difference was found in the antigenic structure of the 4 laboratory strains investigated. This is interesting since all 4 strains are of bovine origin. The small quantitative differences seen in the concentration of some separate antigens in the 2 strains Teps and Str. 18, as compared with the reference strain 2E, are in accordance with similar observations dealing with closely related strains of other microbes (*Axelsen*).

The appearance of cross-reactions between 39 antigens from the laboratory strain 2E and the goat-pathogenic variant of M. paratuberculosis is in accordance with practical experience. Strains 2E and 316F have for several years been used to prepare antigens and for the production of vaccine against Johne's disease in Norway with good results.

The great number of cross-reacting antigens between M. paratuberculosis and M. avium and BCG are in agreement with previous findings (*Fischer* 1951, *Jensen* 1956, *Tuboly* 1965). Using Grabar and Williams' classic immunoelectrophoresis method, *Tuboly* identified 10 different antigens in M. paratuberculosis. Of these, 6 and 5 had common determinants with antigens from M. avium and BCG, respectively. Diagnostic observations also

indicate an antigenic relationship between these organisms. Typical examples are the strong reactions to avian and bovine tuberculin in animals suffering from Johne's disease. This fact has been a limiting factor as regards the use of vaccination in the control of Johne's disease in many countries.

Crossed immunoelectrophoresis with heterologous antiserum in the intermediate gel is a very sensitive method when investigating possible cross-reactions between various antigen fractions. In the present investigation problems sometimes arose as regards the identification of the lines in the intermediate gel. Varying the concentration of antiserum in the intermediate gel made the task easier, as some lines which precipitated right down at the base line at high antiserum concentration were only partly drawn into the intermediate gel at lower concentration. However, in the trials with antisera in the intermediate gel, it was sometimes impossible to identify as many precipitation lines as in the reference system with saline in the intermediate gel. The number of cross-reacting antigens between the reference strain and M. avium, BCG and the pathogenic strains of M. paratuberculosis examined may therefore be somewhat higher than shown in Table 2.

One of the aims of the present investigations was to identify species-specific antigens as regards M. paratuberculosis. Previously attempts have been made to isolate and prepare pure antigens from this organism, using chemical extraction agents such as phenol, alcohol and urea, often combined with chromatographic or other preparative methods. The various fractions obtained in this way have, however, proved to be deficient either as regards sensitivity or specificity (*Annau* 1959, *Rice et al.* 1959, 1960, *Yugi et al.* 1966 a, b).

One of the most interesting of the antigens described in the present investigation is antigen No. 17. This did not react with either anti-BCG or anti-M. avium serum, but did react with antisera against both the pathogenic strains of M. paratuberculosis investigated. Another characteristic antigen is antigen No. 20. This showed marked cross-reaction with antisera against the 2 pathogenic strains of M. paratuberculosis, but on the other hand only a very weak cross-reaction with M. avium and BCG. Antigen No. 16 seems to be partly species-specific. It probably consists of 2 different fractions. Neither of these reacted with antiserum against M. avium or BCG, while 1 of them reacted

with antiserum against M. ptb. OI and both with antiserum against M.ptb. CN. Antigen No. 26 and probably antigen No. 18 seem to be specific for the reference strain 2E as reactions with any of the heterologous antisera used were not found. As regards the antigen pattern in M. paratuberculosis, a further study of the immunological significance of the above-mentioned antigens would be of great interest.

In the present investigation only cross-reactions between M. paratuberculosis and 2 other mycobacterial species were studied. The results, however, stress the complicated antigenic situation in mycobacteria. This is of decisive significance as regards the diagnosis and classification of mycobacterial infections. Interesting in this connection are the atypical or anonymous mycobacteria. They are often assumed to be the cause of non-specific reactions met with when employing immunodiagnostic methods in relation to mycobacterial infections (*Fodstad* 1977). Antigens from strains of atypical mycobacteria isolated from wild animals give more than 20 precipitation lines in CIE with antiserum against both M. paratuberculosis and M. avium (*Fodstad & Gunnarsson* 1978, unpublished data). This emphasizes the need for careful analysis of the antigen structure in different mycobacterial species, in order to develop more specific diagnosis methods than those available at present.

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SAMMENDRAG

Analyse av antigener hos Mycobacterium paratuberculosis.

Ved hjelp av krysset immun-elektroforese (CIE) og krysset lineær immun-elektroforese (CLIE) er det utført sammenlignende undersøkelser av antigener fra forskjellige stammer og varianter av Mycobacterium paratuberculosis samt kryss-reaksjoner mellom disse og stammer av Mycobacterium avium og BCG. I hver av fire bovine laboratoriestammer av Mycobacterium paratuberculosis ble 44 ulike antigener påvist. Dette er det største antall antigener som hittil er beskrevet i stammer fra dette species. De fire laboratoriestammene brukes rutinemessig i produksjonen av vaksine mot paratuberkulose i Norge og Island. Et viktig siktepunkt i det foreliggende arbeid var derfor å undersøke det antigene slektskap mellom disse stammer og den geitepatogene norske og den islandske variant av M. paratuberculosis. Av de 44 påviste antigener i laboratoriestammene ga henholdsvis 39 og 31 kryssreaksjoner med den norske og islandske variant. Dette resultat er interessant sett på bakgrunn av de gode resultater som er høstet i vaksinasjonsarbeidet mot paratuberkulose hos geiter i Norge.

Det ble påvist 27 og 24 kryss-reagerende antigener mellom M. paratuberculosis og stammer av henholdsvis M. avium og BCG. Dette funn stemmer med kliniske observasjoner.

Et annet mål for de utførte undersøkelser var å påvise eventuelle spesifikke antigener for M. paratuberculosis. Resultatene viser at ett antigen ga markert kryss-reaksjon mellom de undersøkte stammer av M. paratuberculosis men ingen reaksjon med anti-sera mot M. avium og BCG.

De oppnådde resultater understreker de kompliserte antigene forhold i mykobakterier. En klargjøring av disse forhold vil være av avgjørende betydning for såvel sikker diagnostikk som klassifisering av mykobakterielle infeksjoner.

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