Spectrum of Ovine Immunoglobulins* (33445)

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(Introduced by E. V. Morse)
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Several structurally and functionally distinct classes of antibodies have been described for numerous species of animals. The present investigation was designed to elucidate the spectrum and interrelationships of ovine immunoglobulins (Ig) and to establish the basic temporal features of the antibody response of the sheep.

Materials and Methods. Purified ovine Ig were prepared by several methods from whole normal ovine serum (NOS) or immune ovine serum and from serum fractions obtained by ammonium sulfate or low ionic strength precipitation. Two stepwise elution schemes were used for the chromatographic purification of serum proteins on DEAE-cellulose. In the first, proteins were eluted by tris–phosphate buffer, pH 8.6, at molarities of 0.01, 0.04, 0.05, 0.08, and 0.09. In the second, proteins were eluted with 0.02, 0.035, 0.05, 0.1 and 0.15 *M* phosphate buffers and with 1 *M* NaCl, at pH 6.3. Serum was also separated by gel filtration on Sephadex G-200 using tris–HCl buffer at pH 8 with 1 *M* NaCl and by preparative zone electrophoresis (1).

Antisera to whole ovine serum and purified Ig were prepared in rabbits by the multiple injection of antigen with complete or incomplete Freund's adjuvant. Sera were rendered specific for one or more classes of Ig by stepwise absorption with appropriate ovine Ig.

Microimmunoelectrophoresis (IEP) (2) and immunodiffusion (3) were used to characterize the antigenic relationships of Ig. Radioimmunoelectrophoresis (RIEP) (4), and passive hemagglutination (HA) (5) were used to characterize the ovine antibody response to a protein antigen, human γ-globulin. Microscopic agglutination (MA) (6), and immunodiffusion tests were also used to study antileptospiral antibody of sheep hyperimmunized with *Leptospira pomona.*

Results and discussion. Four major classes of ovine Ig (IgG_1, IgG_2, IgM and IgA) were detected (Figs. 1 and 2). In addition, one, and perhaps two, minor subclasses (delta and epsilon components of IgG) were detected (Fig. 1). The designations adapted for ovine Ig were largely established by analogy with human Ig and may require modification following further study.

The IgG_2 and IgG_1 classes appeared to be the predominant species of ovine Ig in NOS. The IgG_2 class was characterized by its low anionic binding properties (eluted with 0.02 *M* phosphate or 0.04 *M* tris–phosphate buffer), and intermediate (7S) position of elution from Sephadex G-200. It had a wide electrophoretic mobility in agar or preparative electrophoresis. A greater degree of charge heterogeneity than recognized for most species was indicated by the trailing of this protein throughout the chromatogram, as recently reported by others (7).

The IgG_1 class was electrophoretically faster than IgG_2 although equally heterogeneous in charge and anionic binding capacity (eluted by 0.05 *M* phosphate and 0.08 *M* tris buffer). It also appeared to be a 7S globulin on the basis of its elution from G-200.

The IgM class had a higher anionic binding capacity (eluted with 0.15 *M* phosphate) than either of the two IgG classes. It was eluted from G-200 like a high molecular weight (19S) globulin, was electrophoretically faster than IgG_2 and largely overlapped the electrophoretic mobility of the faster portion of the IgG_2 globulin.

The IgA class was the electrophoretically fastest of the four Ig although it overlapped with both IgG_1 and IgM in its electrophoretic and anionic binding properties (eluted with 0.1 *M* phosphate buffer). It was eluted from

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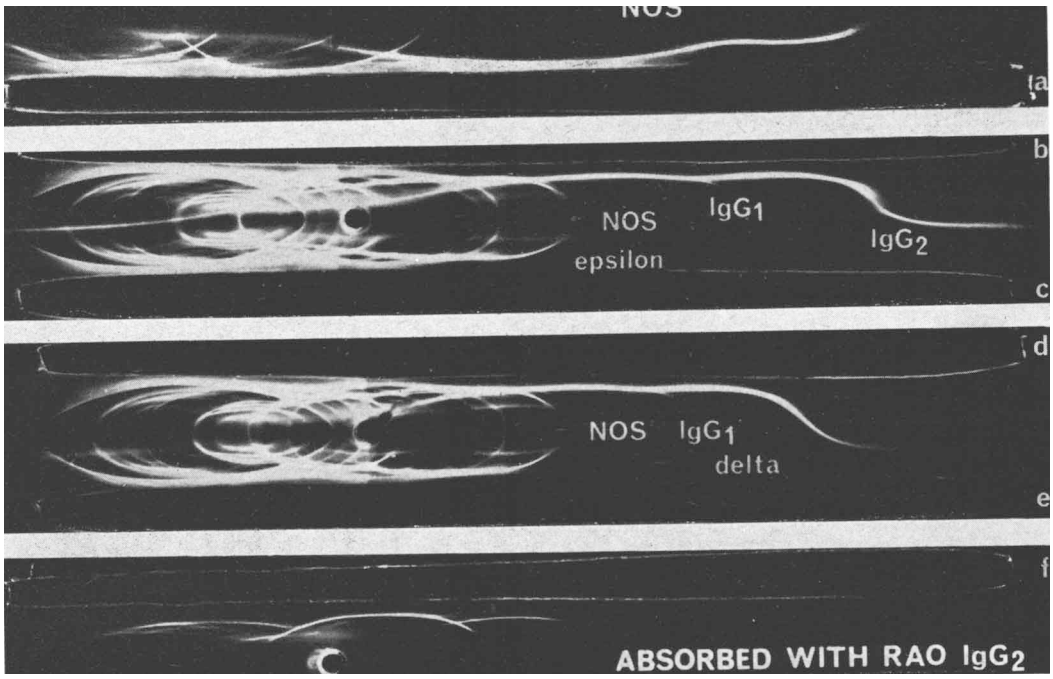


FIG. 1. Immunoelectrophoretic analysis of ovine Ig. Spurs indicative of unique antigenic determinants were observed when normal ovine serum (NOS) was developed with rabbit antiovine sera (RAOS) containing antibodies specific for either IgG_2 (a) or IgG_1 (b, d). Exhaustive absorption of RAOS with IgG_1 (c) or IgG_2 (e) resulted in abolition of the portion of the arc contributed by that specific Ig and diminution of the remainder of the IgG arc, presumably due to the removal of antibodies to the common (L chain) determinants or cross-contamination of the antigens used for absorption. Absorption of an antileptospiral immune serum with RAO-Ig removed all Ig lines (f) and decreased the MA titer by several logs. Delta and epsilon lines, apparently minor Ig, were observed in the positions indicated on the original slides but are not readily visible on the plate.

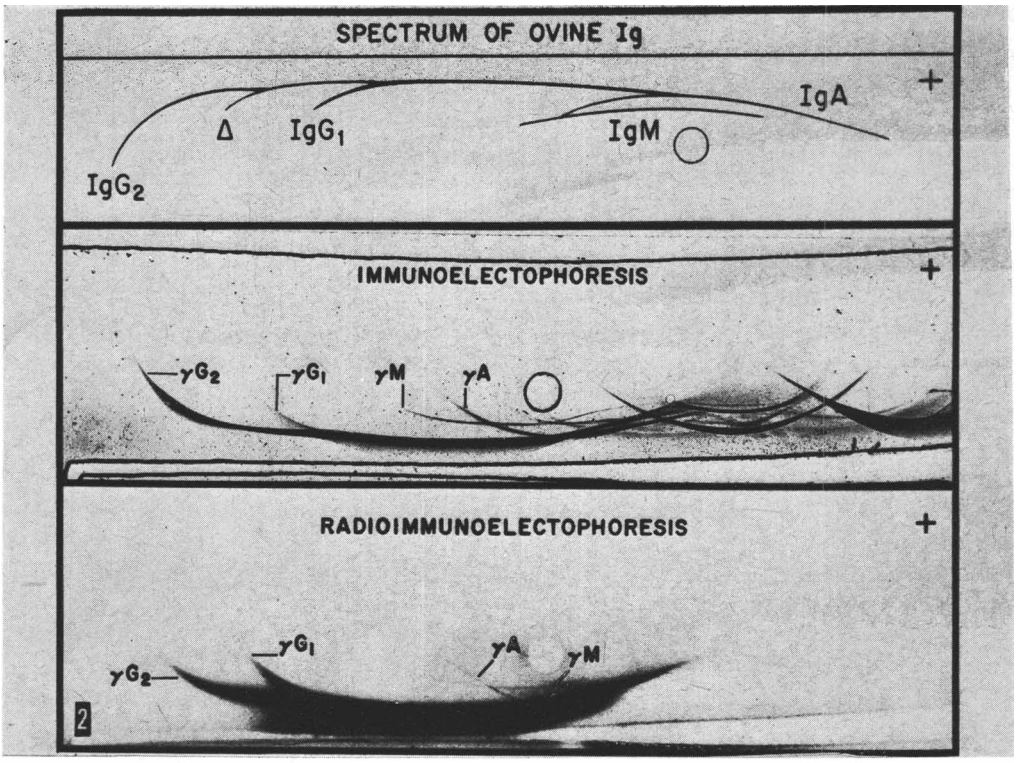
G-200 in a position intermediate to IgG and IgM.

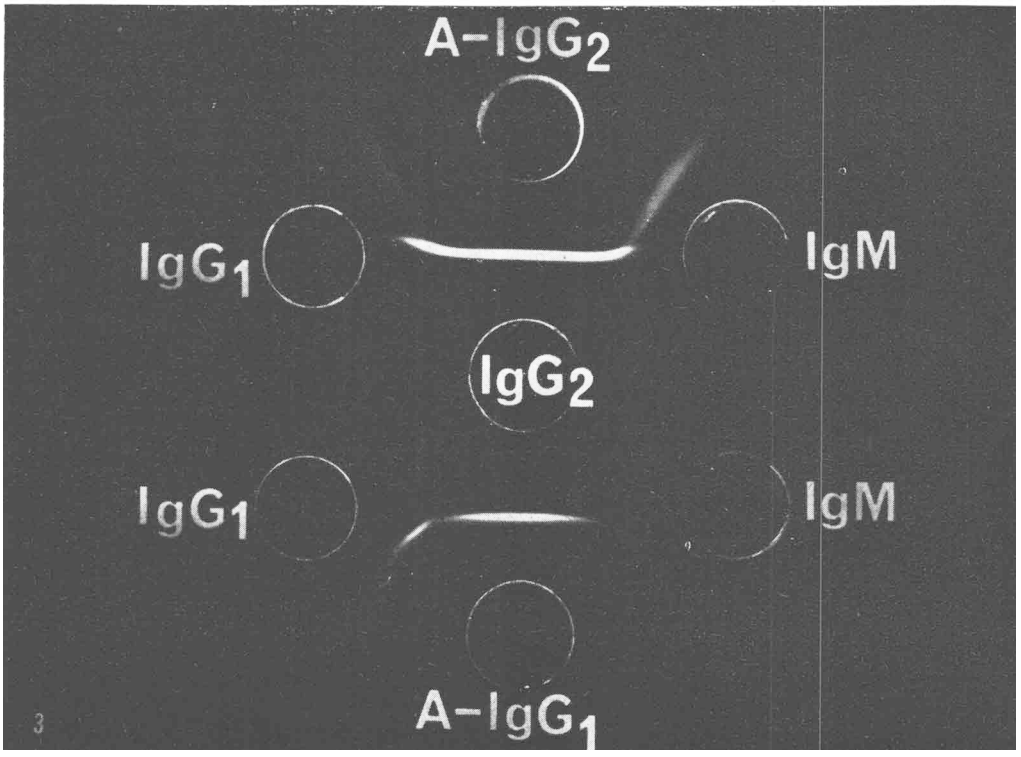
All four Ig, by IEP analysis, appeared to be antigenically related (Figs. 1 and 2) and had a pattern like that seen for other species and, in part, reported previously for the sheep (8, 9). In addition, each of the four classes appeared to have additional antigenic determinants. The common and unique determinants were presumably a property of the L and H polypeptide chains of the respective molecules since selective absorption with purified Ig of one or more classes removed antibodies to those but not all classes of Ig. The existence of both common and unique determinants was also indicated by immunodiffusion tests of the individual purified Ig (Fig. 3).

All four classes of Ig had antibody activity as indicated by the binding of ^{125}I-labeled HGG by the RIEP method (Fig. 2). The antibody formed first after immunization

FIG. 2. Schematic representation of the spectrum of ovine Ig and typical immunoelectrophoretic pattern of an ovine anti-HGG serum after development with a rabbit antiserum. Typical radioautographic localization of HGG-I^{125} by specific ovine Ig is also shown.

FIG. 3. Antigenic relationships of ovine Ig. Distinct as well as common antigenic determinants of IgG_2 and IgG_1 are indicated by spurring across the line formed with the common determinants when specific anti-IgG_2 or IgG_1 serum was used for development.


SPECTRUM OF OVINE Ig
IgA
Δ
IgG1
IgM
IgG2
+
IMMUNOELECTOPHORESIS
γG2
γG1
γM
γA
+
RADIOIMMUNOELECTOPHORESIS
γG1
γA
γM
γG2
+
2


A-IgG2
IgG1
IgM
IgG2
IgG1
IgM
A-IgG1
3

with alum-precipitated HGG was predominantly IgG_1 and IgM. With higher doses of antigen, IgG_2 antibody appeared early in the response and, in most cases, diminished more rapidly than IgG_1. The IgM antibody was most prominent in the primary response but its presence, in low levels, was also indicated in early secondary sera by reduction with 2-mercaptoethanol. The IgA antibodies arose somewhat later in the primary response than IgG_1 and IgM classes and often persisted until late in the response.

The delta and epsilon minor Ig classes were detected only by their relationship to the major IgG precipitin arc by IEP. No antigen binding by these arcs was detected even when an excess of labeled antigen was assured by diffusing the antigen from two directions.

The antibody response to HGG was also evaluated by passive hemagglutination, homologous skin fixation and radial immunodiffusion tests (10). These methods generally supported the conclusions suggested by RIEP and indicated many similarities of the antiprotein responses of the rabbit and sheep. However, several differences were apparent. The IgG_2 and IgG_1 classes of antibody appeared to be the predominant molecular species in the rabbit and sheep, respectively. In addition, the sheep appeared to form relatively lower levels of precipitating antibody than the rabbit. As noted previously (11) the IgM component of the response appeared to persist longer in the sheep than in the rabbit. The existence of homocytotropic ovine antibody, which did not appear to correlate with any of the four Ig classes described, indicated the existence of at least a fifth Ig class possibly analogous to human IgE.

The functional heterogeneity of ovine antibodies was also substantiated by studies of antileptospiral antibodies. The participation of IgM or IgA antibody was indicated by diminution of the MA titer after reduction of the test serum with 2-mercaptoethanol. Chromatographically purified IgG_1, IgG_2, and IgM globulins all had MA activity. The IgM and IgG_1 antibodies appeared to be the major source of antileptospiral activity in both the column peaks and specifically purified antibody eluted from leptospiral cells by CsCl.

Summary. The presence of three distinct ovine Ig (IgG_1, IgG_2, and IgM) reported by others was confirmed and the presence of an additional Ig class, presumably IgA, was demonstrated. A fifth Ig class, possibly analogous to human IgE, was indicated by the detection of homocytotropic antibody activity which did not appear to be attributable to any of the other classes.

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