

Identification and Quantitation of Equine Serum and Secretory Immunoglobulin A

T. C. McGUIRE AND T. B. CRAWFORD

Department of Veterinary Pathology, Washington State University, Pullman, Washington 99163

Received for publication 24 February 1972

Immunoglobulin A (IgA) was demonstrated in equine serum and secretions. This immunoglobulin had a molecular weight extending from 150,000 to 700,000 and reacted with specific antihuman alpha-chain antiserum. Antigenic determinants specific for secretory IgA were demonstrated and found to be absent on serum IgA. Antigen binding activity was detected in IgA from tears. Purified IgA was antigenically distinct from equine IgG, IgM, IgG(T), and aggregating immunoglobulin. Quantitative studies demonstrated that IgA was the predominant immunoglobulin in tears and milk but not in colostrum. The electrophoretic mobility, size, presence of secretory component, and reaction with specific antihuman alpha-chain antiserum demonstrates that this immunoglobulin is the equine homologue of human IgA.

Eight antigenically distinct immunoglobulins have been identified in purified anti- β -lactoside antibody isolated from horse serum (8, 9, 17, 18). They were designated immunoglobulin Ga (IgGa), IgGb, IgGc, IgA (T component), IOS $_{\gamma 1}$, IgM, and two others with $\gamma 1$ mobility were not classified (17). An immunoglobulin of $\gamma 1$ mobility with heterogeneous sedimentation properties caused by salt-dissociable noncovalently-linked aggregates of 6.3S globulin has been isolated from hyperimmune, horse antipneumococcal sera (21). Subsequent workers used the designation aggregating immunoglobulin (AI) (31) $\gamma 1$ component (5) IgB (20) and demonstrated that it was antigenically distinct from IgG, IgM, and IgG(T). The relationship between AI and the $\gamma 1$ antilactoside antibodies described by Rockey (17) is not clear. In the past, a number of workers classified the T protein of horse diphtheria and tetanus antitoxin (22) as IgA (6, 8, 16, 17). Weir et al. (29), however, demonstrated partial homology between the C-terminal amino acid sequences of the heavy chains of T protein and IgG. Antigenic studies demonstrated identity of Fab fragments and light chains of T protein and IgG and also that some cross-reaction occurred with the Fc fragments (28). On the basis of these studies, it was concluded that horse T protein represented an IgG subclass rather than an equivalent of IgA, and this conclusion suggested the designation of IgG(T) (29).

Audibert and Sandor (2) isolated an immunoglobulin from horse colostrum and milk which they considered to be IgA, based on electropho-

retic mobility and carbohydrate content. In contrast, Genco et al. (4) examined the immunoglobulins of horse parotid fluid and colostrum and found no evidence for a secretory immune system in the horse. Rouse and Ingram (19) examined horse colostrum and milk and found no evidence for a selective secretion of $\gamma 1$ immunoglobulin. Hurlimann and Darling found that salivary glands in vitro synthesized an immunoglobulin which appeared analogous to IgA but did not react with antihuman IgA (7). Vaerman et al. (27) identified an immunoglobulin in horse serum and milk which showed an immunological cross-reaction with antihuman alpha-chain antisera. Additional work demonstrated that the size and electrophoretic mobility of this immunoglobulin was similar to human secretory IgA and that it was the predominant immunoglobulin produced in the lamina propria of the equine intestine (27; J. P. Vaerman, Ph.D. thesis, Université Catholique de Louvain, Belgium, 1970). The purpose of this investigation was to examine quantitatively and qualitatively the immunoglobulins of equine serum and secretions in order to further establish and characterize the IgA secretory system.

MATERIALS AND METHODS

Secretions. Intestinal washings, parotid salivary fluid, bronchial washings, and sweat were collected from mature horses. Salivary flow was stimulated by dilute acetic acid, and sweat was collected after vigorous exercise. Intestinal and bronchial secretions were washed out with phosphate-buffered saline (PBS)

at necropsy. The samples were centrifuged, and supernatant fluids were concentrated $\times 20$ by vacuum dialysis. Colostrum was obtained from a Shetland pony on the day of parturition and 3 days after parturition. Milk was taken at 16 and 182 days after parturition. Tears were collected from 10 mature Shetland ponies.

Isolation of immunoglobulins. Secretory IgA was purified from 20 ml of equine tears which were subjected to serial 50 and 45% saturated ammonium sulfate (SAS) precipitations. The precipitate was dissolved, equilibrated with 0.03 M sodium phosphate, pH 8.0, applied to a triethylaminoethyl (TEAE)-cellulose column, and eluted with the same buffer. After the initial protein peak, a sodium phosphate gradient of 0.03 M, pH 8.0 to 0.25 M, pH 4.0 was applied. Eluted fractions were evaluated by immunoelectrophoresis (IEP) against rabbit anti-whole tears. Those fractions containing an immunoglobulin of beta mobility were pooled, concentrated, and applied to a Sephadex G-200 column (2.5 \times 90 cm) equilibrated with 0.3 M NaCl and 0.01 M sodium phosphate, pH 7.4. Fractions of 4.5 ml were collected and examined by immunodiffusion and IEP.

IgG (12), IgM (31), and IgG(T) (12) were isolated from equine serum by techniques which have been described previously. Heavy and light chains from purified IgG were obtained by reduction, alkylation (17), and subsequent separation on Sephadex G-100 in 0.1 M acetate buffer, pH 5.

AI was purified from serum of a horse hyperimmunized with pneumococcal polysaccharide, type 3, obtained from David Axelrod, New York State Health Laboratories, Albany, N.Y. An euglobulin precipitate was prepared (31) and separated on a TEAE-cellulose column as described for the separation of IgA. Fractions containing AI were detected by IEP against antiequine whole serum and further purified by separation on Sephadex G-200 equilibrated with 0.6 M NaCl and 0.01 M sodium phosphate, pH 7.4.

Antisera. Two rabbits and one guinea pig were immunized with purified IgA. The first injection of 1 mg of IgA in 1 ml of complete Freund's adjuvant was given in the footpads and was followed 2 weeks later by an intramuscular injection of 1 mg of IgA in 1 ml of incomplete Freund's adjuvant. Two weeks after the second injection, four daily doses of 1 mg each were given intravenously, and the animals were bled 7 days after the last injection. Antisera to equine light chain and heavy chain of IgG, IgM, IgG(T), AI, equine whole tears, gamma globulin (precipitated with 40% SAS), and whole serum were produced in the same way. All antisera were evaluated initially by IEP. Antisera to IgA, IgM, IgG(T), and AI absorbed with IgG produced only one line with equine serum in immunodiffusion and IEP. These antisera were used in single radial immunodiffusion to quantitate immunoglobulin levels in serum and secretions (14). Immunoglobulins purified as described above were used as standards.

Six antihuman alpha-chain antisera were obtained from three different commercial sources. Only one reacted in immunodiffusion with equine serum and

purified IgA (obtained from Meloy Laboratories, Mel Labs, Inc., Biological Products Division, Springfield, Va., batch no. GHA05). Monospecific anti-equine IgM and AI ($\gamma 1$) was kindly supplied by Peter Allen, University of Rochester, Rochester, N. Y., for reference purposes.

Tears and sera were collected from two horses immunized with 2,4-dinitrophenol-bovine gamma globulin (DNP-BGG) prepared as described below. An initial subcutaneous injection of 75 mg of DNP-BGG in complete Freund's adjuvant was followed in 6 weeks by 200 mg intravenously over 2 consecutive days. Serum was collected at 2 and 7 weeks and tears were collected at 17 weeks after the initial injection.

Molecular weight determination. Preparations of purified equine IgA and samples of whole serum, tears, colostrum, and milk were chromatographed on columns of Sephadex G-200 (2.5 \times 90 cm) calibrated with protein standards of known molecular weights and elution characteristics as established by Andrews (1). The standards used were chymotrypsinogen, ovalbumin, bovine serum albumin, catalase, rabbit IgG, horse spleen ferritin, and blue dextran. Detection of the standards and purified IgA was by optical density at 280 nm. IgA in fractions from secretions and serum was detected by immunodiffusion of the fractions against a monospecific antiequine IgA antiserum.

Preparation and radiolabeling of DNP-BGG. DNP-BGG was prepared by using a previously described technique (3). The chloramine-T method of labeling with ^{125}I , as described by McConahey and Dixon (11), when applied to DNP-BGG was found to result in very low uptake of radioactive label. Consequently, for radioimmuno-electrophoresis, 5 mg of BGG was labeled with ^{125}I by using the chloramine-T method prior to addition of the DNP group. The radiolabeled BGG then reacted with dinitrobenzene sulfonate. Four mg of ^{125}I -BGG in a volume of 10 ml was dialyzed against 2% K_2CO_3 and 100 mg of DNB- SO_4 was then added to the protein. After incubation for 24 hr at 37 C, the DNP- ^{125}I -BGG was dialyzed free of unreacted DNB- SO_4 .

Radioimmuno-electrophoresis. Radioimmuno-electrophoresis (RIEP) was performed as described by Yagi et al. (30). Samples of serum or secretions were subjected to electrophoresis on agar-coated slides and reacted with antiequine serum. The slides were washed for 1 week in PBS and immersed in a 0.5- μg solution of radiolabeled DNP-BGG (specific activity, 0.13 $\mu\text{Ci}/\mu\text{g}$) per ml in PBS. After 2 hr of incubation at room temperature, the slides were washed for an additional week in PBS. They were then dried and stained with Amido Schwartz, and film was applied (Kodak medical X-ray film, RB-56). Exposure time ranged from 2 to 14 days.

RESULTS

An immunoglobulin with a beta mobility was purified from equine tears by SAS precipitation, TEAE-cellulose, and Sephadex G-200 column chromatography that gave a single line by IEP and immunodiffusion with antiequine serum and

tear antisera. This immunoglobulin reacted with antiequine light-chain antisera and antihuman alpha-chain antiserum in immunodiffusion. The purified equine IgA (Fig. 1) was antigenically distinct from equine IgG (Fig. 2), IgM (Fig. 3), IgG(T) (Fig. 4), and AI (Fig. 5) as determined by analysis in IEP with purified immunoglobulins and monospecific antisera.

Antisera from two rabbits and a guinea pig immunized with purified IgA reacted with only IgA and IgG in IEP. After absorption of these antisera with IgG, only one line against whole serum and secretions could be detected in immunodiffusion and IEP (Fig. 1). Monospecific antiequine IgA antiserum gave a line of identity with specific antihuman alpha-chain antiserum when the antisera were reacted with purified equine IgA (Fig. 6) and equine serum. An apparent line of identity occurred between equine serum and human serum when reacted with specific antihuman alpha-chain antiserum. The strength of the reaction with human serum was much greater than with equine serum, making interpretation and photography difficult. Only the guinea pig antiserum produced the characteristic spur of secretory component (SC) when reacted with equine serum and secretions (Fig. 7).

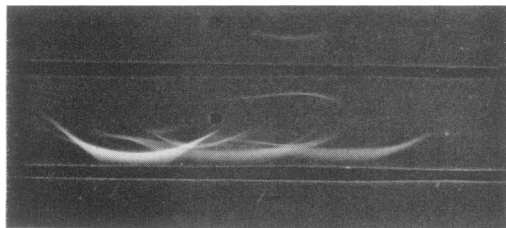


FIG. 1. The top and bottom wells contain purified IgA, and the middle well contains whole equine serum. Monospecific anti-IgA is in the top trough, and antiequine serum is in the bottom trough. The cathode is on the right side.

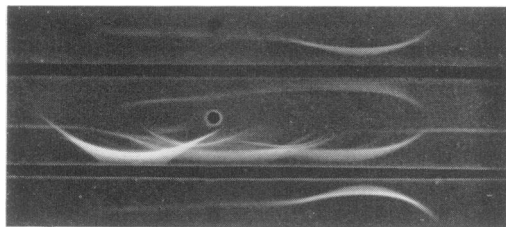


FIG. 2. The top and bottom wells contain purified IgG, and the middle well contains whole equine serum. Monospecific anti-IgG is in the top trough, and antiequine serum is in the bottom trough. The cathode is on the right side.

After this antiserum was absorbed with equine serum to produce specific antiserum to SC, no reaction occurred with serum, and only one line was obtained with most secretions (Fig. 7).

IgA was demonstrated in intestinal secretions, parotid salivary fluid, bronchial mucus, sweat, tears, colostrum, milk, and serum by immunodiffusion with monospecific antiequine antiserum.

DNP-BGG binding activity was demonstrated in the IgA arc of tears by RIEP. Tears collected 17 weeks after the initial injection of DNP-BGG were subjected to electrophoresis and developed with monospecific antiequine IgA. The IgA arc from anti-DNP-BGG tears contained specific

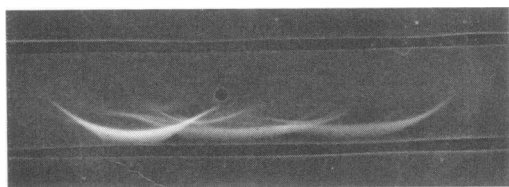


FIG. 3. The well contains whole equine serum. The top trough has monospecific anti-IgM, whereas the bottom well is filled with antiequine serum. The cathode is on the right side.

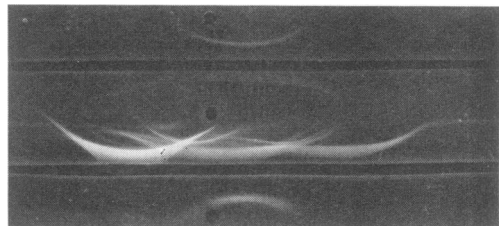


FIG. 4. The top and bottom wells contain purified IgG(T), and the middle well contains whole equine serum. Monospecific anti-IgG(T) is in the top trough, and antiequine serum is in the bottom trough. The cathode is on the right side.

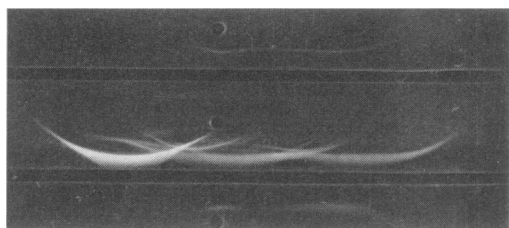


FIG. 5. The top and bottom wells contain purified, aggregating immunoglobulin (AI) and the middle well contains whole equine serum. Monospecific anti-AI is in the top trough, and antiequine serum is in the bottom trough. The cathode is on the right side.

binding activity for DNP-BGG. No activity was present in the IgA arc from nonimmunized horses.

A plot of the elution volumes and the log molecular weights of the standards on Sephadex G-200 gel filtration yielded a straight line. IgA eluted from the calibrated columns over a broad range, indicating heterogeneity in molecular size. The distribution of IgA in both secretions and serum extended from 150,000 to about 700,000 molecular weight. The IgA in the secretions (tears, milk, and colostrum) appeared to have a dis-

tribution weighted towards the heavier species (Fig. 8) with smaller amounts of low-molecular-weight IgA than serum. Bound SC was found in the heavier fractions of the secretion separations (over 350,000 molecular weight). No SC was found in the serum fractions. Antigenic activity apparently representing free SC was detected in milk, and SC size was considerably smaller than that of IgG.

Serum levels of IgG, IgM, IgA, IgG(T), and AI are presented in Table 1. The mean level of IgA was 153 mg/100 ml, which was slightly higher than that of IgM (120 mg/100 ml). A mean serum IgA to IgG ratio of 0.114 was obtained. Levels of these same immunoglobulins were determined in tears from four ponies (Table 2). In these secretions IgA was the predominant immunoglobulin with IgA to IgG ratios ranging from 4.0 to 13.1. Samples of mammary secretions were examined on 1, 3, 16, and 182 days after parturition (Table 3). On days 1 and 3, IgG was present in the highest quantities of the 5 immunoglobulins

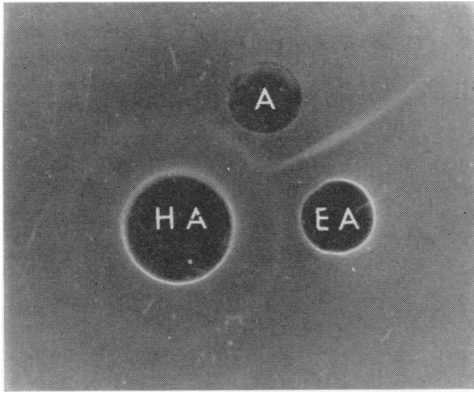


FIG. 6. Demonstration of a line of identity caused by monospecific antiequine IgA (EA) and specific anti-human alpha-chain antisera (HA) when reacted with purified equine IgA (A). The antihuman alpha-chain antiserum well was filled four times, and the reaction with equine IgA extended beyond the halo on the left side, but this did not show in the photograph.

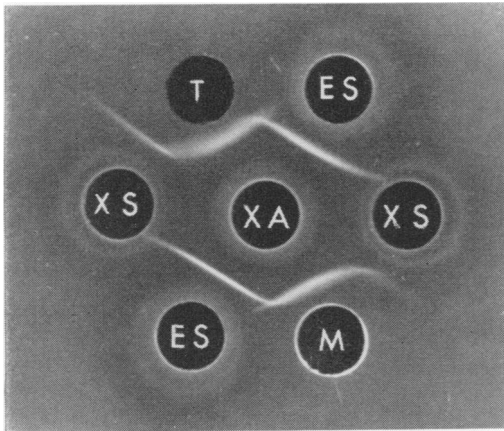


FIG. 7. Demonstration of antigenic determinants (secretory component) in tears (T) and milk (M), but not in equine serum (ES), when reacted with monospecific antiequine secretory IgA (XA). Specific antiserum component (XS) reacts with tears and milk, but not with equine serum.

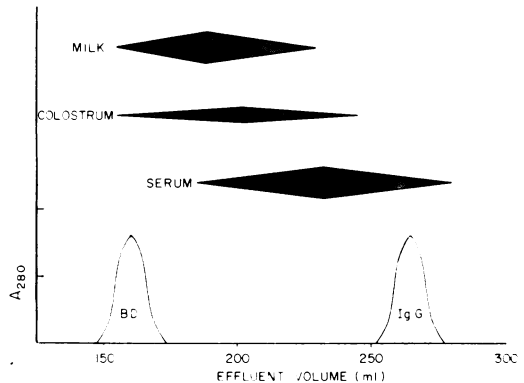


FIG. 8. Sephadex G-200 elution profile of IgA in equine milk, colostrum, and serum. Secretions and serum were fractionated, and the IgA was localized immunologically. BD, Dextran blue; IgG, rabbit IgG. The mean molecular weight of IgA was higher in secretions than in serum.

TABLE 1. Summary of serum immunoglobulin levels from 20 Shetland ponies

Immunoglobulin	Mean	Standard deviation	Range
IgG	1,334 ^a	350	720-1,920
IgM	120	31	82-200
IgA	153	86	60-350
IgG(T)	821	301	214-1,500
AI	39	28	9-104

^a Immunoglobulin levels are expressed in mg/100 ml as determined by single radial immunodiffusion.

TABLE 2. *Immunoglobulin levels in tears from Shetland ponies*

Horse no.	IgG	IgM	IgA	IgG(T)	AI	IgA to IgG ratio
118	13 ^a	5	170	2	N ^b	13.1
99	13	N	150	2	N	11.6
59	20	N	80	7	N	4.0
80	16	N	140	1	N	8.8

^a Immunoglobulin levels expressed in mg/100 ml as determined by single radial immunodiffusion.

^b N, Negative.

TABLE 3. *Immunoglobulin levels of colostrum and milk from a Shetland pony*

Days after parturition	IgG	IgM	IgA	IgG (T)	AI	IgA to IgG ratio
1 (Colostrum)	210 ^a	22	20	100	10	0.01
3 (Colostrum)	80	15	28	40	N	0.34
16 (Milk)	28	7	80	8	N	2.85
182 (Milk)	14	N ^b	130	2	N	9.3

^a Immunoglobulin levels are expressed in mg/100 ml as determined by single radial immunodiffusion.

^b N, Negative.

measured. However, with time after parturition, IgG levels decreased and IgA levels increased until IgA was the predominant immunoglobulin. At 182 days after parturition the IgA to IgG ratio was 9.3. IgG(Y) and AI were detected in colostrum on day 1. Their levels rapidly decreased in subsequent samples (Table 3).

DISCUSSION

IgA with immunological cross-reactivity to human IgA has been identified in a number of mammalian species (10, 13, 15, 24-27). T protein of equine serum (22) was erroneously considered to be the homologue of human IgA (6, 8, 16, 17). Subsequent examinations of immunoglobulins in secretions failed to detect equine IgA, and it was concluded that the horse did not have a secretory immunoglobulin analogous to human IgA (4, 19). Our results establish that the horse does indeed possess a secretory IgA system homologous to that in man by confirming the presence of common antigenic determinants with human IgA (26, 27) and demonstrating the presence of SC on secretory IgA.

In contrast to human serum IgA, which exists largely in the form of 7S monomer (23), IgA of equine serum was detected in fractions ranging from a molecular weight of 150,000 to about 700,000. Large-molecular-weight forms of serum

IgA have previously been observed in the dog (24), pig (25), cow (10, 15), goat and sheep (13), and the horse (27). The mean molecular weight of equine serum IgA was found to be about 350,000, suggesting a dimer as the predominant species. Similar conclusions have been arrived at for other species (10, 13, 15, 24, 25). The size of equine IgA from secretions was greater than from serum. Much secretory IgA was found in the exclusion volume of Sephadex G-200. Colostrum contained a more disperse population of IgA molecules with a broader range of molecular weights than did milk. This may reflect the greater contribution of serum to colostrum IgA as opposed to milk from later in lactation. Presumably, in milk the majority of the IgA is formed *in situ* and is complexed with SC. Similar findings have been reported in the cow (15). Our demonstration of the presence of an antigenic determinant on purified IgA from horse tears, which was not present on serum IgA, indicates that SC also occurs in the horse. Evidence also indicates that free SC occurs in horse milk, as has been shown with bovine milk (10). Studies with an equine immunoglobulin secreted *in vitro* by salivary tissue had additional antigenic determinants which were thought to represent SC (7).

Quantitative studies demonstrated that IgA was the predominant immunoglobulin in tears and milk, but not in colostrum. The IgA-IgG ratio in colostrum was 0.01 on the day of parturition; 182 days later it had increased to 9.3. The mean serum level of IgA was 153 mg/100 ml, which was slightly greater than that of IgM, 120 mg/100 ml. Antigen-binding activity was demonstrated in the IgA of tears from a horse immunized with DNP-BGG; however, no studies have been done on the functional role of IgA in equine secretions. The participation of the horse secretory immune system in the protection of epithelial surfaces awaits further study. The demonstration of a secretory IgA immune system in other species strengthens the concept that it is a defense mechanism common to most, if not all, mammals.

ACKNOWLEDGMENTS

This work was supported in part by grants AI-07471 and AI-09237 from the National Institute of Allergy and Infectious Diseases, grant RR-05465 from the National Institutes of Health, and the Veterinary Sciences Research Division, Agricultural Research Service.

The assistance of Barrie Grant with the collection of secretions and the technical assistance of Susan Quist and Julie Brauner are gratefully acknowledged.

ADDENDUM IN PROOF

Since submission of this manuscript, other investigators have also detected secretory component on equine secretory immunoglobulin A and in the free

form (J. J. Pahud and J. P. Mach. 1972. Equine secretory IgA and secretory component. *Int. Arch. Allergy* 42:175-186).

LITERATURE CITED

1. Andrews, P. 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. *Biochem. J.* 91:222-233.
2. Audibert, F., and G. M. Sandor. 1968. Nature de la fraction antitoxine des immunosérums de cheval. *C. R. Acad. Sci. (Paris)* 267:457-458.
3. Eisen, H. N. 1964. *Methods in medical research.* Yearbook Medical Publishers, Chicago.
4. Genco, R., L. Yecies, and F. Karush. 1969. The immunoglobulins of equine colostrum and parotid fluid. *J. Immunol.* 103:437-444.
5. Helms, C. M., and P. Z. Allen. 1970. Studies on equine immunoglobulins. II. Antigenic interrelationships among horse IgG, IgG(T), and antipneumococcal γ 1-component. *J. Immunol.* 105:1253-1263.
6. Hill, W. C., and J. J. Cebra. 1965. Horse anti-SI immunoglobulins. I. Properties of γ M-antibody. *Biochemistry* 4:2575-2584.
7. Hurlimann, J., and H. Darling. 1971. In vitro synthesis of immunoglobulin-A by salivary glands from animals of different species. *Immunology* 21:101-110.
8. Klinman, N. R., J. H. Rockey, G. Frauenberger, and F. Karush. 1966. Equine anti-haptene antibody. III. The comparative properties of γ G and γ A antibodies. *J. Immunol.* 96:587-595.
9. Kinman, N. R., J. H. Rockey, and F. Karush. 1965. Equine anti-haptene antibody. II. The γ G (7S γ) components and their specific interaction. *Immunochemistry* 2:51-60.
10. Mach, J. P., and J. J. Pahud. 1971. Secretory IgA, a major immunoglobulin in most bovine external secretions. *J. Immunol.* 106:552-563.
11. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy Appl. Immunol.* 29:185-189.
12. McGuire, T. C., J. Van Hoosier, and J. B. Henson. 1971. The complement-fixation reaction in equine infectious anemia. Demonstration of inhibition by IgG(T). *J. Immunol.* 107:1738-1744.
13. Pahud, J. J., and J. P. Mach. 1970. Identification of secretory IgA, free secretory piece and serum IgA in the ovine and caprine species. *Immunochemistry* 7:679-686.
14. Perryman, L. E., T. C. McGuire, K. L. Banks, and J. B. Henson. 1971. Decreased C3 levels in a chronic virus infection: equine infectious anemia. *J. Immunol.* 106:1074-1078.
15. Porter, P., and D. E. Noakes. 1970. Immunoglobulin IgA in bovine serum and external secretions. *Biochim. Biophys. Acta* 214:107-116.
16. Raynaud, M., S. Iscaki, and R. Mangalo. 1965. Separation chromatographique des γ G et γ A antitoxines antidiphthériques de cheval. *Ann. Inst. Pasteur* 109:525-551.
17. Rockey, J. H. 1967. Equine antihaptene antibody. The subunits and fragments of anti- β -lactoside antibody. *J. Exp. Med.* 125:249-275.
18. Rockey, J. H., N. R. Klinman, and F. Karush. 1964. Equine antihaptene antibody. I. 7SB₂A- and 10S γ 1-globulin components of purified anti- β -lactoside antibody. *J. Exp. Med.* 120:589-609.
19. Rouse, B. T., and D. G. Ingram. 1970. The total protein and immunoglobulin profile of equine colostrum and milk. *Immunology* 19:901-907.
20. Sandor, G., and F. Audibert. 1970. Deux fonctions physiologiques de l'immunité humorale et les deux protéines qui l'assument dans le serum de cheval. *C. R. Acad. Sci. (Paris)* 270:1538-1540.
21. Sandor, G., S. Korack, and P. Mattern. 1964. 7S globulin immunologically identical to 19S gamma-1 (beta-2)-M-globulin, a new protein of horse serum. *Nature (London)* 204:795-796 (1964).
22. Schultze, H. E., H. Haupt, K. Heide, N. Neimburger, and H. G. Schwick. 1965. Comparative investigations of purified diphtheria and tetanus T-components and their fragments. *Immunochemistry* 2:273-284.
23. Tomasi, T. B. 1970. Structure and function of mucosal antibodies. *Annu. Rev. Med.* 21:281-298.
24. Vaerman, J. P., and J. F. Heremans. 1968. The immunoglobulins of the dog. I. Identification of canine immunoglobulins homologous to human IgA and IgM. *Immunochemistry* 5:425-532.
25. Vaerman, J. P., and J. F. Heremans. 1970. Immunoglobulin A in the pig. I. Preliminary characterization of normal pig serum IgA. *Int. Arch. Allergy* 38:561-572.
26. Vaerman, J. P., J. F. Heremans, and G. Van Kerckhoven. 1969. Identification of IgA in several mammalian species. *J. Immunol.* 103:1421-1423.
27. Vaerman, J. P., P. Querinjean, and J. F. Heremans. 1971. Studies on the IgA system of the horse. *Immunology* 21:443-454.
28. Weir, R. C., and R. R. Porter. 1966. Comparison of the structure of immunoglobulins from horse serum. *Biochem. J.* 100:63-68.
29. Weir, R. C., R. R. Porter, and D. Givol. 1966. Comparison of the C-terminal amino-acid sequence of two horse immunoglobulins IgG and IgG(T). *Nature (London)* 212:205-206.
30. Yagi, Y., P. Maier, D. Pressman, C. Arbesman, and R. Reisman. 1963. The presence of the ragweed-binding antibodies in the β 2A-, β 2M- and γ -globulins of the sensitive individuals. *J. Immunol.* 91:83-89.
31. Zolla, S., and J. W. Goodman. 1968. An aggregating immunoglobulin in hyperimmune equine anti-pneumococcal sera. *J. Immunol.* 100:880-897.