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The Transmission of Bovine Anti-*Brucella abortus* Agglutinins Across the Gut of Suckling Rats

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Summary. The transmission across the gut of suckling rats of several types of anti-*Brucella abortus* agglutinins separated from a bovine immune serum by electrophoresis, gel-filtration and chromatography has been investigated.

When the immune serum was subjected to immunoelectrophoresis a minor component distinct from the main classes of immunoglobulins was detected in the γ-region. The mean electrophoretic mobility of this component was considerably lower than that of slow γG. The anti-*B. abortus* agglutinins of the immune serum were tentatively identified with some of the immunoglobulins because of similarities in their electrophoretic and chromatographic behaviour. Sixty to 70 per cent of the antibody activity was due to γM agglutinins. Most of the remaining activity was due to fast γG agglutinins, and less than 5 per cent was due to more slowly migrating γ-globulins. The latter could be the slow γG, the minor slow component, or both.

Chromatographic preparations of the above three types of antibodies were fed to young rats which were killed and bled 4 hours later and their sera titrated for anti-*B. abortus* agglutinins. The γM agglutinins were not transmitted across the gut in detectable amounts. Transmission of the fast γG agglutinins was of a very low order. The slow γG or the minor slow component agglutinins were transmitted readily and were detected in the circulation as incomplete agglutinins. It was not possible to determine whether the incomplete agglutinins appearing in the circulation of fed rats were transmitted preferentially from the preparation administered or whether complete agglutinins suffered a change into the incomplete type during transmission.

The transmission of the agglutinins is discussed in relation to their physicochemical and immunochemical properties.

INTRODUCTION

In young rodents which receive most of their passive immunity from the mother after birth, milk proteins or proteins from serum administered to them by stomach tube are taken into the absorptive cells of the gut non-selectively by pinocytosis (Clark, 1959; Vacek, 1964). Only a small proportion of the absorbed protein is then transmitted into the circulation intact and most of this is γ-globulin; homologous γ-globulin is transmitted more readily than heterologous γ-globulins (Bangham and Terry, 1957; Hemmings, 1962 unpublished results). The proteins absorbed but not transmitted are broken down within the absorptive cells by enzymes which presumably reach the pinocytotic vesicles from

lysosomes when these structures fuse together. The presence of one γ-globulin in a mixture administered orally reduces the transmission of the others to an extent depending on its own species-specificity and on the species-specificity of the others (Morris, 1964). This effect is called interference. A hypothesis to account for selection and interference has been postulated by Brambell *et al.* (Brambell, Halliday and Morris, 1958; Morris, 1964, 1965). It was suggested that the walls of the pinocytotic vesicles of the absorptive cells of the gut contain receptors adapted to the homologous γ-globulin but capable of acting as receptors of heterologous γ-globulins according to the degree of their resemblance to the homologous γ-globulin. During transmission, γ-globulin molecules in the pinocytotic vesicles would be protected from proteolysis by becoming attached to receptors. Competition between γ-globulins for receptors would be the basis of selection and the blocking of receptors by attached γ-globulin would be the basis of interference.

The transmission of rabbit anti-*B. abortus* agglutinins across the gut of young rats has been investigated previously (Morris, 1965). When immune sera containing γG and γM agglutinins of the complete type were administered orally to young rats, γG agglutinins of the incomplete type appeared in their sera shortly afterwards. The qualitative change in the γG agglutinins on feeding, which took place during transmission across the gut, was attributed to a configurational change turning complete antibody into the incomplete type. Such a change would be tenable on the receptor hypothesis. The receptors are adapted to receive and to keep intact the Fc part of the γ-globulin molecule, while the remaining Fab parts which are the active sites in antibody molecules could still be susceptible to alteration. Susceptibility of the rabbit anti-*B. abortus* γG agglutinins to alteration during transmission in the young rat may have been a peculiarity of their type of immunoglobulin, of their species-specificity, or of anti-*B. abortus* agglutinins generally. The present investigation using a bovine anti-*B. abortus* serum was undertaken in an attempt to distinguish between these possibilities and to shed further light on the mechanism of transmission in general.

MATERIALS AND METHODS

Animals

The experimental animals were 12-day-old albino rats of a Wistar strain. They were fed with various serum protein preparations delivered from a tuberculin syringe directly into the stomach through a fine polythene cannula.

Antisera

Bovine anti-*B. abortus* serum (bovine immune serum) was supplied by Dr A. W. Stableforth. Gifts of antisera prepared in rabbits against bovine serum proteins were received from Dr W. A. Hemmings. These antisera reacted strongly against all the major bovine serum proteins as evidenced by the results of immunoelectrophoretic tests (Fig. 2) and were used also in the Coombs titrations for incomplete antibodies. Antisera against bovine serum protein preparations (DEAE-Sephadex A-50 peak I, or γ-globulins separated by Porath zone-electrophoresis) were also prepared in rabbits which received three fortnightly intramuscular injections of antigen in Freund's complete adjuvant (Difco). The rabbits were bled 7 days after the last injection. Adult albino rats provided antisera against bovine γ-globulin (Armour, Fraction II). They received a series of four fortnightly subcutaneous injections of antigen in adjuvant, were killed and bled 10 days after the last injection and their sera pooled.

Antibody titrations

Saline agglutinins were titrated by incubating two-fold serial dilutions of an antibody preparation in 0·9 per cent saline with equal volumes of antigen solution (525×10^7 formol-killed *B. abortus* organisms per millilitre) at 37° for 24 hours. Titres were expressed as the reciprocals of the highest dilutions showing agglutination of antigen. To estimate the proportion of the antibody activity due to 2-mercaptoethanol (2-ME) sensitive γM antibodies, the agglutinin titrations were also carried out in the presence of 0·05 M 2-ME (Fink, Miller, Dorward and LoSpalluto, 1962). Incomplete antibodies were titrated by a modification of the Coombs test (Jones, 1953), where serial dilutions of the antibody preparations were incubated with antigen for 1 hour at 37°; the sensitized antigen was then washed thrice with saline, resuspended in a 1:20 dilution of rabbit anti-bovine-serum-proteins serum, and incubated for 24 hours at 37°.

Concentration quotients

To compare the rates of transmission across the gut in young rats of antibodies from the bovine immune serum, or from fractions prepared from it, the results in feeding experiments were expressed as concentration quotients (CQ), which are the ratios of the titres in the experimental rat sera to the titres of the preparations administered.

Double-diffusion in agar-gel

The method of Ouchterlony (1964) was used with a 1 per cent solution of agar (Oxoid Ionagar No. 3) in 0·9 per cent saline containing 0·5 per cent sodium azide and 0·02 M, pH 7, phosphate buffer.

Immunoelectrophoresis

Grabar's (1964) method was followed using 1 per cent agar (Oxoid Ionagar No. 2) prepared in veronal buffer, pH 8·6, and ionic strength 0·025. Plates were electrophoresed for 2½ hours under a field of 5 V/cm, and developed with antiserum for 24 hours.

Zone-electrophoresis

Preparative electrophoresis of bovine immune serum was carried out according to the method of Porath (1956). Samples of 3 or 5 ml were exhaustively dialysed against 0·0115 M borate buffer, pH 8·5, and applied to an $85 \times 2{\cdot}5$ cm column of powdered cellulose equilibrated with the same buffer. Electrophoresis at 1000 V and 20 mA was continued for 60 hours at 5°. The same buffer was used for elution; eluate was monitored on an ultraviolet absorptiometer (LKB Uvicord Model 4701A) and then collected in 3 ml fractions in a Beaumaris fraction collector. The percentage transmittance of the eluate at 2537 Å was traced on a recorder (LKB Model 6520A).

Gel-filtration

The proteins of bovine immune serum were separated according to their molecular weights on Sephadex G-200 (Pharmacia, Uppsala, Sweden). Samples of 3 ml of serum were applied to the Sephadex which was equilibrated with 0·1 M tris, pH 8, in 0·2 M saline and contained in a $50 \times 3{\cdot}5$ cm column. The same buffer was used for elution and the eluate was collected as before.

Chromatography

Bovine immune serum was fractionated on columns of DEAE-Sephadex A-50 medium (Pharmacia, Uppsala, Sweden) according to a slightly modified method of Murphy and co-workers (Murphy, Aalund, Osebold and Carroll, 1964; Murphy, Osebold and Aalund, 1965). The chromatography medium (0·5 g/ml of serum sample) was equilibrated with 0·02 M phosphate buffer, pH 8, and serum samples were initially eluted from it with this buffer (150 ml for a 5-ml serum sample, 350 ml for a 20-ml serum sample) and then with a buffer of increasing molarity. The latter was delivered from a cylindrical reservoir equipped with a magnetic stirrer, containing initially 0·02 M buffer (30 ml/ml of serum sample) and into which an equal volume of 0·3 M buffer was siphoned from a second reservoir identical to the first. The column eluate was collected in a Beaumaris fraction collector and the transmittance of each fraction at 280 mμ was measured in a Unicam SP.500 Spectrophotometer. The salt molarity of the first and each succeeding tenth fraction was estimated from their electrical resistance measured on a Wheatstone bridge.

Concentration of protein solutions

Various fractions collected during zone-electrophoresis, gel-filtration or chromatographic fractionation of bovine immune serum were pooled and concentrated to the starting serum sample volume either by dialysis at 4° against a 5 per cent solution of carbowax in 0·9 per cent saline or against saline under negative pressure. Since serious losses were experienced with the first method it was subsequently replaced by the second.

RESULTS

ELECTROPHORETIC RESULTS

A sample of 5 ml of bovine immune serum was subjected to zone-electrophoresis. The distribution of protein and of anti-*B. abortus* activity in the electrophoretic fractions is shown in Fig. 1. It was estimated from the volumes and titres of the fractions that the loss of antibody activity during electrophoresis was negligible. The antibody activity showed a symmetrical distribution extending from the fast β- to the slow γ-region, with the saline titres equalling or exceeding the Coombs titres in the β- and fast γ-regions, and lower than the Coombs titres in the slow γ-regions. This indicated that a large proportion of the antibody activity in the electrophoretically faster fractions was attributable to γM agglutinins which are easily dislodged from the antigen during the washing procedure of the Coombs titrations (Morris, 1965).

Fractions 35, 40, 46 and 50 were concentrated to small volumes and subjected to immunoelectrophoresis and agar-gel precipitin tests (Figs. 2 and 3). The γG-globulins reacted with the rabbit anti-bovine serum-proteins serum as a single antigenic component having a continuous range of mobility from the fast β- to the slow γ-region. Their reaction with rat anti-bovine γ-globulin serum, however, indicated the existence of at least two antigenically distinct slow γG components. γM and γA were limited to fractions 35, 40 and 46.

The remaining electrophoretic fractions were pooled as shown in Fig. 1, and the ratio of the antibody activity of each pool relative to that of the initial sample of immune serum was, immune serum: pool E_1: pool E_2: pool E_3 = 5:1:2:2. Each pool was concentrated by dialysis against carbowax. Since serious losses in antibody activity were experienced with this method the pools were concentrated to smaller volumes than the

initial immune serum sample in order to maintain the above ratio. Volumes of 0·2 ml of each of the concentrated pools or of immune serum were fed separately to different young rats, which were killed and bled 4 hours later and their sera titrated (Table 1). Saline

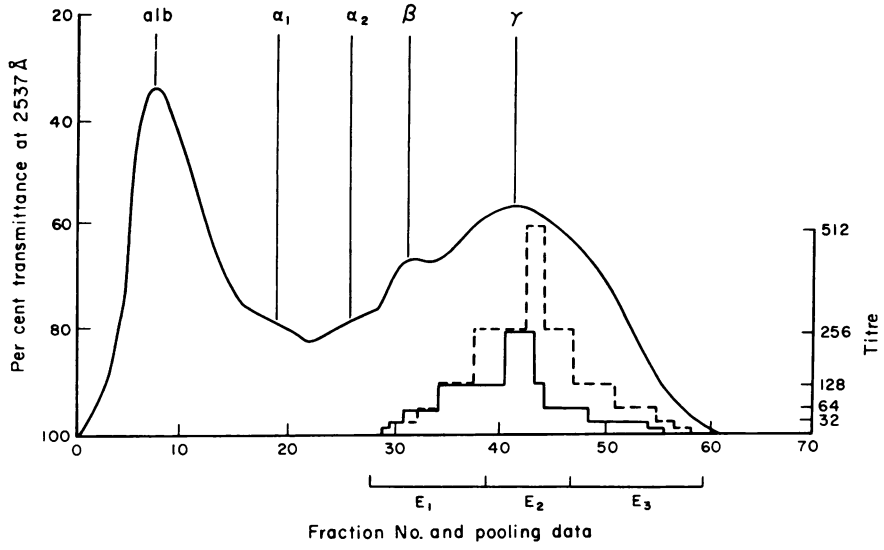

FIG. 1. Zone-electrophoresis of bovine anti-*B. abortus* serum. The histogram shows the saline (——) and Coombs (- - -) titres of the eluted fraction.

TABLE 1

THE RESULTS OF FEEDING YOUNG RATS WITH ELECTROPHORETIC FRACTIONS E_1, E_2 AND E_3 OF BOVINE ANTI-*B. abortus* SERUM

Serum or preparation tested	Anti-*B. abortus* titre		Concentration quotient	
	Saline	Coombs	Saline	Coombs
Bovine anti-*B. abortus*	2048	4096	—	—
Fraction E_1	1024	2048	—	—
Fraction E_2	1024	2048	—	—
Fraction E_3	512	1024	—	—
Sera of rats fed with bovine anti-*B. abortus*	<4(3)	8(2) 16(1)	<1/512	1/512 1/256
Sera of rats fed with E_1	<4(4)	<4(4)	<1/256	<1/512
Sera of rats fed with E_2	<4(4)	<4(4)	<1/256	<1/512
Sera of rats fed with E_3	<4(4)	4(1) 8(3)	<1/128	1/256 1/128

Figures in parentheses give the number of animals reacting.

agglutinins could not be detected in the sera of rats fed with immune serum, but incomplete agglutinins were present in low titres. The antibodies transmitted across the gut were evidently the minority characterized by the lowest electrophoretic mobilities, presumably the slow γG.

COMBINED GEL-FILTRATION AND ELECTROPHORETIC RESULTS

It has already been indicated that the antibodies in the bovine immune serum excluded from entering the circulations of fed rats were fast γG, γA and/or γM. The antibodies

associated wth the γM were separated from the others by subjecting the immune serum (3 ml) to gel-filtration on Sephadex (Fig. 4). It was estimated from the volumes and titres of the eluted fractions that about 67 per cent of the antibody activity of the immune serum was due to γM agglutinins. The eluted fractions were pooled as shown in Fig. 4, and

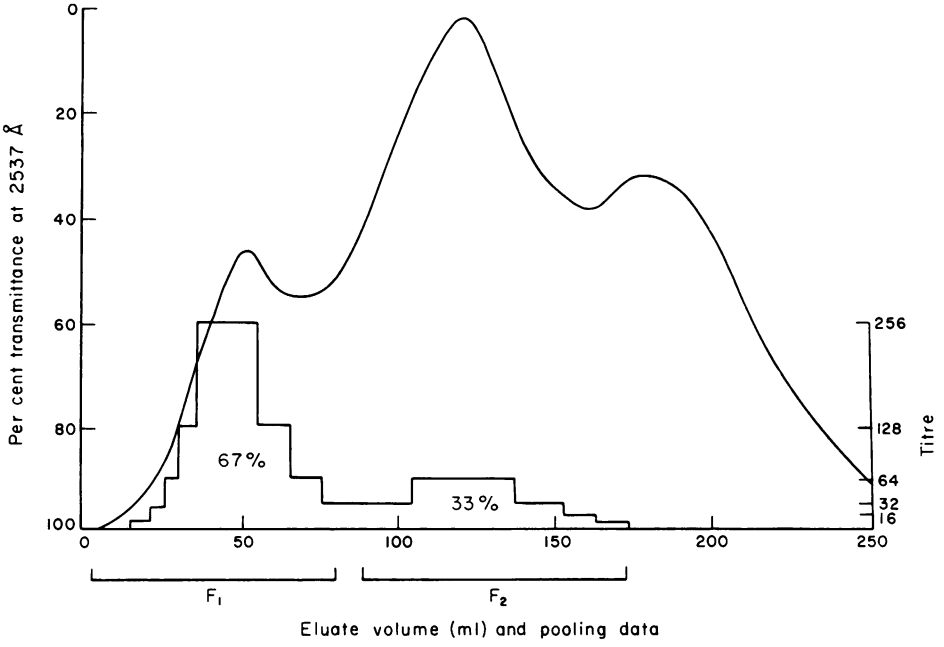

FIG. 4. The chromatography of bovine anti-*B. abortus* serum on Sephadex G-200. The histogram shows the saline titres of the effluent fractions.

TABLE 2

THE RESULTS OF FEEDING YOUNG RATS WITH FRACTIONS F_1 AND F_2 OF BOVINE ANTI-*B. abortus* SERUM SEPARATED ON SEPHADEX G-200

Serum or preparation tested	Anti-*B. abortus* titre		Concentration quotient	
	Saline	Coombs	Saline	Coombs
Bovine anti-*B. abortus*	4096	8192	—	—
Fraction F_1	4096	2048	—	—
Fraction F_2	2048	4096	—	—
Sera of rats fed with bovine anti-*B. abortus*	<4(6)	8(1) 16(5)	<1/1024	1/1024 1/512
Sera of rats fed with F_1	<4(6)	<4(6)	<1/1024	<1/512
Sera of rats fed with F_2	<4(6)	8(2) 16(4)	<1/512	1/512 1/256

Figures in parentheses give the number of animals reacting.

each pool was concentrated by pressure dialysis to volumes of 3 ml. Each concentrated pool (F_1 and F_2) and bovine immune serum were then fed separately to different young rats at doses of 0·3 ml. The anti-*B. abortus* titres of the rats' sera 4 hours later are given in Table 2. Evidently the γM agglutinins of the immune serum were not transmitted across the gut in detectable amounts.

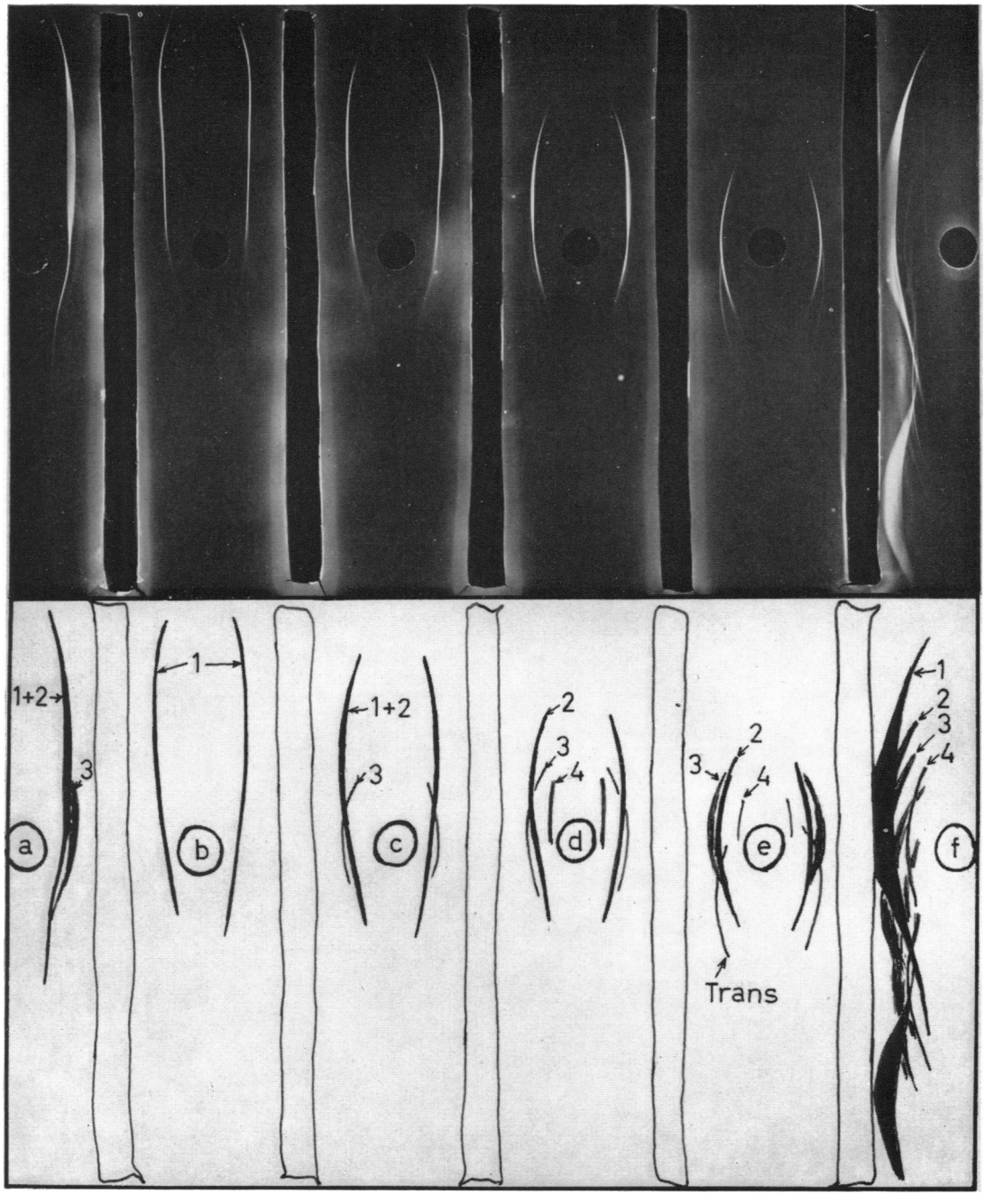

FIG. 2. The immunoelectrophoresis of: (a) Armour bovine γ-globulin, (b–e) electrophoretic fractions 50, 46, 40, and 35 respectively of bovine anti-*B. abortus* serum (see Fig. 1), (f) bovine anti-*B. abortus* serum. The troughs contained rabbit anti-bovine serum-proteins serum. 1, Slow γG; 2, fast γG; 3, γA; 4, γM; Trans, transferrin.

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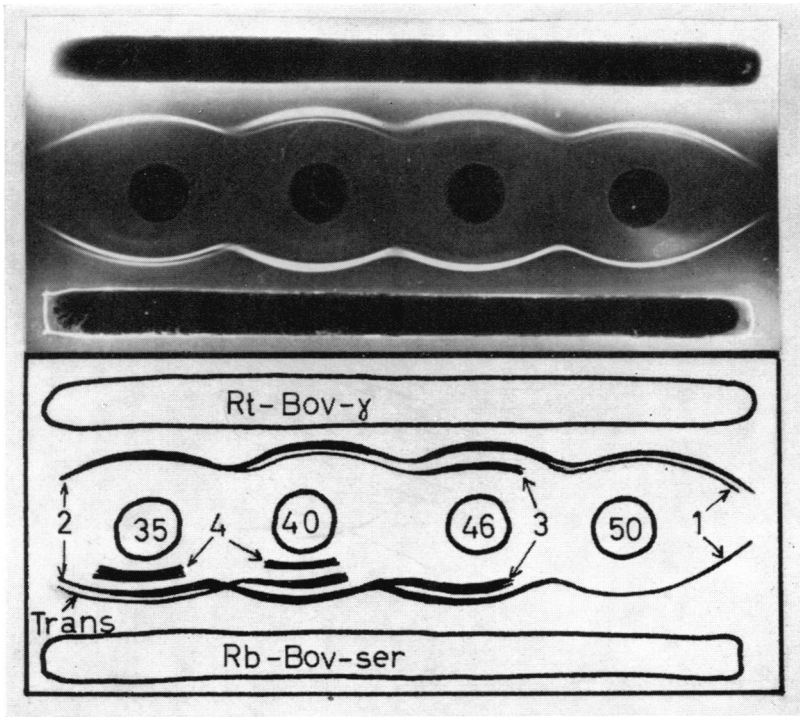

FIG. 3. Agar-gel precipitin test of electrophoretic fractions 35, 40, 46 and 50 of bovine anti-*B. abortus* serum (see Fig. 1). The troughs contained rat anti-bovine γ-globulin serum (Rt-Bov-γ) or rabbit anti-bovine serum-proteins serum (Rb-Bov-ser). 1, Slow γG; 2, fast γG; 3, γA; 4, γM; Trans, transferrin.

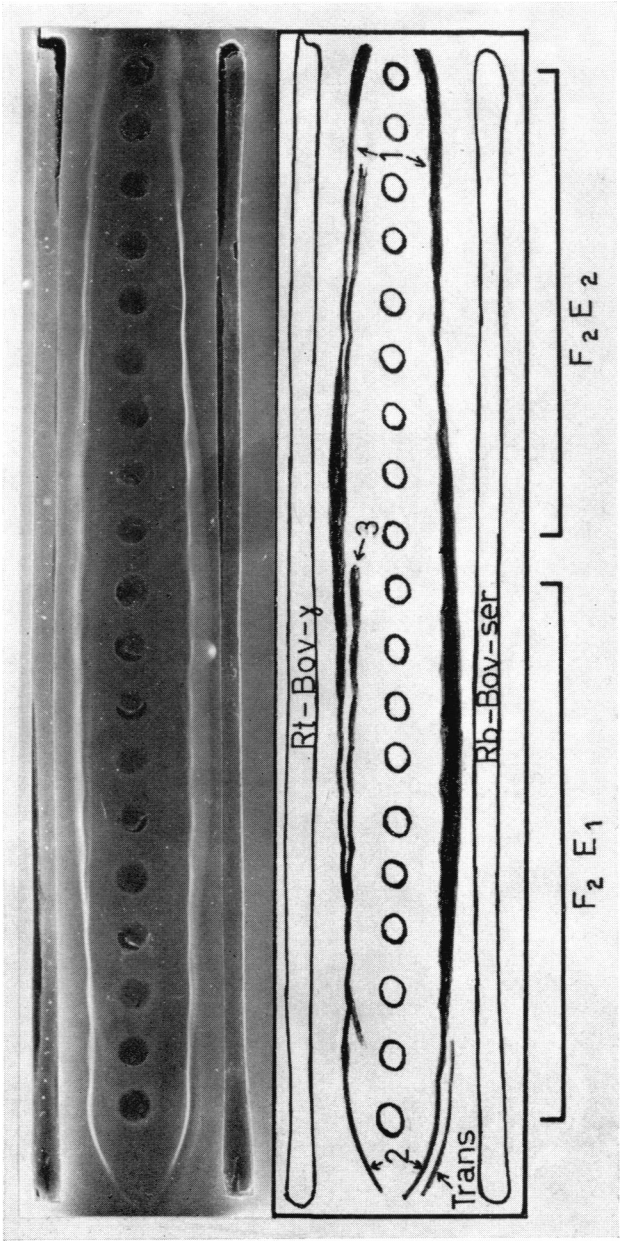

FIG. 6. Agar-gel precipitin test of the alternate electrophoretic fractions 21–57 of the F2 preparation of bovine anti-*B. abortus* serum (see Fig. 5). The troughs contained rat anti-bovine γ-globulin serum (Rt-Bov-γ) or rabbit anti-bovine serum-proteins serum (Rb-Bov-ser). 1, Slow γG; 2, fast γG; 3, γA; Trans, transferrin.

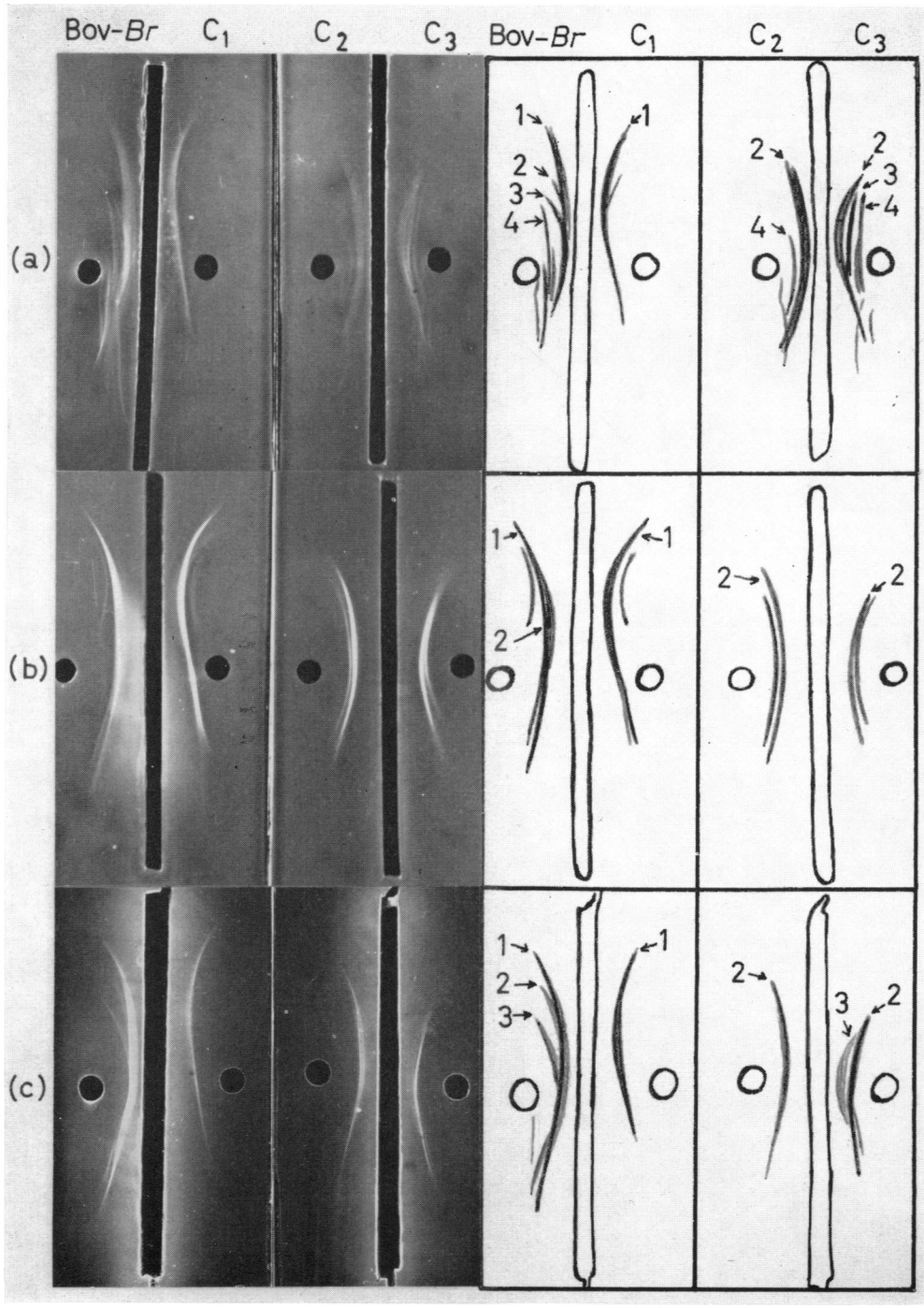

FIG. 8. Immunoelectrophoresis of bovine anti-*B. abortus* serum (Bov-*Br*), and of C_1, C_2 and C_3. The troughs contained: (a) a rabbit antiserum against a Porath zone-electrophoresis preparation of bovine γ-globulins, (b) a rabbit antiserum against C_1, or (c) a pooled rats' antiserum against Armour bovine γ-globulin. 1, Slow γG: 2, fast γG; 3, γA; 4, γM.

This fractionation of bovine immune serum was repeated, and the concentrated pool F_2 was subjected to zone-electrophoresis (Fig. 5). The distribution of antibody activity in the eluted fractions lacked symmetry and showed a gradual tailing off to the slow γ-region. An agar-gel precipitin test of the antibody reactive fractions (Fig. 6) indicated the presence of γG in all, whereas the γA was confined to the electrophoretically faster fractions.

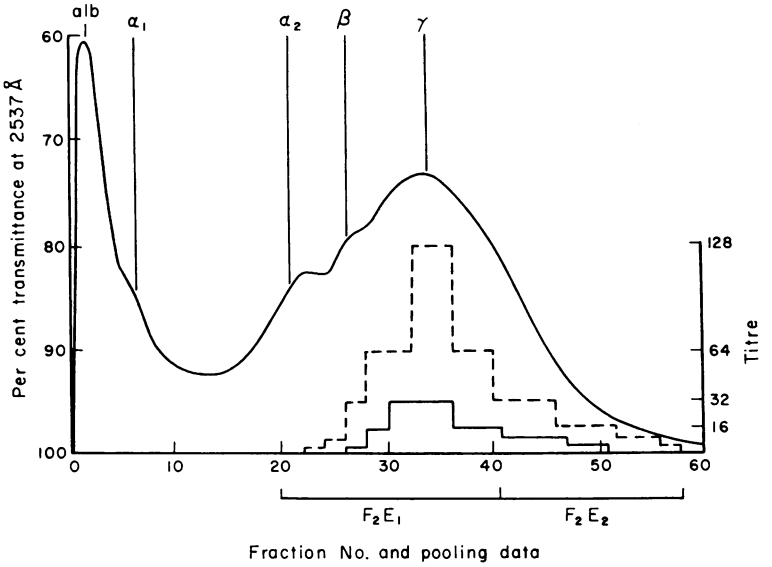

FIG. 5. Zone-electrophoresis of the gel-filtration fraction F_2 of bovine anti-*B. abortus* serum (see Fig. 4). The histogram shows the saline (——) and Coombs (- - -) titres of the effluent fractions.

TABLE 3

THE RESULTS OF FEEDING YOUNG RATS WITH THE F_2E_1 AND F_2E_2 PREPARATIONS SEPARATED FROM BOVINE ANTI-*B. abortus* SERUM BY GEL FILTRATION AND ELECTROPHORESIS

Serum or preparation tested	Anti-*B. abortus* titre		Concentration quotient	
	Saline	Coombs	Saline	Coombs
Bovine anti-*B. abortus*	4096	8192	—	—
Fraction F_2E_1	1024	4096	—	—
Fraction F_2E_2	512	2048	—	—
Sera of rats fed with F_2E_1	<4(4)	<4(4)	<1/256	<1/1024
Sera of rats fed with F_2E_2	<4(4)	8(4)	<1/128	1/256
Sera of rats fed with normal bovine serum	<4(4)	<4(4)	—	—

Figures in parentheses give the number of animals reacting.

The slow γG again reacted with rabbit anti-bovine serum-proteins serum as a single antigenic component, but with the rat anti-bovine γ-globulin serum as two antigenically distinct components. The eluted fractions were pooled as shown in Figs. 5 and 6, so that pool F_2E_2 lacked γA. Each pool was concentrated to the original volume of 3 ml and fed separately to young rats in doses of 0·2 ml. The results (Table 3) again show that the slow γG but not the fast γG or γA agglutinins of the bovine immune serum were transmitted in detectable amounts across the gut.

CHROMATOGRAPHY RESULTS

Murphy *et al.* (1964, 1965) successfully separated the slow and fast γG of bovine whey and serum from each other, and from the γA and γM, by chromatography on DEAE-Sephadex A-50. Their method, with slight modifications, was used to fractionate a 5-ml sample of bovine immune serum (Fig. 7). The eluted fractions were titrated against *B. abortus* in saline and in the presence of 2-ME, and by the Coombs technique. The distribution of antibody activity showed three peaks, the first representing 3 per cent, the second 40 per cent, and the third 57 per cent of the total activity. The bulk of the antibody activity of the third peak was due to 2-ME-sensitive γM agglutinins.

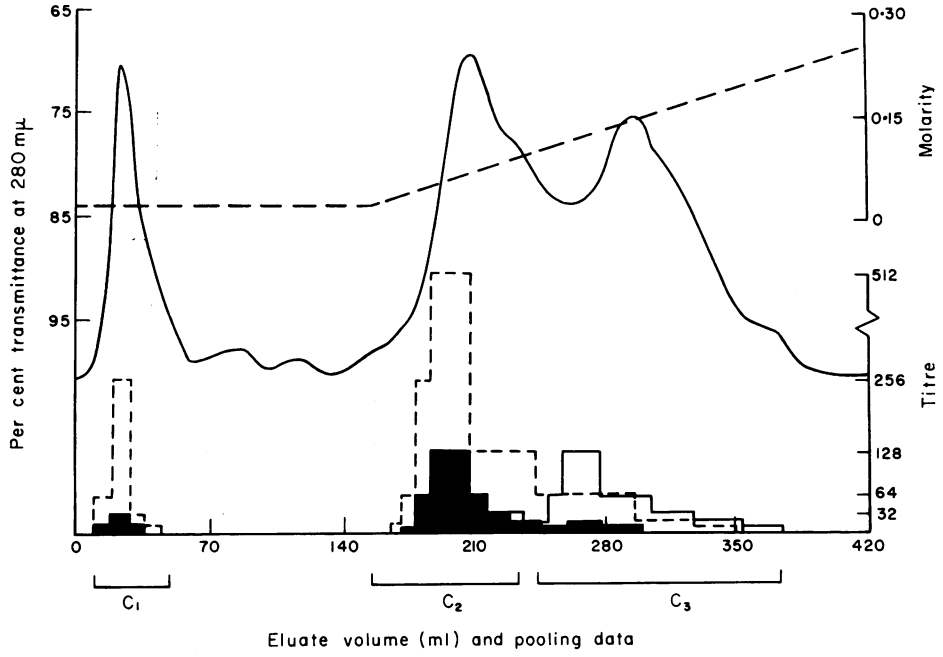

FIG. 7. The chromatography of bovine anti-*B. abortus* serum on DEAE-Sephadex A-50. The histogram shows the saline (solid black plus solid line), 2-ME (solid black) and Coombs (broken line) titres of the effluent fractions.

The chromatographic fractionation of bovine immune serum was repeated on a larger sample (20 ml), when the elution pattern was very similar to that obtained previously. Fractions containing the three eluted peaks of antibody activity were combined separately, as indicated in Fig. 7 for convenience, and concentrated to volumes of 5 ml. The anti-*B. abortus* titres of the concentrated pools (C_1, C_2 and C_3) are given in Table 4. The Coombs titre of C_1 exceeded the saline titre consistently in titrations, so that the preparation may have contained an appreciable amount of incomplete agglutinins. Most of the activity of C_2 was due to incomplete agglutinins, since the Coombs titre greatly exceeded the saline titre and a prozone lacking agglutination of antigen was evident in the saline titration. Treatment of C_3 with 2-ME reduced the saline titre drastically. Evidently most of the antibody activity was due to γM agglutinins. The activity remaining after the reduction of the γM could conceivably have been due to γA agglutinins.

Preparations C_1, C_2 and C_3 were diluted 1:4 with the veronal buffer used in immunoelectrophoresis and subjected to electrophoresis. The electrophoretic plates were developed with an antiserum from a rabbit injected with a Porath zone-electrophoresis preparation of bovine γ-globulins, an antiserum from a rabbit injected with C_1, or a pooled

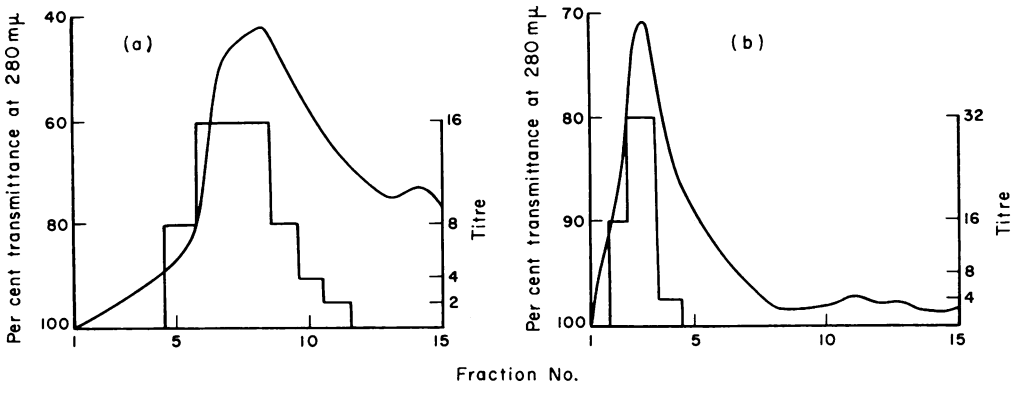

FIG. 9. The chromatography of: (a) 20 ml, or (b) 5 ml sample of bovine anti-*B. abortus* serum on DEAE-Sephadex A-50, showing the elution of the first peak protein (curve) and saline agglutinins (histogram).

TABLE 4

THE RESULTS OF FEEDING YOUNG RATS WITH THE C_1, C_2 AND C_3 PREPARATIONS SEPARATED FROM BOVINE ANTI-*B. abortus* SERUM BY CHROMATOGRAPHY ON DEAE-SEPHADEX A–50

Serum or preparation tested	Anti-*B. abortus* titre			Concentration quotient	
	Saline	Coombs	2-ME	Saline	Coombs
Bovine anti-*B. abortus*	4096	8192	2048	—	—
Bovine normal serum	4	8	—	—	—
Fraction C_1	512	1024	256	—	—
Fraction C_2	4096	32768	2048	—	—
Fraction C_3	8192	4096	256	—	—
Sera of rats fed with bovine anti-*B. abortus*	<4(6)	8(1) 16(3) 32(2)	—	<1/1024	1/1024 1/512 1/256
Sera of rats fed with bovine normal serum	<4(4)	<4(4)	—	—	—
Sera of rats fed with C_1	<2(6)	8(1) 16(4) 32(1)	—	<1/256	1/128 1/64 1/32
Sera of rats fed with C_2	<2(6)	8(1) 16(4) 32(1)	—	<1/2048	1/4096 1/2048 1/1024
Sera of rats fed with C_3	<2(6)	<4(6)	—	<1/4096	<1/1024

Figures in parentheses give the number of animals reacting.

antiserum from rats injected with Armour bovine γ-globulin (see Fig. 8). The protein of C_1 reacted with one of the rabbit antisera as a double antigenic component (Fig. 8b). A minor component, distinct from the classical slow γG, moved considerably slower under electrophoresis than the bulk of the proteins in this preparation. The anti-*B. abortus*

activity of C_1 could be associated with either or both of these components. The immunoglobulin of C_2, which was almost exclusively fast γG, reacted with the rabbit anti-C_1 serum as two antigenically distinct components having identical mobilities (Fig. 8b). It is possible that the antiserum was distinguishing between types of molecules having dissimilar light polypeptide chains. Some γM was also detected in C_2 (Fig. 8a). The γM and γA of the bovine immune serum were concentrated in C_3 (Fig. 8a and c), although much of the immunoglobulin in this preparation was fast γG. All the γ-globulin seemed to be reacting as doubly antigenic components against the rabbit anti-bovine γ-globulin serum (Fig. 8a).

During the chromatography of bovine immune serum, the first peak agglutinins were eluted more rapidly than the bulk of the protein representing this peak (Fig. 9). It appeared that most of the antibody activity was associated with a type of γ-globulin which formed a minor part of the first elution peak protein.

Preparations C_1, C_2 and C_3, and bovine normal and immune sera were fed separately to young rats at doses of 0·3 ml. The anti-*B. abortus* titres of the rats' sera 4 hours later are given in Table 4. The results confirm the previous conclusion that the antibodies of the bovine immune serum which were transmitted in appreciable quantities across the gut of fed rats were mostly those characterized by the lowest electrophoretic mobilities. The antibodies appearing in the circulation, however, were incomplete agglutinins. Incomplete agglutinins of the fast γG class were also transmitted, but to a markedly lesser extent.

DISCUSSION

The antibodies of the bovine anti-*B. abortus* serum used in this investigation have been tentatively identified with some of the immunoglobulins from similarities in their electrophoretic and chromatographic behaviour. Definite identification of the antibodies failed, since they could not be eluted from sensitized antigen in sufficient quantities for detailed immunoelectrophoretic studies.

The main γ-globulin classes of mammalian sera are the γG, γA and γM. In guinea-pig (Benacerraf, Ovary, Bloch and Franklin, 1963), sheep (Silverstein, Thorbecke, Kraner and Lukes, 1963; Aalund, Osebold and Murphy, 1965) and mouse (Fahey, Wunderlich and Mishell, 1964) the γG separates into two distinct immunoelectrophoretic arcs, fast γG (7S γ_1) and slow γG (7S γ_2), which represent components having common and distinct antigenic determinants in their heavy polypeptide chains. Great heterogeneity exists amongst these main classes, notably in the slow γG, of which sub-classes differing in the antigenicity of their heavy chains have been found in man, mouse and horse (for references see Fleischman, 1966; Lichter and Drey, 1964). In man each γ-globulin class and sub-class can be divided into two groups according to the antigenic type of light chain (K or L) in the globulin molecule. γ-Globulins of each main class have been identified provisionally in bovine serum (Murphy *et al.*, 1965). Murphy *et al.* also separated the γG of bovine serum into fast and slow components by chromatography on DEAE-Sephadex. These components probably correspond to the fast γG and slow γG of other species. Although the work of Murphy *et al.* provided no direct evidence for heterogeneity within these classes, the Fab and Fc fragments of these components were chromatographically and immunoelectrophoretically complex. The present study provides immunoelectrophoretic evidence for the existence in bovine immune serum of at least two sub-classes or groups of fast γG and of slow γG.

When preparation C_1, which was separated chromatographically from bovine anti-

B. abortus serum and which corresponded to the slow γG of Murphy *et al.* (1965), was electrophoresed and diffused against a rabbit anti-C_1 serum, two distinct arcs appeared in the slow γ-region. The main arc simulated the classical slow γG of serum, whereas the minor arc corresponded to an electrophoretically slower component. The latter was not developed by reaction with antisera prepared in a rabbit or rats injected with a mixture of bovine γ-globulins. When preparation C_2, which corresponded to the fast γG of Murphy *et al*, was electrophoresed and diffused against rabbit anti-C_1 serum twin parallel arcs were developed in the fast γ-region. These arcs possibly represent groups of molecules having dissimilar light chains, analogous to the K and L chains in man. A similar duality of antigenicity was indicated amongst the other γ-globulins when C_1, C_2 and C_3 were electrophoresed and diffused against the rabbit anti-bovine γ-globulins serum. This heterogeneity amongst the bovine γ-globulins renders the identification of the anti-*B. abortus* agglutinins of the bovine immune serum used more difficult.

The behaviour of the anti-*B. abortus* agglutinins of the bovine immune serum under chromatography and gel-filtration, and their extreme susceptibility to reduction by 2-ME at neutral pH, indicated that most of them (60–70 per cent) were γM agglutinins. These antibodies were not transmitted in significant quantities across the gut of young rats. This is in agreement with previous results showing that the corresponding antibodies of rabbit origin were not transmitted (Morris, 1965).

Very little if any of the anti-*B. abortus* activity of the bovine immune serum could be attributed to γA agglutinins, so that the transmission of this protein across the gut remains to be elucidated.

The remainder of the anti-*B. abortus* activity of the bovine immune serum was mostly due to fast γG agglutinins, and it appeared from the results of saline and Coombs titrations of these antibodies that a large proportion of them were incomplete agglutinins. The transmission of these antibodies across the gut of young rats was of a very low order. Less than 5 per cent of the total antibody activity of the immune serum was found in the serum fraction C_1 eluted from DEAE-Sephadex by the buffer of lowest molarity. The antibody activity of this fraction was eluted more rapidly than the bulk of the protein. Since the speed of elution of the γG globulins from DEAE-Sephadex varies inversely with their electrophoretic mobilities (Murphy *et al.*, 1965), much of the antibody activity of C_1 may be associated with the minor protein component having the lower mean electrophoretic mobility. The Coombs titre of C_1 was consistently higher than the saline titre, so that an appreciable part of the antibody activity must have been due to incomplete antibodies. When young rats were fed with C_1, incomplete agglutinins appeared in their circulations at relatively high CQ values.

In a previous investigation of the permeability of the young rat gut to anti-*B. abortus* agglutinins of rabbit origin, it was found that γG complete agglutinins were transmitted very much more readily than γM agglutinins. Since the electrophoretic mobilities and the molecular size of these agglutinins were interrelated, it was impossible to determine which of these parameters, if any, was related to transmission. It was suggested, however, that since the γG agglutinins from different rabbits were transmitted to different extents, transmission may have depended on the presence of genetically determined transmission sites within the molecules analogous to or identical with the sites which confer antigenicity. The possibility of distinguishing definitely between these alternatives in the light of the present results is precluded by the inability to identify the anti-*B. abortus* agglutinins of the bovine immune serum. However, two possibilities are apparent: (a) The antibodies which

were transmitted across the young rat gut were mainly the classical slow γG agglutinins. These differ from the fast γG agglutinins (which were not transmitted) mainly in their electrophoretic mobility. They are similar in molecular weight and, together with their papain digestion fragments, have a major degree of common antigenicity (Murphy *et al.*, 1965). (b) The antibodies which were transmitted were associated with the minor component of C_1. The peculiarities which set this component apart from the other slow γG globulins are so slight that its detection by ultracentrifugation or immunoelectrophoresis using a variety of antisera escaped Murphy *et al.* The parameters distinguishing this component most clearly from the fast γG is, again, its electrophoretic mobility.

It is unlikely from these observations that the molecular size of the bovine agglutinins is related to their transmission across the gut. The agglutinins transmitted are characterized by a very low electrophoretic mobility. Bangham and Terry (1957) found that very little albumin from homologous or heterologous sera was transmitted intact across the gut of suckling rats, whilst the globulins transmitted were mainly γ-globulins; the homologous γ-globulins were transmitted preferentially. The part played, if any, by the net charge of the protein molecules in their transmission across the gut is difficult to visualize, unless a gross selection between the serum proteins is effected by a differential attraction of the molecules to the walls of the pinocytotic vesicles of the absorptive cells (see 'Introduction'), which could occur if these surfaces were predominantly negatively charged. Selection between γ-globulins according to their species of origin must be due to more subtle differences in the molecules. Possibly these differences involve relatively small areas of the polypeptide chains of the Fc fragment, for Weir, Porter and Givol (1966) have shown that the C-terminal octadecapeptides of these fragments derived from the γG-globulins of widely different species resemble each other as closely as those derived from γG sub-classes of a single species. According to these authors these similarities will undoubtedly extend much further from the C-terminal end of the chain.

When rabbit anti-*B. abortus* sera containing complete γG agglutinins were fed to young rats, incomplete γG agglutinins appeared in their circulations (Morris, 1965). The incomplete antibodies appearing in the sera of fed rats partially reverted to the complete type on gel-filtration and osmotic concentration. It is impossible to determine from the present results whether the incomplete agglutinins appearing in the circulation of young rats fed with bovine immune serum were absorbed selectively and unchanged from the doses administered, or whether complete agglutinins suffered a change into incomplete types during transmission. The *B. abortus* agglutinin titre of the pooled serum of fed young rats was so low that, following chromatography on DEAE-Sephadex, the eluted fractions even after concentration did not react with *B. abortus* antigen, thus precluding comparison with the results for the rabbit immune sera.

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