SECRETORY IMMUNOGLOBULINS OF THE DUCK *(Anas platyrrhynchos domesticus)*

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"SECRETORY IMMUNOGLOBULINS OF THE DUCK *(Anas platyrhynchos domesticus)*"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society

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CERTIFICATE

Certified that this thesis, entitled "SECRETORY IMMUNOGLOBULINS OF THE DUCK *(Anas platyrrhynchos domesticus)*" is a record of research work done independently by Sri A.V Harikumar under my guidance and supervision and that it has not previously formed the basis for the award of any degree fellowship or associateship to him

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ABBREVIATIONS

NDV		New Castle Disease Virus
Abs		Antibodies
Ig(s)	-	Immunoglobulin(s)
kDa		kilo Daltons
D	-	Daltons
VN	-	Virus neutralization
SIg		Serum immunoglobulins
TIg	-	Tracheal immunoglobulins
Test Ig		Testicular immunoglobulins
OIg		Oviduct immunoglobulins
IIg		Intestinal immunoglobulins
HI		Haemagglutination inhibition
IM		Intramuscular
IN		Intranasal
SC		Subcutaneous
SAS		Saturated ammonium sulphate solution
AGPT		Agar gel precipitation test
PVP		Poly vinyl pyrolidone
RADSIg		Rabbit antiduck serum immunoglobulin
RA1		Rabbit anti first peak fraction
RA2		Rabbit anti second peak fraction
STAT		Standard tube agglutination test

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Introduction

INTRODUCTION

Many of the infectious or contagious diseases prevalent among chicken are not seen to occur in ducks Interestingly the habitat of the ducks in general is apparently filthy and the environs are such that they are always at a greater risk of exposure to a multitude of infectious agents causing diseases in other avian species Moreover several workers have documented the deficiency of duck immunoglobulins in primary and secondary binding reactions (Higgins, 1971, Toth and Norcross, 1981a, b c) with few exceptions (Calnek, 1978, Higgins, 1989)

Despite the aforesaid facts, why the ducks are found to have an inherent insusceptibility or in other words relatively greater resistance to some of the avian infectious diseases remains as a paradox The reasons attributable to a shorter spectrum of diseases is yet to be fully understood and explained

Many workers studied the immunoglobulin profile of duck serum by immunoelectrophoresis and reported it to contain IgM and two subclasses of IgG, the 7 8s and 5 7s, differing in sedimentation coefficients (Grey, 1963, 1967a and b, Unanue and Dixon, 1965, Toth a nd Norcross, 1981a)

Eventhough IgA has been demonstrated in chicken, pigeon and turkey, the presence of the same in ducks is still obscure

The above workers used White Pekin, Mallard or Muscovy ducks for their study Similar reports on Desi or Desi x Khaki Cambell ducks are few Hence, the present study was undertaken with a view to elucidate the immunoglobulin profile of Desi and Desi x Khaki Cambell ducks and to relate the levels of these immunoglobulins in serum and various secretions when immunized by a bacterial *(S typhimurium)* and a viral (NDV) antigen, in order to give a better understanding of the role of these immunoglobulins in warding away infections To accomplish this, the following techniques were employed

a Separation and purification of immunoglobulin classes from serum bile, tracheal washings and intestinal washings

b Determination of the type of immunoglobulins in serum bile, tracheal, intestinal and oviduct washings

c Quantification of immunoglobulin classes in serum, bile, tracheal washings, testicular extract and oviduct washings of ducks immunized with a bacterial *(S typhimurium)* and a viral (NDV) antigen, which were administered through various routes and doses

Review of Literature

REVIEW OF LITERATURE

Reports on duck immunoglobulins have appeared in the literature during the past 40 years Earliest observations were from a series of reports in which duck antibodies (Abs) to kidney tissue were employed to initiate experimental glomerulonephritis in rats The delayed onset of glomerulonephritis induced by duck Abs (3-20 days) compared to rapid onset when rabbit Abs were used, in spite of the fact that they were shown to bind to kidney tissue immediately, could be attributed to the inability of duck Abs to bind with the complement (Higgins and Warr, 1993)

Unanue and Dixon (1965) while studying the interaction of nephrotoxic Abs with tissues of rat found ducks to possess a 27 25s IgM and two antigenically related forms of IgG (7 3s and 7 8s)

Grey (1967a, b) recognized three Igs in ducks, IgM and two low molecular immunoglobulins (Igs) with sedimentation coefficients of 7 8s and 5 7s IgM had antigenic and structural properties similar to that of mammalian IgM (Hadge and Ambrosius, 1984)

As of now, four types of Igs have been identified in ducks IgM, a secretory Ig resembling IgM, a 7 8s IgG and a 5 7s IgG (Higgins and Warr, 1993)

Ng and Higgins (1986) while studying bile Igs of the duck estimated the molecular weight of IgM as 800,000 daltons and that of 'H' chain to be 86,000 daltons They also found IgM to be present in serum in very low concentrations at hatching which gradually increased to adult concentration (1-2 mg/ml) by about 10 days of age

Studies by Zimmerman *et al* (1971) revealed that of the two IgGs, the larger one was the 7 8s IgG, with a molecular weight of 178-200 kDa, the heavy chains being 62 67 kDa and light chains, 22-25 kDa, with a carbohydrate content of 5 per cent The smaller IgG was the 5 7s IgG having a molecular weight of about 118 kDa with heavy chain of 35-42 kDa and light chains of 22-25 kDa and a carbohydrate content of only 0 6 per cent The 7 8s IgG was found to possess additional antigenic determinants over 5 7 IgG (Zimmerman *et al* 1971, Pamela and Higgins, 1986) It was also reported that the 7 8s IgG could be transmitted through yolk sac while 5 7s IgG could not (Lin and Higgins, 1990)

Grey (1967a) found that White Pekin ducks had about equal amounts of 7 8s and 5 7s IgG, while in Muscovy ducks, the 7 8s IgG predominated The same was reported by Zimmerman *et al* (1971)

Toth and Norcross (1981b) observed that duck Igs appeared to be inherently deficient in immunological reaction like agglutination and precipitation

Ng and Higgins (1986) found bile Igs of the duck to be of a single class with a molecular weight of 8,90,000 Da which resembled serum IgM but carried additional determinants They reported that duck biliary Ig was secreted independently of serum IgM They also reported that Ig was not present in bile until around 25 days of age which then rapidly increased to reach adult levels (around 10 mg/ml) by 55 to 60 days of age

Hadge and Ambrosius (1988b) opined that biliary Igs of Anseriformian and gallinaceous birds did not belong to the same class

Hadge and Ambrosius (1988a) observed that biliary Igs were present in relatively high amounts (4 5-15 mg/ml) in chicken, turkey, duck and goose

Hadge and Ambrosius (1988b) studied the antigenic properties of biliary Igs of chicken, turkey, duck and goose and found a high degree of antigenic relationsnip between chicken and turkey biliary Igs and concluded that in Anseriform birds IgM like Igs were secretory Igs, while in galliforms, biliary Igs had special antigenic properties The characteristics or existence of duck IgA are still

obscure Eventhough Grey (1963) designated a duck serum protein as IgA on the basis of it's electrophoretic characteristics it was later identified as a minor duck IgG (Toth and Norcross, 1981a) Parry and Aitken (1975) also failed to detect any cross reacting homologous protein in sera or secretions (Saliva and Bile) of ducks on doing immuno diffusion tests using rabbit and pheasant antisera monospecific for fowl IgA At the same time homologous antigens were detected in guinea fowl, quail, turkey and goose

Immunoglobulins of other avian species

Three major Igs have been identified in chicken serum namely IgM, IgG and IgA (Higgins, 1975)

Chhabra and Goel (1980) while studying the concentration and distribution of the three Igs in the sera and tracheal washings of chicken found that the distribution of IgM and IgG irrespective of age was almost normal whereas that of IgA was skew An Ig-like component, the beta component, with a molecular weight of 1,90,000 Da was also identified in chicken serum (Goel, 1984)

Schranner and Losch (1986) identified monomeric and polymeric IgM in chicken serum and reported that IgM existed in monomeric (1,84,000 Da) and pentameric states (9,20,000)

Leslie *et al* (1971) opined that chicken had a secretary immunologic system wherein the secretary Ig was of the same class as the predominant serum Ig

Gallagher and Voss (1969) estimated the molecular weight of purified chicken IgG to be between 1,78,000 and 1,79,000 daltons Three possible subclasses of fowl IgG - G1, G2 and G3 - have been identified (Watanabe and Isayama, 1973) Lebacq-Verheyden *et al* (1972) first demonstrated an Ig in chicken which was neither IgM nor IgG and predominated in secretions and provisionally called it IgA While not all properties of this fowl secretory Ig were the same as those of mammalian IgA, there were similarities in the physical and chemical structure and function (Higgins, 1975) Schranner and Losch (1986) observed that serum IgA existed in dimeric (340,000 D) and in monomeric (170,000 D) states

Saif and Dohms (1976) isolated IgG and IgM from turkey serum which had S values of 7 4 and 16 8-16 9 respectively Two molecular forms of IgA with sedimentation coefficient values of 8 5 and 17s were found in turkey serum with a higher concentration of the former (Lim and Maheswaran 1977) Gouswaard *et al* (1977b) identified IgG and IgM from pigeon serum

Immunoglobulins in various organs and secretions

Bienenstock *et al* (1973a) studied the synthesis of IgG IgA and IgM by chicken tissues and found that most number of IgA cells were found in the duodenum, and less further down the intestinal tract The caecal tonsil contained mostly IgG followed by IgM cells Only a few IgA cells were seen in the caecal tonsil Spleen also showed a similar distribution of Igs No positive cells were seen either in crop or Peyer s patches The thymus showed presence of IgG only, while the bursa showed the presence of both IgG and IgM The Harderian gland showed synthesis of primarily IgG and IgM

While studying the development and distribution of the Ig containing cells in chicken, Kincade and Cooper (1971) found cells containing μ and light chains in the lymphoid follicles of the bursa of Fabricius of 14 day old embryos IgG containing cells were initially observed in bursal follicles at 21-days of incubation The above developmental sequence of Ig containing cells was later recapitulated in the spleen, caecal tonsils and the thymus The ratio of IgG to IgM containing cells was greatest in the intestinal wall of immunologically mature birds The medullary regions of bursal follicles frequently stained for both IgG and IgM, which was not observed in lymphoid follicles of other tissues

In secretions of chicken IgA was mainly of high molecular weight (3,50,000-3,60,000 D) The IgA\IgY ratio in all secretions examined was greater than in serum Secretory IgA in chicken bile contained an additional antigenic determinant not found in serum IgA (Leslie and Martin, 1973)

Watanabe and Kobayashi (1974) purified IgA from intestinal secretions of chickens and found the structure of biliary IgA similar to serum type of high polymeric IgA with a molecular weight of 8,00,000 to 9,00,000 Daltons, but lacking a secretory component On the other hand, IgA in chicken intestinal secretions was quite different from biliary IgA, but similar to mammalian secretory IgA since it was associated with a protein homologous to mammalian secretory component, and had a value of 30,000 to 50,000 Daltons But Peppard *et al* (1986) reported that chicken bile protein had functional and biochemical properties similar to those of mammalian secretory component

Watanabe *et al* (1975) studied the distribution of secretory component in chicken and concluded that the same could be detected only in intestinal secretions and had a molecular weight of 3,50,000 daltons with a sedimentation coefficient of 11 2

Leslie *et al* (1971) traced the presence of IgY in chicken seminal plasma tracheo bronchial washes, alimentary tract washes and faeces and found that these did not contain any additional antigenic determinant to that of serum IgY IgM in trace amounts was detected in seminal plasma, crop washes and duodenal washes A 10 8s component was also identified in seminal plasma

Chhabra and Goel (1980) studied the normal profile of Igs in sera and tracheal washings of chicken and found IgG to be predominant in the tracheal washings The IgG-IgA ratio was higher in tracheal washings compared to that of serum, which indicated local IgA production in the chicken respiratory tract

Saif and Dohms (1974) identified and characterized Igs in various secretions of turkey and reported that the precipitate from bile by ammonium sulphate showed two major peaks on gel filtration chromatography of which the second fraction showed one component with beta mobility and partial identity with purified IgM and IgG but could not characterize it IgG was found in all preparations examined except large intestinal washings IgM was found in uterine fluid, oviduct scrapings, small intestinal scrapings and washings, bile lacrimal secretions tracheal washings and saliva but could not be detected in egg yolk, albumen, large intestinal scrapings and washings

Goudswaard *et al* (1977) isolated and characterized IgG IgM and IgA in body fluids, eggs and intraocular tissues of turkeys and observed IgG to be most abundant Ig in serum while in egg white intestinal secretions and bile, it was IgA IgM was detected in serum and egg white and IgG in the egg yolk, vitrous body and aqueous body

Lim and Maheswaran (1977) purified IgA from bile intestinal secretions and serum of turkey and reported that predominant forms of polymeric IgA in bile had respective s values of 16 1 and 15 2 Larger polymers of 22-26s were also present in bile

Parry and Aitken (1977) while studying local immunity in respiratory tract of chicken reported that all secretions contained IgA which atleast in saliva accounted for 85 per cent of its activity, remainder being IgG Flourescent localisation of Ig producing cells revealed large numbers containing IgA in association with upper respiratory tract, particularly in the Harderian gland They concluded that the respiratory tract of chicken possessed an antibody mediated secretory immune system analogous to that of mammalian species

Chicken IgA was determined to be the only immunoglobulin class present in bile which revealed polymeric form (17s-19s) on gel filtration studies The IgA

thus isolated did not show antigenic homology to human IgA (Sanders and Case 1977) But Mockett (1986) demonstrated IgM in chicken bile for the first time, using an immmunoabsorbent prepared from monoclonal antibody for IgM

Higgins and Calnek (1975) conducted a study in genetically resistant and susceptible chicken inoculated with JM strain of Mareks disease virus and found that no significant primary differences existed in the humoral system between genetically susceptible and resistant birds They attributed all differences to the immunosuppressive effects of Marek's Disease virus, which were greater in susceptible birds, apparently due to greater lymphoid damage in these strains

Parry and Aitken (1977) studied the nature, specificity and characteristics of secretory immune response in the respiratory tract of chicken after vaccination with lentogenic and inactive NDV They detected virus neutralizing (VN) activity considerably exceeding transudation levels from serum in lacrymal fluid, saliva and tracheal washings, following infection by both oral and occular routes Heat inactivated virus inoculated into trachea evoked neither serum nor secretory VN activity whereas commercial inactivated virus vaccine in mineral oil adjuvant stimulated high titres of serum antibody and some VN activity in tracheal fluids Antibody titre in secretions

was limited but did not prevent reinfection of trachea when challenged Challenge induced only a repeated primary response of secretory Ab in contrast to an elevation of circulatory Ab titre

Zakay Rones *et al* (1971) observed that when chicken were immunized with BPL inactivated NDV intramuscularly, HI Abs appeared in the serum and lungs, while administration through oral route produced Abs only in lungs Zakay Rones (1972) while studying secretory NDV Abs from chicken respiratory tract purported that local Abs belonged to the IgG type of Ig which was responsible for systemic as well as local immunity in chicken

Szenberg *et al* (1965) reported that the fowl produced IgM Abs in response to particulate antigens (Coliphage C16 and influenza A virus) IgM antibodies were present in the serum not only during the primary response but in an increased amount during secondary and tertiary responses

Higgins *et al* (1988) while studying bile Igs of the duck found antibody activity in bile Ig against influenza A virus at times when no activity was detected in serum IgM

Separation and purification of immunoglobulins

A By salting out

Benedict (1967) observed that immunoglobulins of chicken could be precipitated from serum at room temperature, by addition of 18 per cent, 14 per cent and 14 per cent of sodium sulphate in three successive steps Later various workers precipitated chicken globulins (Higgins, 1976, Parry and Aitken, 1977, Chhabra and Goel, 1980, Nandapalan *et al* 1983) and turkey globulins, Saif and Dohms (1976) using the same technique Toth and Norcross (1981a) used sodium sulphate in two successive concentrations of 50 per cent and 33 per cent respectively to precipitate Igs from duck serum

Zakay-Rones *et al* (1972) precipitated globulins from lung extracts of chicken using Benedict's technique

Watanabe and Kobayashi (1974) precipitated Igs from the bile of chicken with 50 per cent saturation of ammonium sulphate in three successive steps Oviductal mucus and intestinal secretions of chicken were also treated in the same manner to precipitate globulins (Watanabe *et al* 1975) Higgins and Calnek (1975) precipitated fowl immunoglobulins from serum with 50 per cent saturation of ammonium sulphate The same procedure was used by Lim and Maheswaran (1977) to

precipitate bile Igs of turkey and by Hadge and Ambrosius (1988a) to precipitate bile Igs of chicken, turkey, duck and goose

Parry and Aitken (1975) used ammonium sulphate to a final concentration of 45 per cent to precipitate Igs in sera and secretions (bile, lachrymal fluid and saliva) of pheasant, guinea, fowl, turkey, Japanese quail, ducks and pigeons The same procedure was used to precipitate Igs from chicken bile (Sanders and Case, 1977)

Immunoglobulins in turkey intestinal washings were also precipitated with a 45 per cent saturation of ammonium sulphate (Goudswaard *et al*, 1977, Lim and Maheswaran, 1977)

Weir (1967) precipitated gamma globulins of many mammalian sera by the addition of saturated ammonium sulphate solution to the serum to a final concentration of 33 per cent Herbert (1974) used three precipitations in 35 per cent saturation of ammonium sulphate for separation of chicken serum globulins Fei *et al* (1986) precipitated duck serum globulins using 40 per cent saturated ammonium sulphate

B Purification of immunoglobulins by chromatography

Szenberg *et al* (1965) separated IgM and IgG fractions from chicken sera using Sephadex G-200 column (65 x 4 8 cm) The first peak was found to be IgM and the second, IgG Zakay-Rones *et al* (1972) analysed the nature of the Abs extracted from lungs of chicken immunized intranasally with NDV by chromatography on Sephadex G-200 column and observed two peaks of which the second peak appeared comparable in terms of HI activity to the second peak obtained when immune sera was run through Sephadex G-200 column

Zimmerman *et al* (1971) fractionated sera from adult Pekin and Muscovy ducks in two steps, starch block electrophoresis followed by application of the gamma-globulins to an upward flow Sephadex G-200 or Bio-gel P-300 column from which they were eluted with PBS They observed IgM to appear in the void volumn 7 8s IgG in the second peak and 5 7s IgG in the third Pure samples of 7 8s IgG were obtained by pooling the leading edge of the second peak and 5 7s IgG from centre of the third peak

Leslie *et al* (1971) passed chicken seminal plasma tracheo bronchial and alimentary tract washes through Sephadex G 200 column and obtained four peaks for seminal plasma of which the second peak eluted in a position similar to the serum IgY Elution patterns were also observed for

tracheo bronchial and alimentary tract washings and the presence of IgY in these secretions were confirmed

Bienenstock *et al* (1973b) separated IgA of a high molecular weight from Igs precipitated from bile and duodenal washings of chicken which eluted immediately after the void volume on Sephadex G-200 column On the other hand high molecular weight Ig from chicken serum eluted mainly in the void volume

Watanabe and Kobayashi (1974) purified IgA from chicken bile and intestinal secretions by using Sephadex G 200 gel filtration and DEAE cellulose chromatography

Grey (1967a) separated the duck gamma globulin fractions obtained by preparative starch block electrophoresis, using Sephadex G-200 gel filtiation in a 2 5 x 1 00 cm column with a buffer composed of IM NaCL and 0 1 M tris pH 8 Three elution peaks were observed, the first corresponding to the gamma M fraction along with varying amounts of lipid and aggregated material Two incompletely resolved peaks followed the first peak, representing the 7 8s and 5 7s globulin fractions respectively He also observed that the relative heights of the first and second peaks varied with the species of duck and state of immunization Normal Muscovy ducks always had a predominant second peak and a rather minor third peak,

whereas normal Pekin and Mallard ducks had second and third peaks of roughly equal height Upon hyperimmunization, all ducks demonstrated a major third peak and a minor second peak When individual peaks were pooled, concentrated and rerun on the same column, purified immunoglobulins were obtained

Saif and Dohms (1976) passed the serum globulins of turkey through a series of 2 Sephadex G-200 columns (2 5 x 45 and 2 5 x 100 cm) Most of the IgM along with minor contaminants was obtained by pooling the ascending and initial descending first peak reactions The second peak fractions contained IgG Final purification of IgM and IgG was done by DEAE chromatography

Goudswaard *et al* (1977a) passed Igs separated from serum egg white, egg yolk of turkey through Sephadex G-200 columns to fractionate them

Parry and Aitken (1977) could recover IgA from chicken bile by first running the bile dialysed against borate buffer saline, pH 8 2, through a Sephadex G-50 column The first peak obtained from this was further fractionated on Sephadex G-200, the first peak of which contained IgA in a relatively pure form as judged by double diffusion and immunoelectrophoresis

Sanders and Case (1976) obtained an IgA peak when chicken bile Ig was applied onto a Sephadex G-200 column (2 8 x 150 cm) The peak thus obtained was further purified by DEAE Sepahdex chromatography

Lim and Maheswaran (1977) fractionated turkey serum globulins through a Sephadex G-200 column with an upward flow IgG was obtained from eluates of the second peak

Chhabra and Goel (1980) obtained purified IgG from sodium sulphate precipitated serum globulins of chicken, by subjecting the second peak obtained by gel filtration chromatography through a DEAE-Sephadex G-50 column IgM was obtained by batch elution from sepharose-4 B anti Ig immunoabsorbant IgA from chicken bile was purified by zone electrophoresis in Pevikon C-870 Though IgG and IgA were obtained in pure form, IgM was contaminated with a maximum 4 per cent of IgG

Toth and Norcross (1981a) fractionated pooled serum samples from ducks on a 2 5 x 90 cm and 1 5 x 41 cm Sephadex G-200 column with a 0 05 Tris buffer, pH 8, containing 0 001 EDTA to obtain IgM and IgG which was further purified by immunoabsorbant chromatography

Ng and Higgins (1986) purified IgM 7 8s - IgG and 5 7s IgG from duck serum by salt precipitation followed by gel filtration through Sephadex G-200 (2 5 x 100 cm) column The

different peaks obtained were recycled through the same column till each peak gave a single line in AGP against the rabbit antiserum to whole duck serum

Nandapalan *et al* (1983) fractionated Igs from chicken serum on Sehadex G-200 column and obtained two peaks The first peak largely composed of IgM and Alpha 2-macroglobulin IgG eluted in the second peak which was further purified by ion exchange chromatography on DEAE 52 They also found that IgG and IgM preparations aggregated on freeze thawing and necessitated dialysis in 4 M urea

Schranner and Losch (1986) obtained 6 peaks on column chromatography of chicken serum through a column of Ultrogel A6 (1 6 x 100 cm) using 0 1 M Tris Hcl, 15 M NaCl, 5 m M aminocaproic acid, 0 025 NaN3 (pH 8) as the buffer First peak contained gamma lipoproteins only IgA and IgM were identified in the second peak, along with traces of gamma lipoprotein and glycoproteins The bulk of polymeric IgA was found to elute in the third peak IgA and IgM in monomeric states were represented in the fourth peak Fei *et al* (1986) chromatographed ammonium sulphate precipitated duck globulins through Sephacryl S-300 column to obtain 7 8s globulins

Ahrestani *et al* (1987) subjected chicken serum gamma globulins to DEAE cellulose chromatography and the IgG thus

obtained was further purified on Sephadex G-200 using Tris-Hcl buffer (0 01 M, pH 8)

Primary and secondary binding reactions of duck and other avian immunoglobulins

In context of immunity to infectious diseases, primary binding of duck antibodies was demonstrated by such assays as the enzyme linked immunosorbant assay (Hatfield *et al* 1987), radioimmunoassay (Vickery *et al*, 1989), virus neutralization (Toth, 1971) and anti-globulin bacterial agglutination test (Sari and Thain, 1983) Higgins (1971) reported failure of duck sera in the HI test to Newcastle diseases and influenza viruses But they found that sera of duck with little or no activity to intact virus often inhibit the haemmaglutination of virus disrupted by ether

Calnek (1978) found that duck serum was effective in the HI test for antibodies to the adenovirus EDS-76

The secondary functional deficiencies of duck Igs extend to direct agglutination and precipitation in which duck antibodies are invariably weak with rare exceptions Toth and Norcross (1981a, b, c) demonstrated weak secondary activities of duck antisera to viruses, proteins and heterologous erythrocytes Precipitation of protein antigens occurred to BSA and bovine gammaglobulins only in three of 62 ducks, all at 7 days after inoculation (Toth and

Norcross, 1981b) Duck antisera were effective in virus neutralization but not precipitation (Toth and Norcross 1981a)

Both 7 8s and 5 7s IgG were found to be active in indirect haemmaglutination, with about equal efficiency only exception being that the 5 7s IgG was found to be very sensitive to reduction by 2-ME while 7 8s IgG antibodies were relatively resistant (Grey, 1967 b)

Litman *et al* (1973) immunized ducks with DNP-Brucella and confirmed the presence of anti-DNP antibodies in sera by AGPT Higgins (1989) used DNP-human IgG (DNP-HIgG) to stimulate duck antibodies to DNP While rabbits inoculated with DNP-HIgG produced precipitins to DNP and HIgG, ducks did not produce precipitins to DNP but did produce precipitins to the HIgG carrier The precipitins, unlike chicken IgY, did not require high NaCl, and were antigenically similar to 5 7s IgG, but carried weak additional determinants Erntell *et al* (1988) showed that duck serum inhibited non-specific binding of radiolabelled mammalian Igs to staphylococcal protein-A and streptococcal proteins

Zakay-Rones *et al* (1971) determined the level of serum HI antibodies to NDV in two groups of chickens, one immunized by intramuscular route and the other by intranasal route and

found the level of serum HI antibodies to be higher in the former On the contrary, no appreciable HI activity was detected in lung extracts in chickens after intramuscular (IM) immunization whereas HI activity in lung extracts after intranasal (IN) immunization reached to a maximum of 1 160

Electrophoretic studies on duck proteins

Electrophoresis is defined as the movement of charged particles in solution under the influence of an electrical field

Five precipitin bands were observed in paper electrophoresis and 14 or more bands were seen in starch or acrylamide gel electrophoresis of duck serum (Kaminski and Gajos, 1964)

Unanue and Dixon (1965) reported that analytical ultra centrifuge studies of duck Ig preparations obtained by preparative electrophoresis yielded three peaks with sedimentation values of 5 89, 7 43 and 16 93 the first two peak proteins migrated in the gamma-2 zone of immunoelectrophoretic test, while the third peak protein migrated in the gamma-1 zone

Grey (1967a) observed that immunoelectrophoresis of starch block isolated gamma-globulin from duck serum showed three antigenically distinct proteins in the gamm-globulin

region One extended directly from the wall similar to mammalian gamma-M globulin, while the other two were located in the region where mammalian gamma-G globulins were usually found on immuno-electrophoresis

Immunoelectrophoretic studies by Grey (1967b) revealed that duck 5 7s protein formed a precipitin band very close to the antiserum through, while the more antigenically complete and more slowly diffusing 7 8s protein formed a band behind it

Toth and Norcross (1981a) observed that duck IgM was an electrophoretically heterogenous protein with components migrating slower than IgM of other species The cathodal tip of the duck IgM lines extended into the gamma-2 migration zone In immunoelectrophoresis using duck whole serum and its antiserum they also detected that besides the IgM and other major IgG arcs, there occurred another arc also in the form of a thin line immediately within the curve of the major IgG line and merging with it towards the cathodal end This was presumed to be a minor IgG arc Their studies also revealed that in one-day old duck serum a prominent duck IgG arc developed by both anti-duck serum and anti-duck IgG But no IgM line was seen In 14 day old duck serum, a shorter line instead of the typical elongated duck IgG arc appeared in the gamma-2 migration zone close to the trough This line was recognized as duck IgG by anti-duck IgG A

well separated weak line not merging with the IgG arc was recognized as duck IgM by anti IgM Similar but considerably weaker lines were also observed for the seven day old duck serum

In a study on the electrophoretic mobility of biliary Igs of chicken turkey duck and goose, Hadge and Ambrosius (1988a) demonstrated that chicken and turkey immunoglobulins were beta 1 and alpha-2 globulins while that from duck and goose were beta 2 globulins

Quantification of proteins

Quantitative methods for the determination of individual serum proteins are important tools in immunological studies Eventhough the quantitation of chicken serum protein fractions had been dealt with in detail by many workers, there are only a few such reports on the serum protein fractions of ducks A variety of methods have been employed for the quantification of avian serum proteins, which include salting out with sodium sulphate electrophoresis and radial immunodiffusion Of these radial immunodiffusion method developed by Mancini *et al* (1965) had proved valuable for quantification of individual immunoglobulins

By radial immunodiffusion technique, Lerner *et al* (1971) found that at hatching, chicks had about 150 mg% circulating IgG derived from yolk This quantity declined to 100 mg% by second week after which the level rose until the 14th week, when a value of 515-550 mg% was attained In contrast to IgG, no circulating IgM was present at hatching, but after the first week measurable quantities appeared IgM level was influenced by specific immunisation with sheep cells to a greater extent than were IgG levels By the 24th week the mean IgM level of the immunized controls, 480 mg% was almost twice that of the non immunized birds, 280 mg%

Leslie and Martin (1973) estimated the relative quantities of IqM IgY and IgA in serum and various secretions of chicken by radial immunodiffusion technique and found that serum contained predominantly IgY and about twice as much as IgM as IgA The concentration of IgA in bile was much greater than any other sample examined The IgA/IgY ratio in duodenal secretions tracheo bronchial secretions and seminal plasma was several fold greater than that of serum

Chhabra and Goel (1980) quantitated the immunoglobulins in chicken serum and tracheal washings by radial immunodiffusion method and found mean values of 1 35 mg/ml IgM 5 09 mg/ml, IgG and 0 31 mg/ml IgA in serum and mean

values of 0 14 mg/ml IgG, 0 03 mg/ml IgA with only trace amounts of IgM

Ng and Higgins (1986) quantitated immunoglobulins in bile and sera of healthy unvaccinated ducklings every few days between hatching and 72 days of age Igs were quantitated using yolk absorbed RAD-bile Ig, RAD-IgM and bile absorbed RAD-7 8s IgG Serum IgM was detected in low concentrations in 1-day old ducklings Levels increased to 1 4 mg/ml by 20 25 days and remained stable thereafter IgG occurred at 2 7 7 6 mg/ml in 1 and 2-day old ducklings decreased to about 1 mg/ml by 10 days of age, then progressively increased to 2 mg/ml during the remainder of the experiment Bile Ig was first detected at 26 days of age Between 38 and 50 days of age, the bile Ig levels rose sharply, attaining concentrations of 1-10 mg/ml

Materials and Methods

MATERIALS AND METHODS

Materials

Protein estimation Biuret reagent

1 Reagent I (alkaline sodium potassium tartarate solution)

$KNaC_4H_4O_6 4H_2O$	12 g
1N NaOH	200 ml
KI	5 g
Distilled water	upto 1000 ml

2 Reagent II (5% copper sulphate solution)

$CuSO_4$ $5H_2O$	5 g
Distilled water	100 ml

Precipitations of immunoglobulins

1 Saturated ammonium sulphate (SAS) solution

SAS was prepared by adding 760 g of ammonium sulphate (BDH) to one litre of triple distilled water and heating to 50°C for 30 minutes in a water bath, with continuous stirring to dissolve It was filtered while still hot to

remove insoluble impurities and then cooled to room temperature The pH was adjusted to 7 0 with ammonium hydroxide solution just prior to use

2 Ammonium hydroxide solution

3 Physiological saline

4 Borate buffered saline, pH 8 5

Five parts of Borate buffer was added to ninety five parts of saline

Borate buffer

Boric acid	6 184 g
Borax	9 536 g
Nacl	4 384 g
Distilled water	1000 ml

Added the above reagents to one litre volumetric flask containing 600 800 ml of distilled water and shaken until a complete solution of the contents was achieved Added distilled water to make up to one litre and mixed by additional shaking Used an aliquot of the solution to check the pH

5 Dialysis bags (Sigma)

Column chromatography

1 Sephadex G 200 (Sigma)

2 Tris Hcl Nacl buffer pH 8 0

Tris (hydroxymethyl) aminomethane 0 1 M 912 11 g/litre) sodium chloride 1 0 M (58 45 g/litre) and 0 02% sodium azide in two third volume of buffer using distilled water pH was adjusted to 8 0 by adding 1N Hcl and the volume was made upto one litre by distilled water The buffer solution was filtered through Whatman No 1 filter paper before use

3 UV-Vis Spectrophotometer

Immunoelectrophoresis

1 Tris barbital buffer

Barbitone sodium	9 9 g
Tris (hydroxy methdyl) amino methane	17 7 g
Sodium azide	0 3 g
Distilled water	2000 ml

pH was adjusted to 8 6 with 1N hydrochloride acid

2 Agar coated slides

Clean slides (2 5 x 7 5 cm) and 5 x 75 cm) were dipped in 1% melted agar in distilled water and dried in air by keeping the slides horizontally over glass rods Dried slides were stored at room temperature until used

3 Buffered agarose

Agarose 0 8 g was boiled in 100 ml Tris barbital buffer until the agar was dissolved completely and then stored at room temperature until used

4 Preparation of agarose gel on slides

Agar coated slides were placed on a perfectly horizontal plane and four ml of melted buffered agarose was poured onto each 2 5 x 7 5 cm slides and double the quantity was poured onto each 5 x 7 5 cm slides It was then allowed to form a gel at room temperature These slides were used for immunodiffusion

5 Stain for immunoelectrophoresis

Amido black 10B	1 g
Sodium acetate acetic acid buffer 0 2 M pH 3 6	1000 ml

6 Decolourising solutions for immunoelectrophoresis

Decolouriser 1

Methanol	40 V
Acetic acid	- 10 V
Distilled water	10 V

Decolouriser II

Absolute alchohol	35 V
Acetic acid	5 V
Distilled water	10 V

7 Duck serum samples from experimental and control birds

8 Fractions obtained by Sephadex G 200 chromatography of serum tracheal washings, intestinal washings and bile

9 Anti duck globulins (whole and purified serum fractions) raised in rabbit

Quantification of globulin fractions by radial immunization

1 P B S (pH 7 3)

NaCl	8 00 g
$K HPO_4$	1 21 g
KH_2PO_4	0 34 g
Distilled water	- 1000 ml

2 1 5% agarose gel in P B S

3 Antiserum to globulin fractions obtained by Sephadex G 200 chromatography of duck serum

4 Duck serum samples from experimental and control birds

5 Duck bile samples from experimental and control birds

6 Duck tracheal washings from experimental and control birds

Duck intestinal washing from experimental and control birds

Duck oviduct washings from experimental and control birds

9 Duck testicular homogenate from experimental and control birds

Agglutination test

1 *S typhimurium* culture

S typhimurium isolated in this laboratory from a guinea pig was used

2 Physiological saline

3 Agglutination tubes

Haemagglutination inhibition test

1 Haemagglutination plates

2 Alsevers solution (composition)

Sodium chloride 0 42 g

Sodium citrate	0 8 g
Citric acid	0 55 g
Glucose	2 05 g
Distilled water	100 ml

3 Chicken RBC

Chicken RBC collected in Alsever's was washed three times with normal saline and suspended in the same solution to get a final concentration of 0 5% RBC

4 Physiological saline

5 ND Virus (R_1B strain)

Ducks

Samples were collected from 200 non-descript ducks (3 4 months of age) from a slaughter house at Kottayam Igs obtained from these samples were used for chromatography AGPT and immunoelectrophoresis Ducks for experimental purposes were obtained by local purchase

Bile

Bile was aspirated aseptically using a 18 guage needle Pooled bile was kept at 20°C till use

Intestinal washings

Intestinal washing were collected from the whole intestines immediately after killing of birds and was stored at 20°C till use

Tracheal washings

Respiratory tubes from larynx to tracheal bifurcation was collected and the lower end ligated securely The trachea was then washed with 5 ml cold PBS 0 1 M pH 7 0 the washing thus obtained were pooled and kept at 20°C till use

Oviduct washings

Obtained as for tracheal washings

Testicular homogenate

The testes was triturated in PBS

Sera

Blood obtained from jugular vein section was used to separate sera Pooled sera was kept at 20°C

Methods

Preparation of antigens

Experiments to study immune responses

In order to study the immune responses against both bacterial and viral antigens, when administered through different routes four sets of experiments were conducted for each antigen

Bacterial antigen *(S typhimurium)*

Experiment I

This group comprises 8 control ducks injected with normal saline and four groups of 8 ducks each injected with *Salmonella typhimurium* antigen through two routes and in two different doses namely 1 ml IM 0 5 ml IM 1 ml SC and 0 5 ml SC These birds were sacrificed at the end of the 4 week post inoculation and various samples were collected as described earlier under the materials

Experiment II

In this experiment NDV was used as viral antigen This group also consisted of 8 control ducks injected with normal saline and three groups of 8 ducks each inoculated with NDV (R B strain) antigen through three different routes namely

IM SC and intranasal the dose being 0 5 ml These birds were sacrificed at the end of the 4th week post inoculation and various samples were collected as described previously

1 ***Salmonella typhimurium***

S *typhimurium* organism was cultivated on Mueller Hinton agar medium in large petri dishes The culture was harvested with approximately 15 ml of 0 6% formal saline per dish, scrapings with sterile glass rod It was then filtered through sterile cotton and incubated for 24 hours at 37°C The sterility was tested by inoculating 0 2 ml of this antigen into Mueller-Hinton agar medium and incubating The stock antigen suspension was kept in the refrigerator

Working standard of antigen was made by comparing with 10 McFarland standard (prepared by adding 9 ml of 1% H_2SO_4 and 1 ml of 1% $BaCl_2$), to get approximately 3000 million organisms per ml It was further inactivated by keeping at 56°C for 30 minutes and was used as the antigen for inoculation For agglutination test, a concentration of one billion organisms per ml of antigen was used

Collection of blood and separation of serum

Pooled sample of blood was collected at slaughter 3 4 months of age The blood was allowed to stand for half to

one hour at room temperature for complete clotting keeping the flasks in a slanting position The clot was then carefully detached from the wall of the flasks with a sterile rod and was allowed to stand at 37°C for one hour for serum separation The separated serum was decanted into clean, sterile tubes and was kept in the refrigerator overnight, for further serum separation The following day the separated serum was clarified by centrifugation at 2000 rpm for 5 minutes and was stored in small aliquots of 2 ml in sterile plastic vials Merthiolate (1 in 10,000) was added to all samples as a preservative before deep freezing at 20°C)

Blood from experimental birds also was collected at sacrifice and serum separated and stored as described above

Collection and processing of bile

Bile was collected using sterile syringe and processed as per procedure described by Bienenstock *et al* (1972)

Bile was subjected to high speed centrifugation for 45 minutes The supernatant was dialysed extensively against 2% Nacl in 0 1 M sodium acetate buffer, pH 4 5 to precipitate mucins After centrifugation, the supernatant was kept in deep freeze at 20°C for later use

Processing of tracheal washings

The tracheal washings were collected as described under materials centrifuged at 2000 g for 20 minutes and dialysed against 2% NaCl in 0 1 M sodium acetate buffer pH 4 5 to remove mucins After centrifugation, the supernatant was kept in deep freeze at 20°C for later use

Processing of intestinal washings

Intestinal contents were collected as described under materials and its secretions were separated from the contents by high speed centrifugation for 45 minutes Mucins were removed as described above, centrifuged, and the supernatant was concentrated and kept in deep freeze at 20°C for later use

Processing of oviduct washings

Oviduct washings were collected as described under materials and processed as for intestinal secretions and stored in deep freeze at -20°C until used

Testicular extract

The testes obtained was homogenised in PBS (ten times dilution) using a blender The particulate matter was pelleted out by high speed centrifugation for 45 minutes and

the supernatant was stored in deep freeze at -20°C until use

Estimation of protein concentration

The total protein content in the blood serum, tracheal washings, and intestinal washings were estimated by Biuret method as described by Inchiosa (1964)

Precipitation of globulins

1 Serum

Final concentration of 45% ammonium sulphate was used to precipitate the globulins in the pooled serum samples as per procedure described by Parry and Aitken (1975)

With constant stirring using a magnetic stirrer 200 ml of saturated ammonium sulphate was added dropwise to a 200 ml pooled serum samples The stirring of serum SAS mixture was continued for 30 minutes After adding the last drop of SAS the ensuing precipitate was allowed to stand overnight at 4°C The suspension was then centrifuged in a refrigerated centrifuge at 3000 rpm for 30 minutes The precipitate obtained was dissolved in enough saline to restore the original volume of serum and reprecipitated twice following the above procedure omitting the overnight keeping of the suspension at 4°C The recipitate from the

third precipitation was dissolved in borate buffer saline t a final volume of 100 ml The ammonium sulphate was removed from the precipitate by dialysing against borate buffered saline at 4°C The saline was changed frequently until there was no ammonium sulphate in the dialysate as evidenced by the absence of turbidity on testing with 10% barium chloride solution

The concentration of precipitated proteins was determined by Biuret method (Inchiosa, 1964)

2 Bile

Saturated ammonium sulphate was used to precipitate the globulins from bile and intestinal secretions as per the procedure described by Lim and Maheswaran (1974)

With constant stirring using magnetic stirrer, equal volume of saturated ammonium sulphate was added to 125 ml of bile (50%) to precipitate the globulins The mucins which remained as a floating green viscous substance, were removed The remaining steps were followed as for serum, but using ammonium sulphate at a final concentration of 50% in subsequent precipitations also

3 Intestinal secretions

The processed intestinal secretions (500 ml) was heated at 56°C for 30 minutes to inactivate proteolytic enzyme The intestinal globulins were salted out with a final concentration of 45% ammonium sulphate The procedure was followed as for serum

4 Tracheal washings

The processed tracheal washings (150 ml) was precipitated with 50 per cent saturation of ammonium sulphate as per the procedure described by Watanabe *et al* (1975) The remaining steps were followed as for serum, but for keeping the final concentration of saturated $(NH_4)_2SO_4$ at 50% in subsequent precipitation

Gel-filtration chromatography

Gel filtration chromatography was carried out on Sephadex G-200 column using tris Nacl buffer pH 8 0 as per procedure described by Talwar (1983)

Preparation of the column

Sephadex G 200 in 4 g quantity was suspended in tris Nacl buffer at room temperature, for three days to ensure

proper swelling The slightly turbid supernatant fluid was removed by decantation to get rid of the fines

A small piece of glass wool was placed at the outlet of the glass column having the dimension of 1 5 x 70 cm It was mounted on a stand in vertical position and filled to about one third with tris - Hcl buffer pH 8 0 A moderately thick slurry of Sephadex G-200 was poured down the column surface to avoid the trapping of air bubbles Then a 10 cm layer of the gel particles had formed, the capillary outlet was opened More slurry was added at frequent intervals when the horizontal zone of packed gel reached a level of 65 cm height, a buffer reservoir was connected The column was equilibrated by allowing 2 to 3 column volumes of buffer to pass through the bed Two such columns were prepared

Preparation of the samples

The test globulin samples were equilibrated by dialysis against tris-Nacl buffer at 4°C for 24 hours and brought to room temperature before chromatography

Chromatography

Buffer reservoir was disconnected and the supernatant fluid was allowed to sink almost to the level of gel surface The equilibrated serum globulin sample, 2 ml having a total protein concentration of 1 ml was loaded very slowly

into the gel without disturbing it Two volumes each of 1 5 ml of the buffer were used to wash in any solution adhering to the column, the first being allowed to sink into the gel before adding the second portion A few millilitres of buffer was then slowly added The column was then connected to the buffer reservoir

The chromatography was conducted manually at room temperature The flow rate was 14 ml/h and 2 ml fractions were collected

The absorbance of each fraction was determined at 280 nm using a UV Vis spectrophotometer The readings of absorbane obtained were applied to a regression equation obtained by preparing a standard regression curve with known protein concentrations and its absorbance to obtain the actual protein concentration in each fraction The peaks obtained were concentrated separately using PVP and passed again through Sephadex G 200 column till a single line was obtained for each peak on AGPT against anti duck serum raised in rabbit

Chromatography of bile Igs, tracheal Igs and intestinal Igs were done in a similar manner as that of serum Igs

Production of antisera

For each set of antisera two rabbits were used

a Rabbit antiduck serum immunoglobulin (RADSIg)

Two ml of duck serum diluted 5 times with PBS was homogenised with two ml of Freunds complete adjuvant and two ml each of this emulsion was given intramuscularly to two rabbits Three booster doses of one ml each without adjuvants were given at 7 day intervals intramuscularly and a week later serum from these rabbits was collected and the immunoglobulins were precipitated using $(NH_4)_2SO_4$ The immunoglobulins thus obtained were dialysed against borate buffered saline and stored 20°C as aliquots of 1 ml at 20°C till use

b Rabbit anti 1st peak fraction (RA1)

Purified 2 ml of 1st peak fraction obtained by repeated gel chromatography (having protein concentration of 2 mg/ml) was homogenised with 2 ml of Freund's adjuvant and 2 ml each of the emulsion was inoculated intramuscularly to two rabbits Two booster doses of 1 5 ml each, without adjuvant were given at weekly intervals for three weeks and the serum was collected a week later

d Rabbit anti 2nd peak fraction (RA2)

Rabbit anti 2nd peak fraction obtained by repeated gel chromatography (having protein concentration of 2 mg/ml) was produced by the same method as for RA1

Immunodiffusion

The melted buffered agarose containing sodium azide (1 in 1000) was poured on to glass slide (2 5 x 7 5 cm and 5 x 7 5 cm) and after solidification, wells were cut in a circular fashion on the 5 x 7 5 cm slides so as to get one central well and six peripheral wells On the 2 5 x 7 5 cm slides, three wells were cut in a triangular fashion After charging the slides were kept in a humid chamber at room temperature for 48 hours and examined against light to discern the development of any precipitation line The slides were then washed in two changes of normal saline for 24 hours and subsequently in distilled water for another 24 hours to remove the excess unbound proteins It was then dried at 37°C after keeping a wet filter paper strip on its surface After drying the paper was removed and slides were stained with Amido black for 15 minutes, and decolourized in solutions I and II for 20 minutes each The slides were dried at 37°C for one hour and mounted on DPX

Immunoelectrophoresis

Three ml of melted 0 8 per cent agarose in tris barbiturate buffer was poured on each slide (2 5 cm x 7 5 cm) kept on a levelled surface The agar was allowed to harden for 30 minutes at 4°C Wells and troughs were cut on each slide and the agar was sucked out from the wells only

using a vacuum pump The wells were filled with antigens and a drop of bromphenol blue dye was added to the side of the well as indicator The slides were then placed in the electrophoresis chamber in such a way that the antigen wells were nearer to the cathode than to the anode Contact between the slides and the buffer was effected by filter paper wicks, one on each side of the slide, so that each covered about 1/2 cm of the agarose on the slide Current at a rate of 3 mA per slide was applied and the electrophoresis was continued till the indicator dye reached 1 cm away from the other end of the slide The slides were then taken and the agarose in the troughs were removed carefully The troughs were then filled with the respective antisera (Raising of antisera described elsewhere) Allowed the antisera to diffuse for 20-24 hours at room temperature, keeping the slides in the electrophoretic chamber itself Slides were then processed and stained as far for immunodiffusion

Quantitation of immunoglobulins

Quantitation of immunoglobulin fractions

Single radial immuno diffusion (SRID) technique developed by Mancini *et al* (1965) with slight modification was followed

Agarose gel (0 8%) in PBS was melted and kept at 56°C in a water bath Anti serum against 1st peak (RA1) and second peak (RA2) was warmed to 56°C and added to the agarose gel to obtain a final concentration of 7 per cent of antiserum in the gel These mixtures were overlayered on agar coated slides (7 5 cm x 5 cm) in 8 ml quantities After solidification, wells of 3 mm diameter were punched out with a distance of 12 mm between the wells The wells were then charged with 30 ul of varying dilutions of 1st and 2nd peak of known protein concentration and incubated at 4 C for 24 hours in a humid chamber

Antigen antibody precipitation rings formed around the wells were observed and the diameters measured The slides were washed and stained as in the case of immuno electrophorogram Duplicate determinations of the precipitation ring diameters were made and average values were taken to construct a regression curve and from the regression equation thus obtained, the concentrations of Igs in each sample was determined

The samples of serum, bile, intestinal washings tracheal washings, oviduct washings and testicular homogenate from the control and experimental ducks were used as test samples The test sample of oviduct washings were diluted 2 5 times The tracheal washings, intestinal washings and testicular extracts were concentrated 10 times

to ensure that the ring diameters, if produced are in a measurable range

Serological tests

The methods described be Chang *et al* (1957) with slight modification were followed

a **Bacterial agglutination to detect antibody against *S typhimurium***

The sera, bile, tracheal washings, intestinal washings oviduct washings and testicular extract collected from the experimental group were inactivated at 56°C for 30 minutes to destroy the non-specific agglutinins

Serial two fold dilutions of all the test samples (Serum, bile, tracheal washings, intestinal washings oviduct washings and testicular extract) were prepared by adding 0 8 ml of normal saline to 0 2 ml of the samples in the first tube and 0 5 ml transferred serially to the other tubes each containing 0 5 ml of normal saline A 0 5 ml quantity was then discarded from the last tube Equal quantity (0 5 ml) of bacterial antigen (1 billion organisms per ml) was then added to all the tubes and mixed well After incubation at 37°C for 24 hours, the end titre the highest serum dilution exhibiting visible aggregation of the

test sample was ascertained The titre was expressed as the reciprocal of this dilution

b Haemagglutination Inhibition test (HI)

The beta method of HI test was employed Serial two fold dilutions of the inactivated samples (Sera, bile tracheal washings, intestinal washings, oviduct washings and testicular extract) were made in normal saline by adding 0 6 ml of normal saline to 0 2 ml of sample in the first well, mixing and transferring 0 4 ml of this into the next well which contained 0 4 ml of normal saline This dilution was continued serially till the last well (10th well) from which 0 4 ml of the mixture was discarded Four HA units (0 2 ml) of the virus was added to all the wells After incubation at room temperature for 30 minutes, equal quantity (0 4 ml) of 0 5 per cent suspension of chicken RBC was added to each well and mixed well Simultaneously, RBC and virus controls were made and the readings were taken after 30 minutes of incubation at room temperature

Results

RESULTS

Fractionation of immunoglobulins by chromatography

Fractionation of Igs obtained from serum, bile tracheal washings and intestinal washings by salting out was attempted in this study The peaks obtained in the first chromatographic separation of the salted out protein fractions of the above samples are represented graphically in figures 1 to 4 Two peaks were very conspicuous in the case of serum and tracheal Igs whereas only one peak could be obtained in the case of bile and intestinal Igs

Chromatography of serum Ig

The first peak was obtained between the 8th and the 12th fraction (Fig 1) The second peak was more pronounced and was obtained between the 15th and 27th fractions AGPT of the pooled, concentrated 1st and 2nd peak fractions against antiduck serum raised in rabbit (RADSIg) gave a distinct line for the 1st peak with a spur formation over the line given by the second peak, which was more diffuse (Frame 1) On recycling the pooled, concentrated first peak fractions on Sephadex gel, only a single elution peak was obtained between the 10th and 15th fraction tube (Fig 5)

AGPT of individual fractions were carried out against RADSIg Distinct lines were produced for all fractions from the 10th to 17th tube but was accompanied by another milder line which was least in the line produced by the 10th fraction and became more distinct as the limb ascended but was vague in the 17th fraction (Frame 2) The 10th to 15th fraction was further concentrated and rerun through the same column Here again, only a single elution peak was seen between 11th and 16th fraction tubes (Fig 6) The AGPT of these fractions against RADSIg showed distinct lines for fraction 11 to 14 accompanied by a faint line, but only one distinct precipitation line was formed by the 15th fraction (Frame 3) The void volume was also pooled and concentrated and checked for presence of Igs by AGPT against RADSIg but none could be detected

The second peak fractions (15-27), pooled, concentrated and rerun by Sephadex gel (Fig 7) also gave only a single elution peak, more extended than that obtained for the first peak On AGPT against anti-duck rabbit serum, fractions 11 to 14 gave a distinct line with minor contaminants Fractions 15 to 19 had more number of lines (Frame 4) while fractions 20 to 24 had only a single distinct line (Frame 5) Fractions 10 to 29 when again pooled concentrated and rerun on Sephadex gel (Fig 8) gave a similar wider peak between the 13th and 28th fraction tubes

Chromatography of bile Ig

Only a single peak was obtained between the 12th and 20th fraction (Fig 2) Only fractions 14 to 20 produced precipitation lines on AGPT against RADSIg with the intensity gradually decreasing from 16th fraction (Frame 6) Fractions 32 to 49 were pooled, concentrated and tested by AGPT for the presence of Ig but none was detected

Chromatography of tracheal Ig

Two peaks were obtained, first between the 10th and 17th fraction and the second minor peak between the 22nd upto 31st fraction (Fig 3) The Igs eluted in 2 peaks were found to be different by AGPT (Frame 7) Fraction 14 produced a faint line and fraction 15, a distinct line on AGPT against RADSIg (Frame 8) Fractions from the second peak also produced only a distinct line on AGPT against RADSIg (Frame 9)

Chromatography of intestinal Ig

A single peak was obtained between the 10th and 15th fraction (Fig 4), which gave only a single line on AGPT against RADSIg (Frame 10)

AGPT against RADSIg

1 Serum

Formed three lines of precipitation, of which one was very faint (Frame 11)

2 Tracheal washings

Three distinct lines were observed (Frame 12)

3 Bile

Two distinct lines of precipitation were produced (Frame 13)

4 Intestinal washings

Only a single line was produced (Frame 14)

Relationship between Igs in various secretions and serum as determined by AGPT against RADSIg

Immunodiffusion of whole (WS) serum, Serum Ig (SIg) bile Ig (BIg), intestinal Ig (IIg), Tracheal Ig (TIg) were conducted against RADSIg (Frame 15) All the wells were connected by a line of identity The TIg had 2 additional lines The central line formed by TIg showed identity with BIg The third line formed by TIg was towards the outer

well and did not form any line of identity SIg formed a distinct additional line behind the same line of identity Ig separated from testes and ovary also formed this line of identity but ovarian Ig formed a faint, additional line (Frame 16)

AGPT of Igs from serum and various secretions against RA1

A faint line of identity was formed between BIg, TIg IIg, oviduct Ig (OIg) and SIg A distinct line was formed for SIg which extended upto the edge of the two adjacent wells (Frame 17)

AGPT of Igs from serum and various secretions against RA2

A diffuse line of identity was formed between testicular Ig (Test Ig), SIg, OIg and TIg The line was not present for IIg and BIg (Frame 18)

Immunoelectrophoresis

Serum fractions

Fraction taken from the ascending limb of the first peak gave two arcs which merged towards the cathodal end and bifurcated towards the anodal end with one arm extending more towards the anode

The fraction taken from the middle of the second peak gave a hazy arc in between the well and trough but closer to the latter (Frame 19)

Tracheal Ig

The tracheal Ig formed 3 arcs between the well and trough Two other lines formed in front of the well, towards the trough, of which one extended as a haze towards the anode (Frame 20)

Tracheal fractions

Ascending limb fraction of the first peak from chromatography gave a single arc between the well and the trough and a hazy line in front of it which was hardly detectable

The ascending limb fractions of the first peak gave an arc The fraction from the centre of the second peak also gave only a single arc (Frame 20)

Pooled, concentrated first peak fractions gave two arcs which merged towards the cathodal end and bifurcated toward the anode (Frame 21 1)

Pooled and concentrated second peak gave only a single arc in between the well and trough (Frame 21 2)

Intestinal Ig

Only a single faint arc between the well and trough was produced by the pooled and concentrated peak obtained by gel filtration chromatography (Frame 21 3)

Oviduct Ig

Two separate arcs were obtained between the well and the trough, the larger arc was closer to the trough (Frame 22 1)

Biliary Ig

Produced only a single arc between the well and the trough (Frame 22 2)

Mancini's test for fraction 1 (Using RA 1)

Effect of immunization with *Salmonella typhimurium*

Control

All the test samples except intestinal washings and testicular extract showed precipitation rings against RA1 on substitution of the diameter values in the standard curve (Fig 9) (Frame 23), the serum was found to have fraction 1 concentration of 1477 ug/ml, bile showed a concentration of 270 ug/ml, tracheal washings, 72 ug/ml and oviduct washing 873 39 ug/ml The concentrations of fraction 1 in various

samples in control and all treatment groups are shown in Table 1

Treatment Groups

1 **Intramuscular, 0 5 ml (Frame 24.A)**

The bile, tracheal washings, oviduct washings intestinal washings and serum had concentrations of 1718 06 µg/ml, 75 27 µg/ml, 873 39 µg/ml, 75 27 µg/ml and 2080 06 µg/ml respectively

2 **Intramuscular, 1 ml (Frame 24 B)**

The concentrations of fraction 1 in bile, tracheal washings, oviduct washings, intestinal washings and serum were 1718 06 µg/ml 51 14 µg/ml, 873 39 µg/ml, 172 µg/ml and 2442 µg/ml respectively

3 **Subcutaneous, 1 ml (Frame 25 A)**

The concentrations of fraction 1 in bile, tracheal washings, testicular extracts, intestinal washings and serum were 1718 µg/ml, 75 µg/ml, 51 2 µg/ml, 75 µg/ml and 1959 µg/ml respectively

4 Subcutaneous, 0 5 ml (Frame 25 B)

The concentratıons of fractıon 1 ın bıle, tracheal washıngs, ıntestınal washıngs and serum were 1959 µg/ml 51 14 µg/ml, 75 3 µg/ml, 51 14 µg/ml and 1718 06 µg/ml respectıvely

Effect of ımmunızatıon wıth NDV

Sımılar samplıng lınes were followed as for *Salmonella typhımurıum*

Control

All the samples of sera and secretıons showed the presence of fractıon 1 except testıcular extract and ıntestınal washıngs The concentratıon of fractıon 1 ın bıle, tracheal washıngs, ovıduct washıngs and serum were 270 05 µg/ml, 87 37 µg/ml, 873 37 µg/ml and 1477 µg/ml respectıvely The concentratıon of fractıon 1 ın the control and treatments groups are gıven ın Table 2

1 Subcutaneous, 0 5 ml (Frame 26)

The bıle, tracheal washıngs, testıcular extract and serum showed concentratıons of fractıon 1 as 1597 35 µg/ml 82 µg/ml, 75 3 µg/ml and 1959 34 µg/ml respectıvely No

measurable quantity of this fraction was detected in the intestinal washings

2 Intramuscular, 0 5 ml (Frame 27 A)

The concentrations of fraction 1 were 1115 µg/ml 75 µg/ml, 1235 39 µg/ml and 1959 µg/ml in the bile, tracheal washings, oviduct washings and serum respectively Measurable quantity was not detected in intestinal washings

3 Intranasal, 0 5 ml (Frame 27 B)

The bile, tracheal washings, testicular extract and serum gave concentrations of 1115 µg/ml, 87 34 µg/ml, 75 3 µg/ml and 1597 35 µg/ml respectively

Mancini's test for fraction 2 (using RA2)

Effect of immunization with *S typhimurium*

Quantitation of 2nd fraction was done by Mancini s technique as for fraction 1 The ring diameters obtained were substituted in the regression equation obtained from a standard curve for fraction 2(Graph 10) (Frame 28)

Control

Only serum and tracheal washings showed the presence of fraction 2, the concentrations being 1762 ug/ml and

4 16 µg/ml respectively The concentration of fraction 2 in the control and treatment samples are given in Table 3

Treatment Groups

1 Intramuscular, 1 ml

The tracheal washings, oviduct washings, and serum showed concentrations of 36 µg/ml, 279 91 µg/ml, and 2591 µg/ml respectively

2 Intramuscular, 0 5 ml

The concentration of this fraction was 20 05 µg/ml, 200 47 µg/ml, and 2005 µg/ml in tracheal washings, oviduct washings and serum respectively

3 Subcutaneous, 1 ml

In this experimental group, the concentration of fraction 2 in tracheal washings, testicular extract and serum were 27 9 µg/ml, 12 10 µg/ml and 2194 µg/ml respectively

4 Subcutaneous, 0 5 ml

The concentration of the said fraction in this group in tracheal washings, testicular extract and serum were 12 10 µg/ml, 4 16 µg/ml and 1797 µg/ml respectively

Effect of immunization with NDV

Similar sampling lines were followed as for *Salmonella typhimurium*

Control

Only the tracheal washing and serum showed the presence of fraction 2 with a concentration of 4 16 ug/ml and 1370 ug/ml respectively The concentrations of the said fraction in samples of various treatment groups are given in Table 4

Treatment Groups

1 Intramuscular, 0 5 ml

The tracheal washings, oviduct washings and serum contained a concentration of 20 8 µg/ml, 41 59 µg/ml and 1797 µg/ml respectively

2 Intranasal, 0 5 ml

The concentration of fraction 2 obtained for tracheal washings and serum in this group were 27 91 µg/ml and 1400 µg/ml respectively

3 Subcutaneous, 0 5 ml

The concentrations of fraction 2 obtained for tracheal washings and serum 12 µg/ml and 1796 73 µg/ml respectively

The comparison of quantity of first and second peaks in serum and tracheal washings in ducks treated with *S typhimurium* are given in Fig 11 and Fig 12 respectively Similar comparison between quantities of first and second peaks in serum and tracheal washings in ducks treated with NDV are given in Fig 13 and Fig 14 respectively

Standard tube agglutination test (STAT) of serum and various secretions of *S. typhimurium* treated ducks

Control

No agglutination was obtained for serum or any other samples The agglutination titres obtained for various samples in the experimental group are given in Table 5 The samples collected under each experimental group were pooled before subjecting it to STAT

Treatment Groups

1 Intramuscular, 1 ml

The serum gave an end titre of 1 in 160 and bile 1 in 80 Agglutination was detected in the first agglutination tube (1 in 10) of oviduct washings No agglutination could be detected for tracheal washings (TW) and intestinal washings (IW)

2 Intramuscular 0 5 ml

Serum and bile had agglutination titres of 1 in 80 and 1 in 20 respectively No agglutination was detected in tracheal washings intestinal washings and oviduct washings

3 Subcutaneous 1 ml

Serum and bile gave a titre of 1 in 160 and 1 in 20 respectively Tw Iw and testicular homogenate did not produce any detectable agglutination

4 Subcutaneous, 0 5 ml

Serum and bile gave a titre of only 1 in 20 and 1 in 10 respectively No detectable agglutination was present in tubes containing Tw, Iw and test extract The above secretions and serum from control birds did not produce any agglutination

Haemagglutination inhibition test

HI test was carried out for oviduct washings tracheal washings, bile, serum intestinal washings and testicular homogenate Separately pooled samples from each experimental group were used for the test The results are furnished in Table 6

Control (Frame 29)

No HI was detected in serum and other samples of control birds with the exception of bile which showed a titre of 1 in 64

Treatment Groups

1 Intramuscular, 0 5 ml (Frame 30)

Oviduct washings gave an HI titre of 1 in 16 while serum gave a titre of 1 in 128 and bile 1 in 64

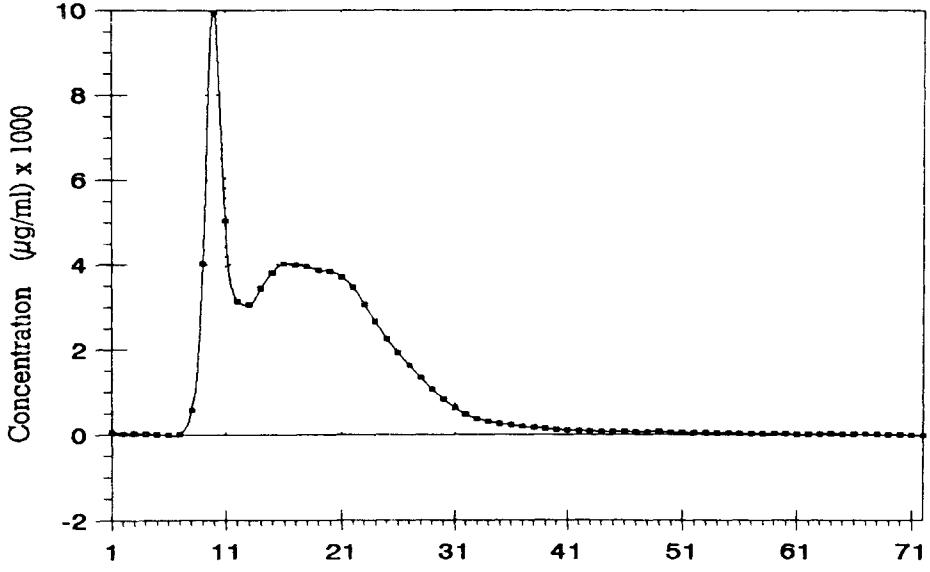
2 Subcutaneous, 0 5 ml (Frame 31)

Serum and bile gave a titre of 1 64 Intestinal washings tracheal washingsand testicular homogenate did not show any HI

3 Intranasal 0 5 ml (Frame 32)

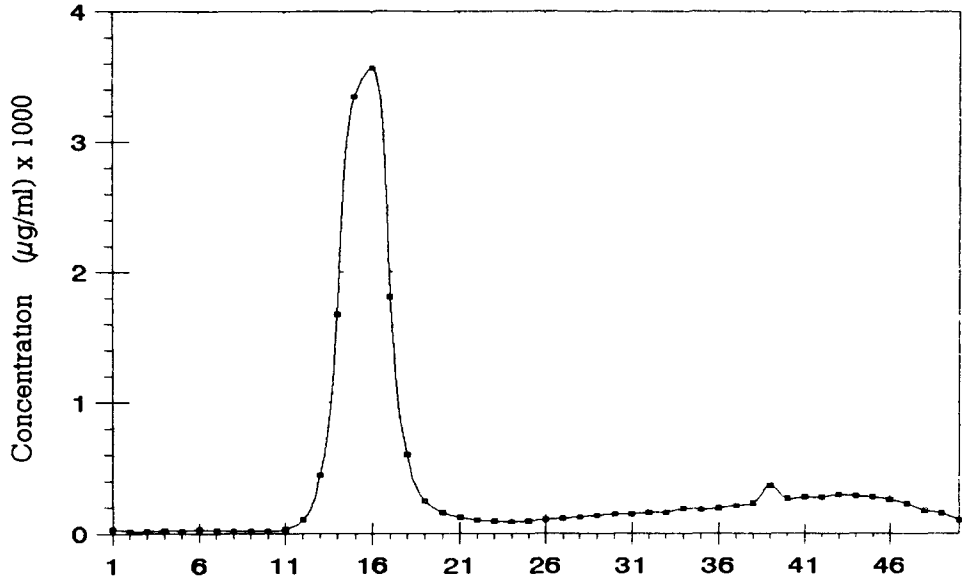
Serum and bile gave a titre of 1 32 Intestinal washings, tracheal washings and testicular homogenate did not show any HI

Fig. 1 ***Sephadex G-200 Chromatogram of serum I_g***



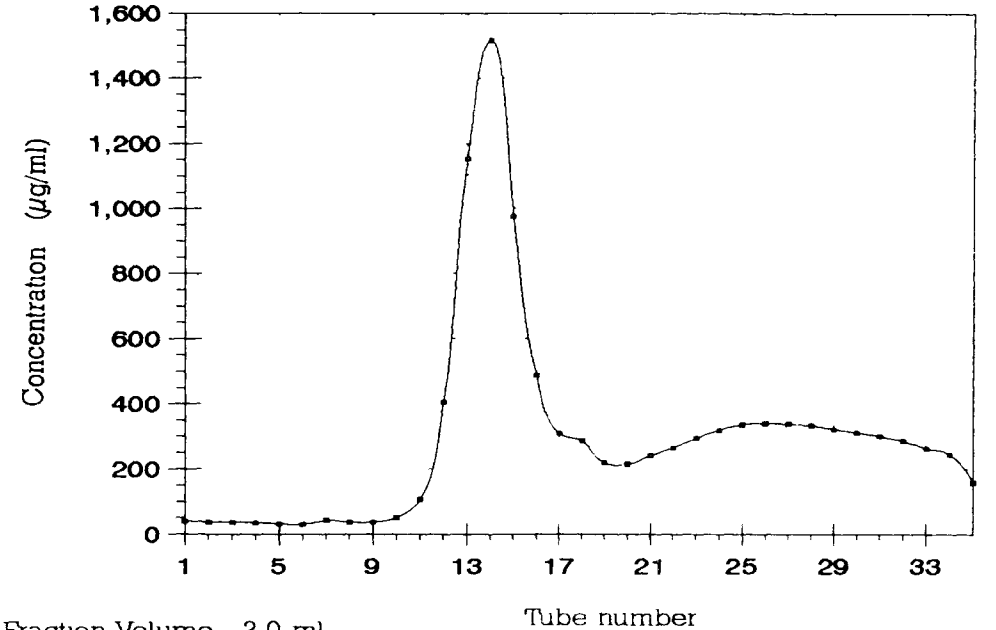
Fraction Volume 2 0 ml

Fig 2 ***Sephadex G-200 Chromatogram of bile I_g***



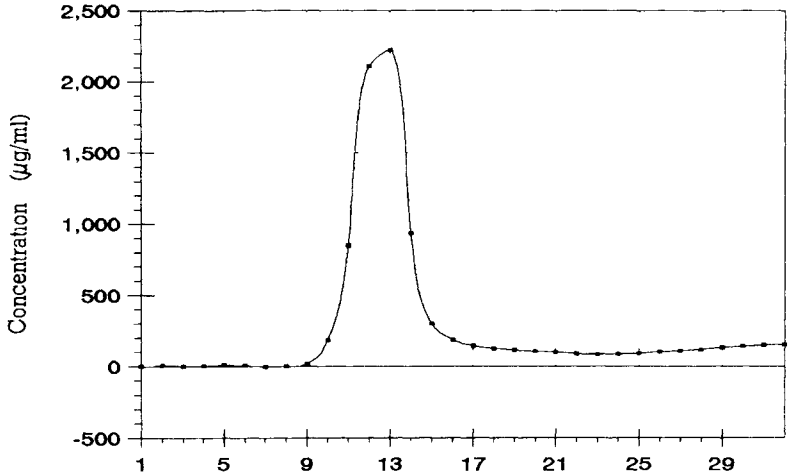
Fraction Volume 2 0 ml

Fig 3 **Sephadex G-200 chromatogram of tracheal I_g**



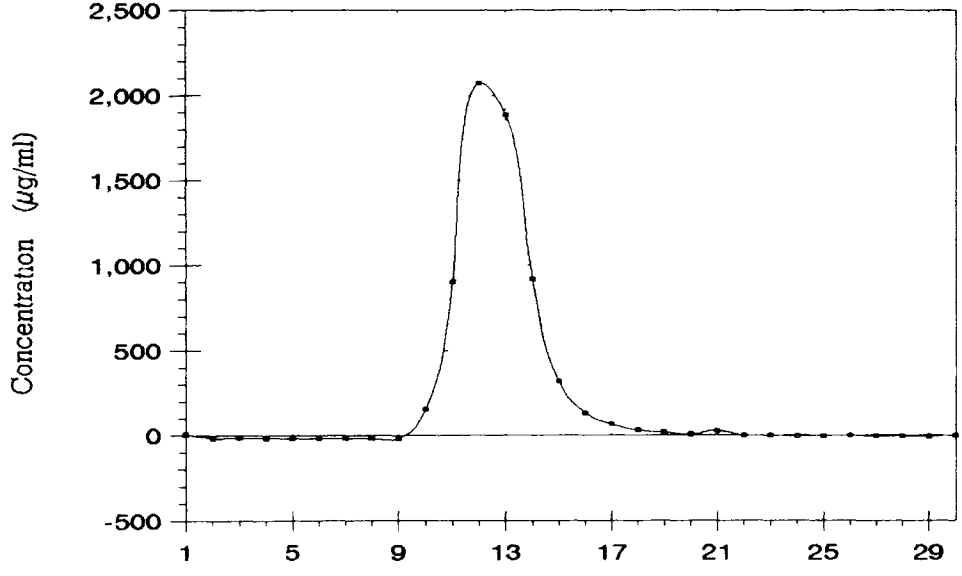
Fraction Volume 2 0 ml

Fig 4 **Sephadex G-200 chromatogram of intestinal I_g**



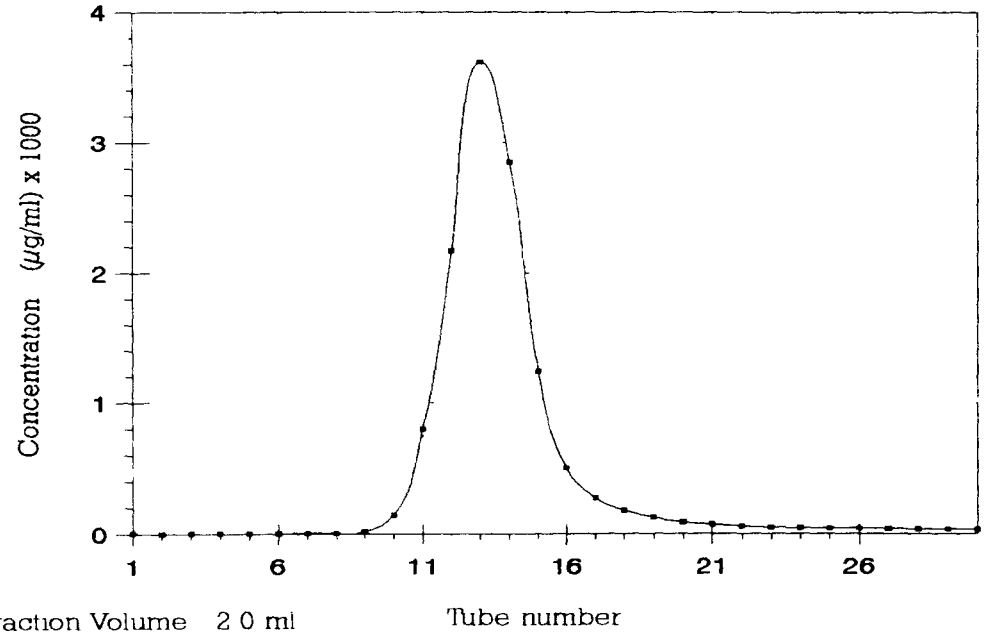
Fraction Volume 2 0 ml

Fig 5 **Chromatogram of first rerun of first peak of serum I_g**



Fraction Volume 2 0 ml

Fig 6 **Chromatogram of second rerun of first peak of serum I_g**



Fraction Volume 2 0 ml

Fig 7 ***Chromatogram of first rerun of second peak of serum I_g***

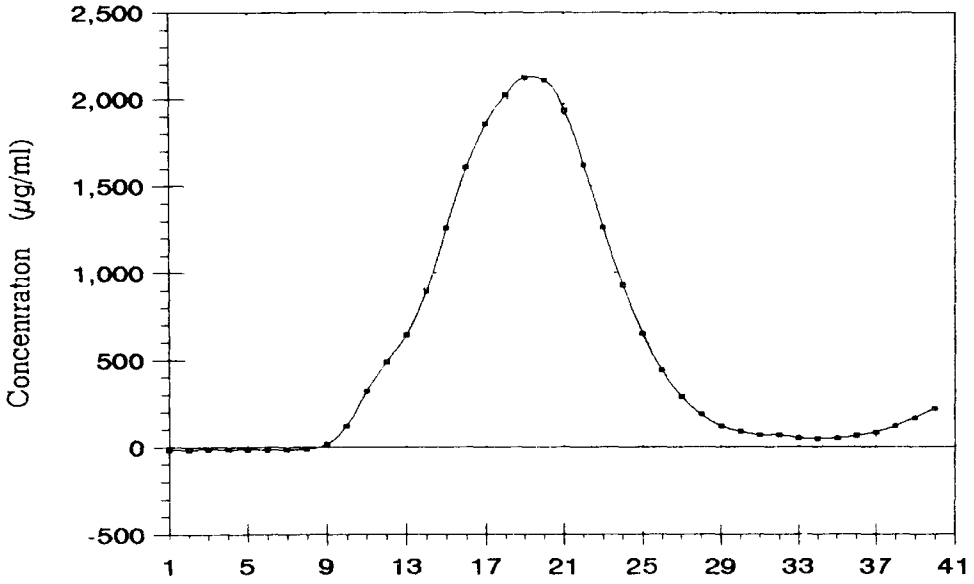
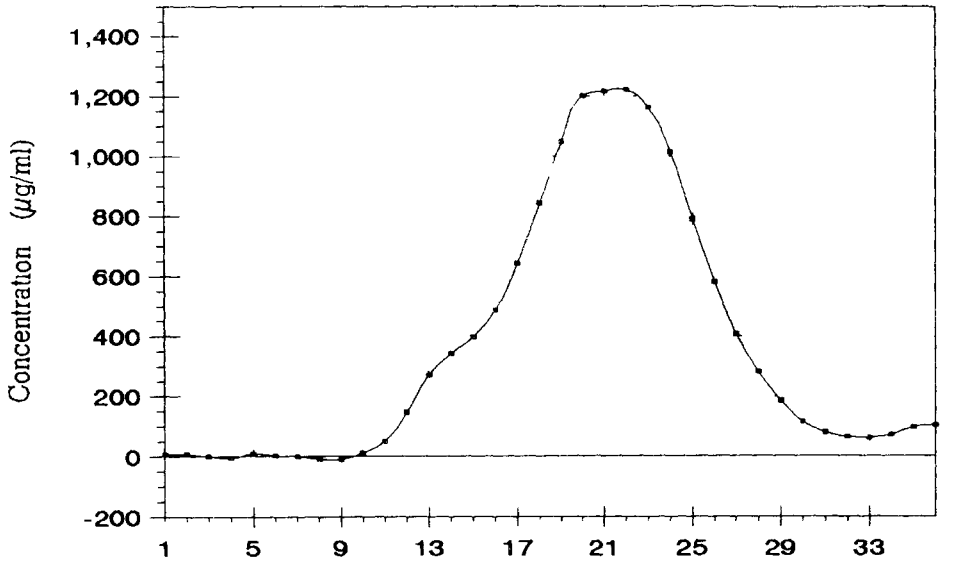


Fig 8 ***Chromatogram of second rerun of second peak of serum I_g***



Fraction Volume 2 0 ml

Fig 9 **Mancini's test - standard curve for first peak**

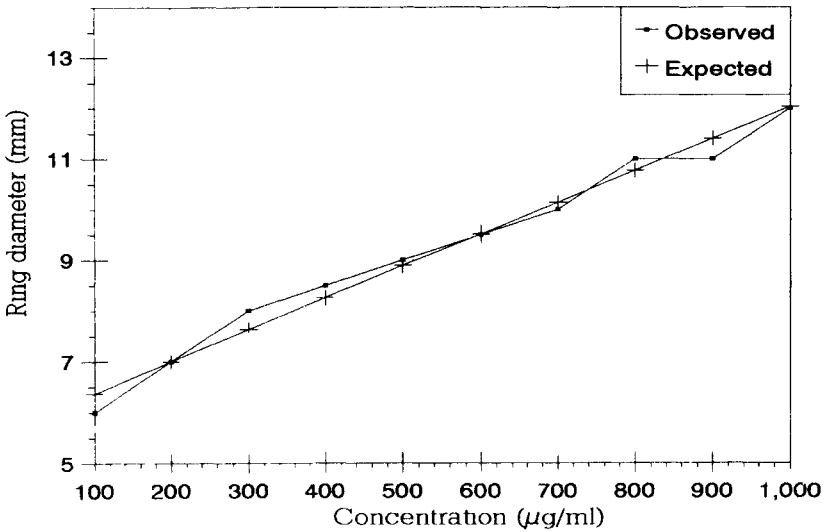
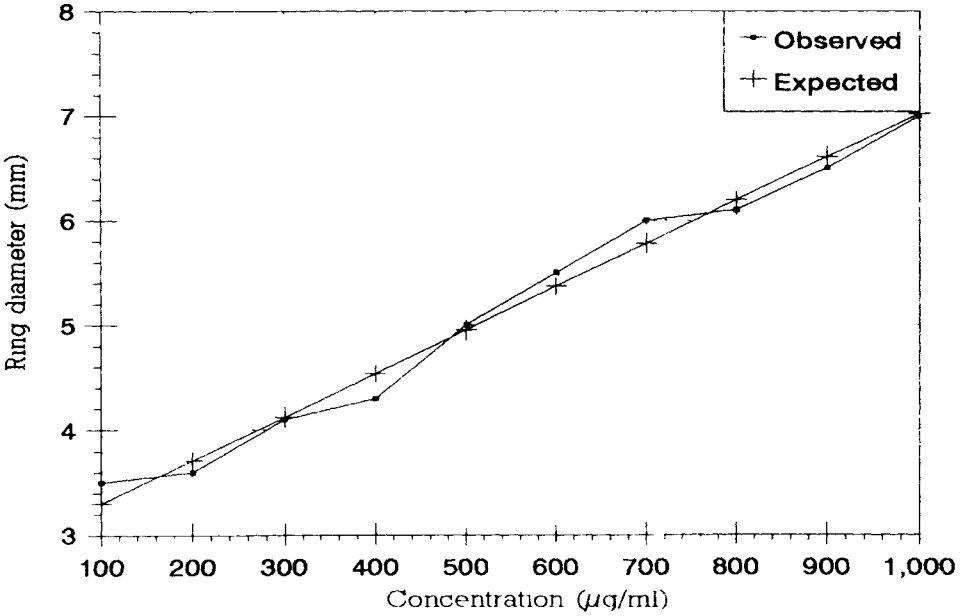


Fig 10 ***Mancini's test - Standard curve for second peak***



Levels of first and second peaks of *S.typhimurium* (STY) treated duck serum

Fig 11

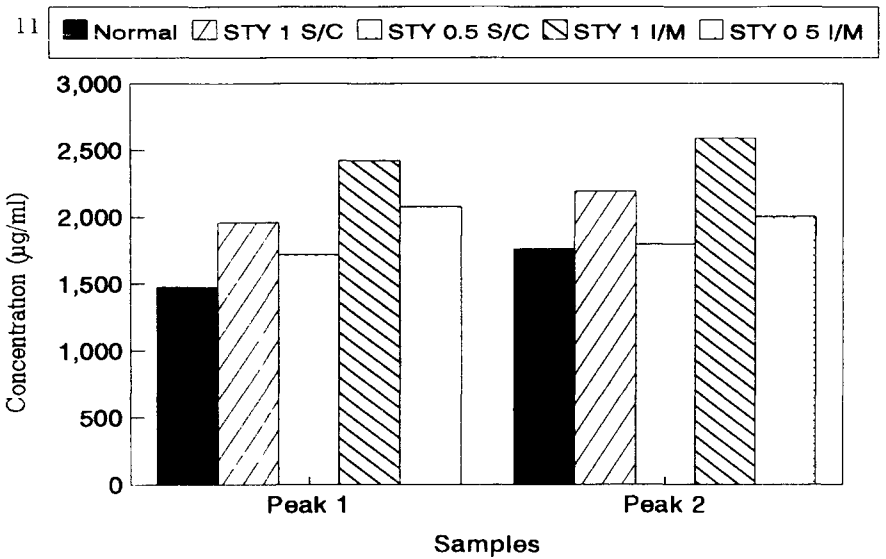
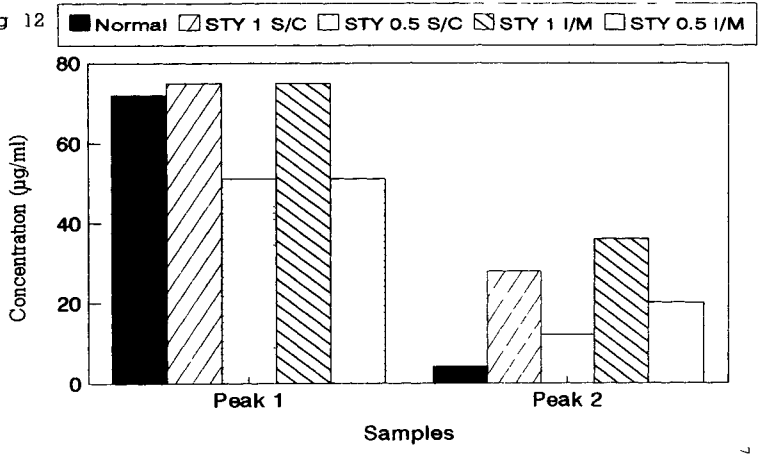
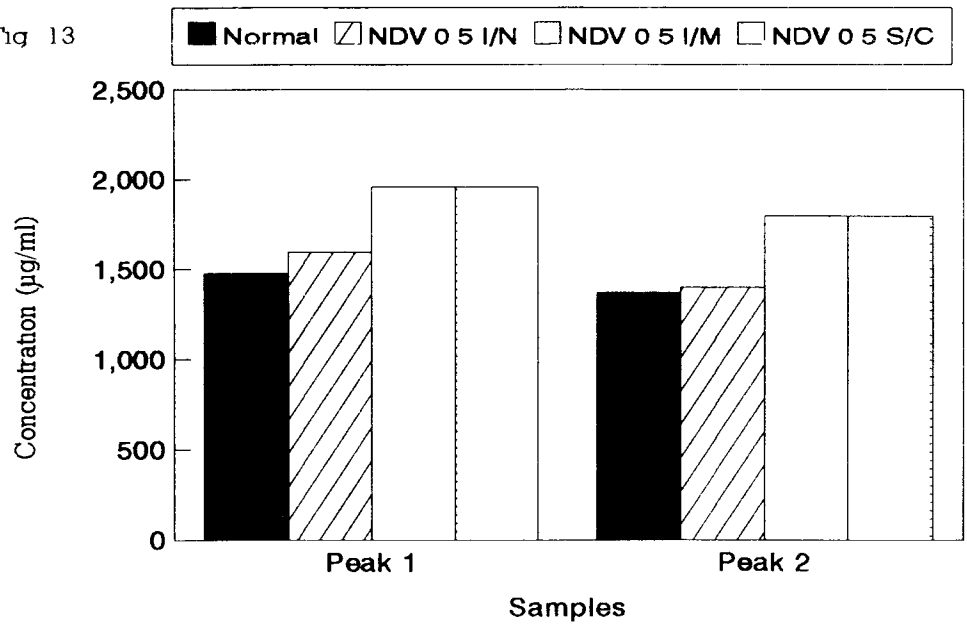


Fig 12


Levels of first and second peaks in tracheal washings of
S.typhimurium (STY) treated ducks
Normal
STY 1 S/C
STY 0.5 S/C
STY 1 I/M
STY 0.5 I/M
Concentration (μg/ml)
80
60
40
20
0
Peak 1
Peak 2
Samples

Levels of first and second peaks of NDV treated duck serum

Fig 13



Levels of first and second peaks in tracheal washings of NDV treated ducks

Fig 14

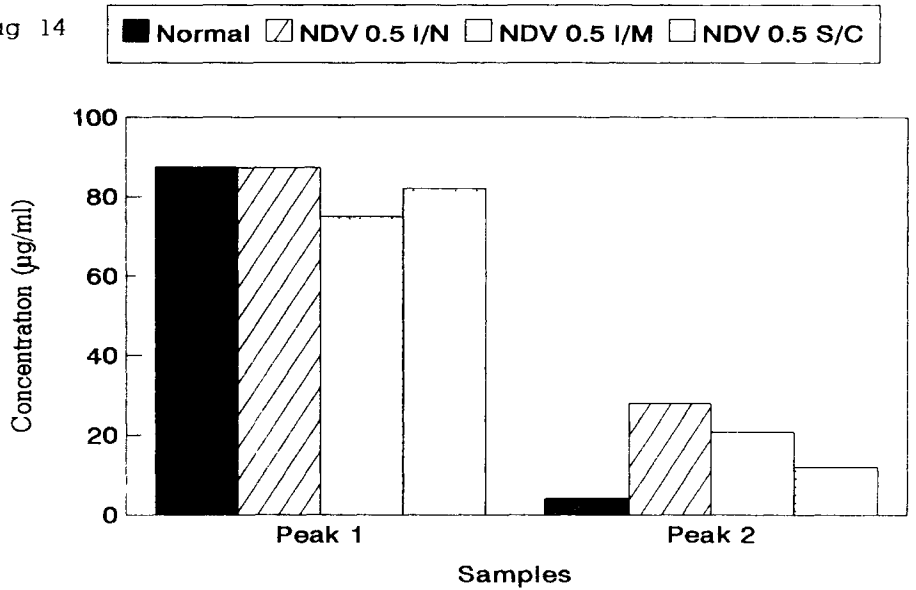


Table 1 Concentration (µg/ml) of fraction 1 in *S typhimurium* treated ducks as estimated by Mancini's method

Sample	Control	Treatment groups				
		1 ml S/C	0 5 ml S/C	0 5 ml I/M	1 ml I M	
Bile	270	1718	1959	1718 06	1718 06	
Tracheal washings	72	75	51 14	75 27	51 14	
* Testicular extract		51 2	75 3			
** Oviduct washings	873 39			873 39	873 39	
Intestinal washings		75	51 14	75 27	172	
Serum	1477	1959	1718 06	2080 06	2442	

* Not sampled in I/M group

** Not sampled in S/C group

Table 2 Concentration (µg/ml) of fraction 1 in NDV treated ducks as estimated by Mancini's method

Sample	Control	Treatment groups		
		0 5 ml I/M	0 5 ml S/C	0 5 ml I/N
Bile	270 05	1115	1597 35	1115
Tracheal washings	87 37	75	82	87 34
* Testicular extract		-	75 3	75 3
** Oviduct washings	873 37	1235 39		
Intestinal washings				
Serum	1477	1959	1959 34	1597 35

* Not sampled in I/M group

** Not sampled in S/C and I/N group

Table 3 Concentration (µg/ml) of fraction 2 in *S typhimurium* treated ducks as estimated by Mancini's method

Sample	Control	Treatment groups			
		1 ml S/C	0 5 ml S/C	1 ml I/M	0 5 ml I/M
Bile					
Tracheal washings	4 16	27 90	12 10	36	20 05
* Testicular extract		12 10	4 16		
** Oviduct washings				279 91	200 4/
Intestinal washings	-				
Serum	1762	2194	1797	2591	2005

* Not sampled in I/M group

** Not sampled in S/C group

Table 4 Concentration (µg/ml) of fraction 2 in NDV treated ducks as estimated by Mancini's method

Sample	Control	Treatment groups 0.5 ml I/N	0.5 ml S/C	0.5 ml I/M
Bile				
Tracheal washings	4 16	27 91	12	20 8
* Testicular extract	-	-	-	
** Oviduct washings				41 59
Intestinal washings				
Serum	1370	1400	1796 73	1797

* Not sampled in I/M group

** Not sampled in S/C and I/N group

Table 5 Standard tube agglutination test of duck samples against *S typhimurium*

Sample	Control	Treatment groups			
		1 ml I/M	0 5 ml I/M	1 ml S/C	0 5 ml S/C
Bile	-	1 80	1 20	1 20	1 10
Tracheal washings	-	-			
Testicular extract	-				
Oviduct washings		1 10			
Intestinal washings					
Serum		1 160	1 80	1 160	1 20

Table 6 Haemagglutination inhibition test of duck samples against NDV

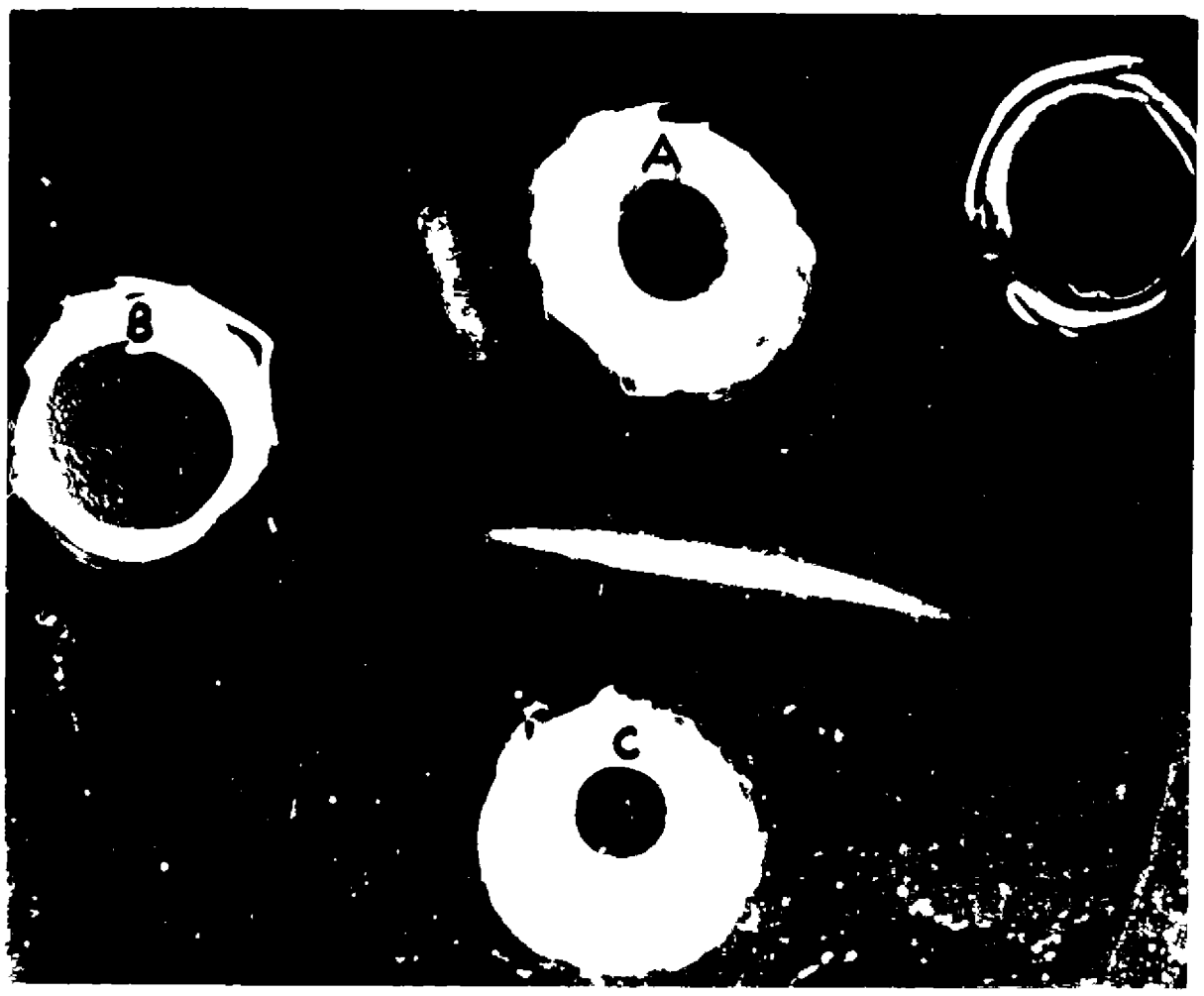
Sample	Control	Treatment groups		
		0 5 ml I/M	0 5 ml S/C	0 5 ml I/N
Bile	1 64	1 64	1 64	1 32
Tracheal washings	-			
Testicular extract				
Oviduct washings		1 16		
Intestinal washings				
Serum		1 128	1 64	1 32

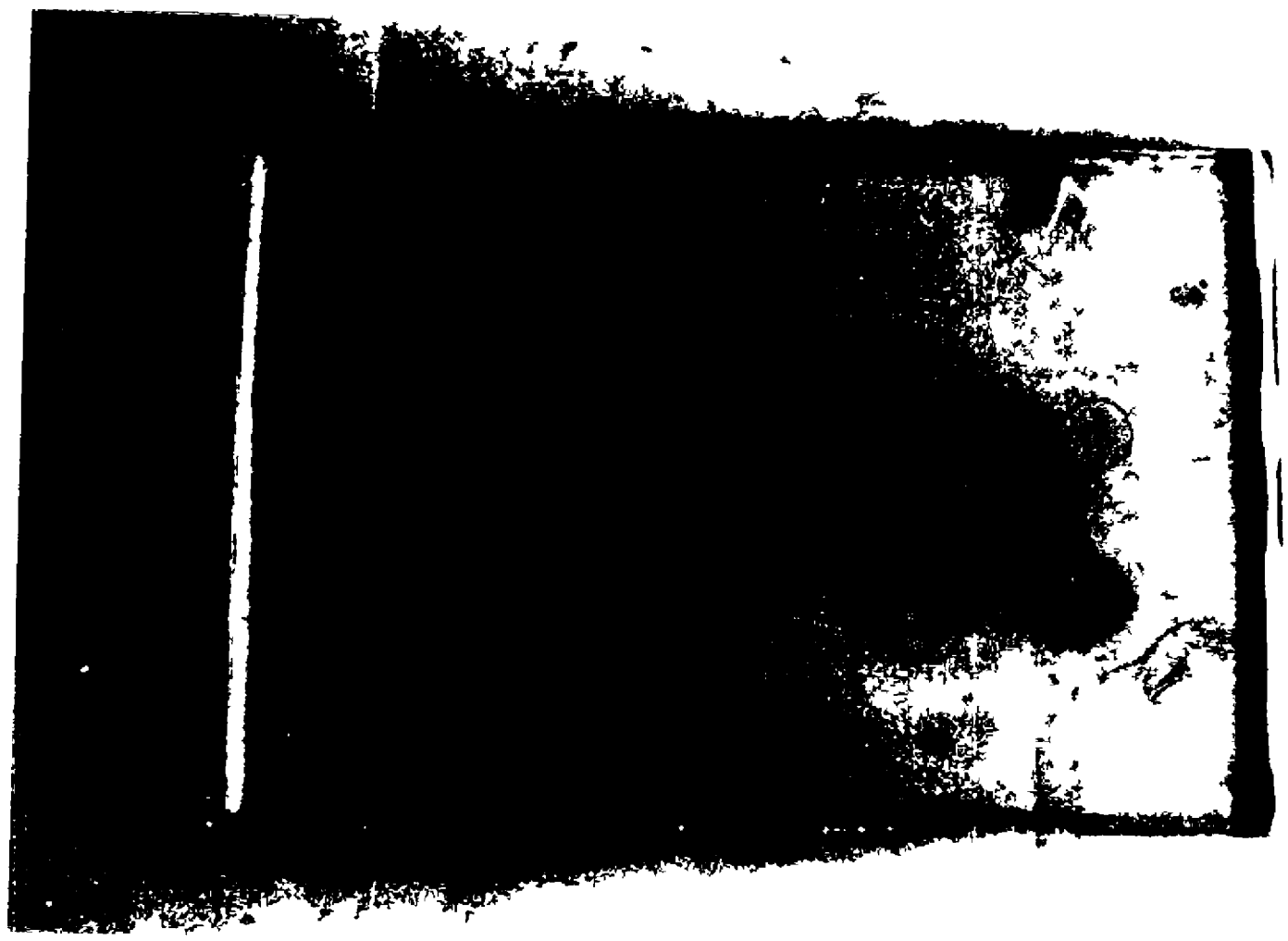
Frame 1 AGPT of 1st and 2nd peaks of serum Ig obtained by chromatography against RADSIg

A RADSIg
B Second peak
C First peak

Frame 2 AGPT of fraction (10-19) obtained on first rerun of first peak of serum Ig against RADSIg

A RADSIg



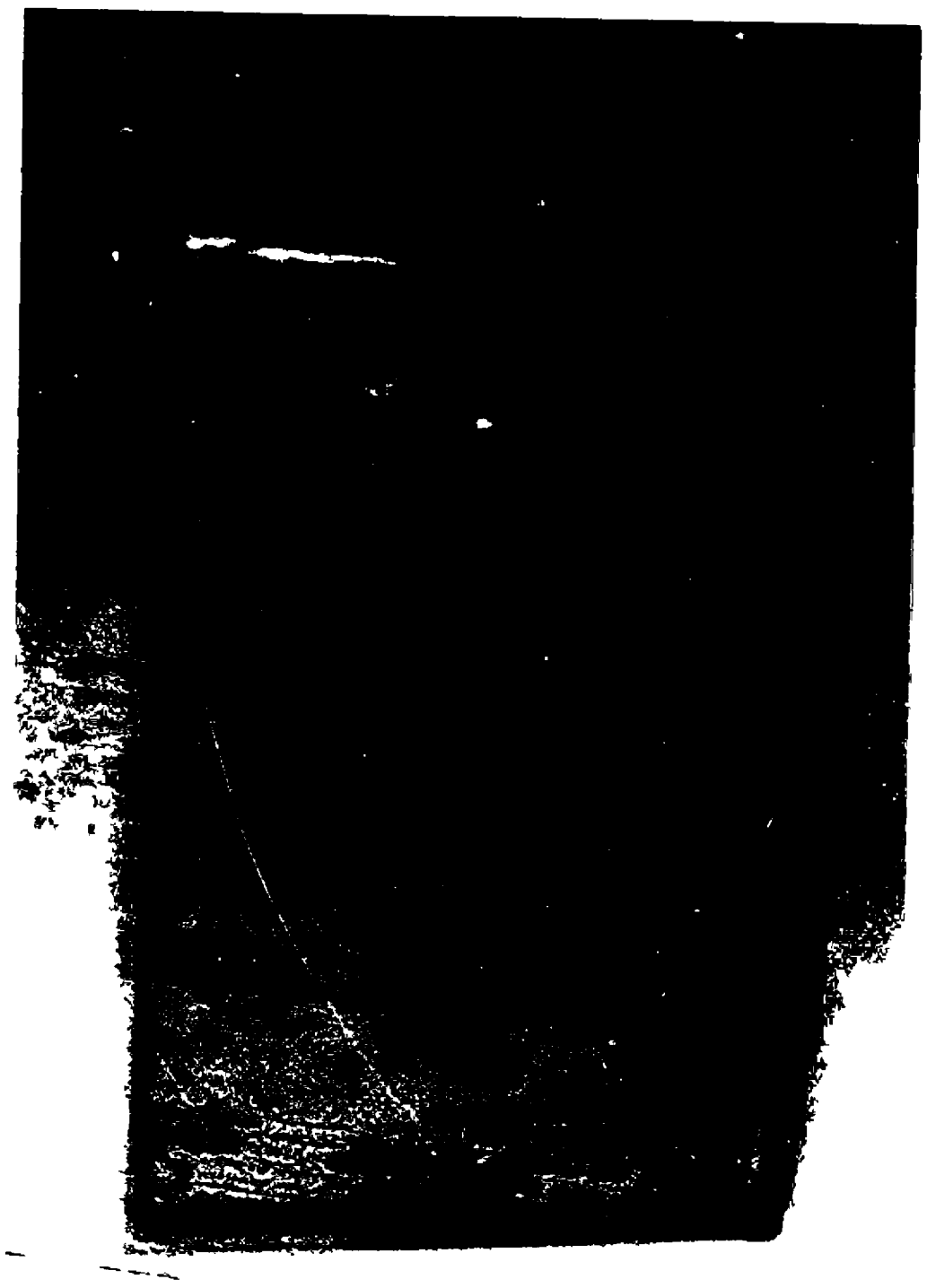


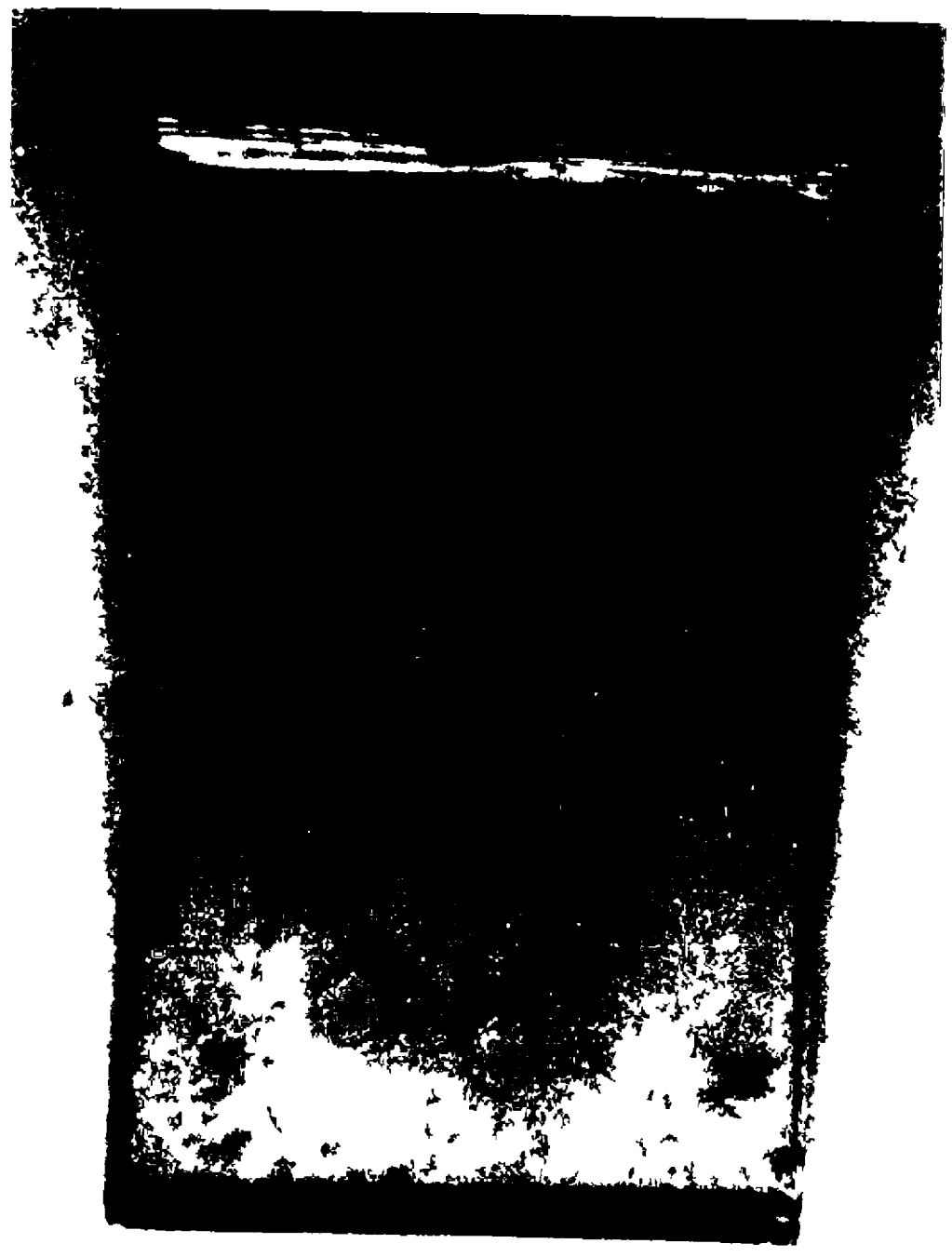
Frame 3 AGPT of fractions (10-19) obtained on second rerun of first peak of serum Ig against RADSIg

A RADSIg

Frame 4 AGPT of fractions (10-19) obtained on first rerun of second peak of serum Ig against RADSIg

A RADSIg



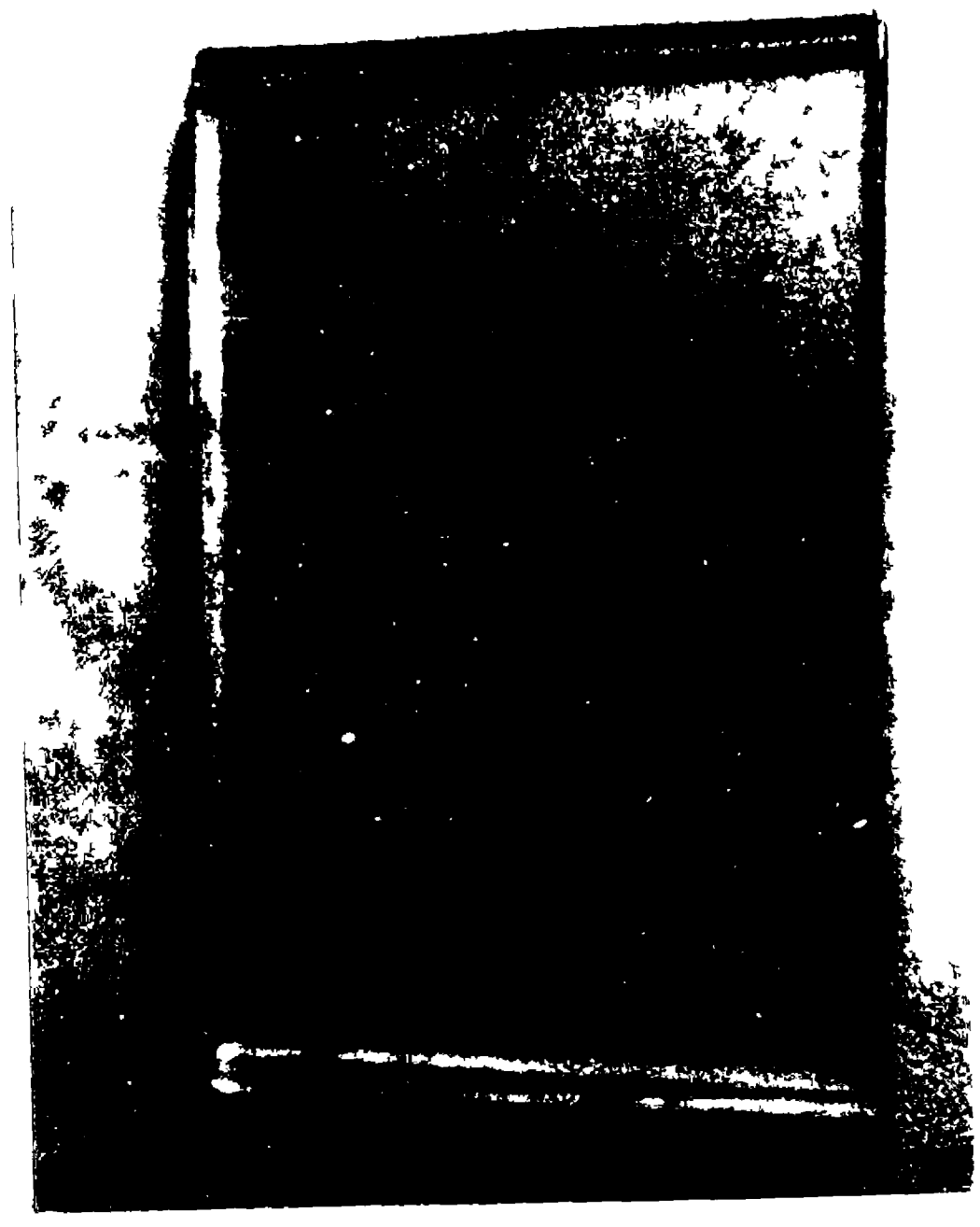


Frame 5 AGPT of fractions (20 29) obtained on first rerun of second peak of serum Ig against RADSIg

A RADSIg

Frame 6 AGPT of fractions (11 20) obtained by chromatography of bile against RADSIg

A RADSIg

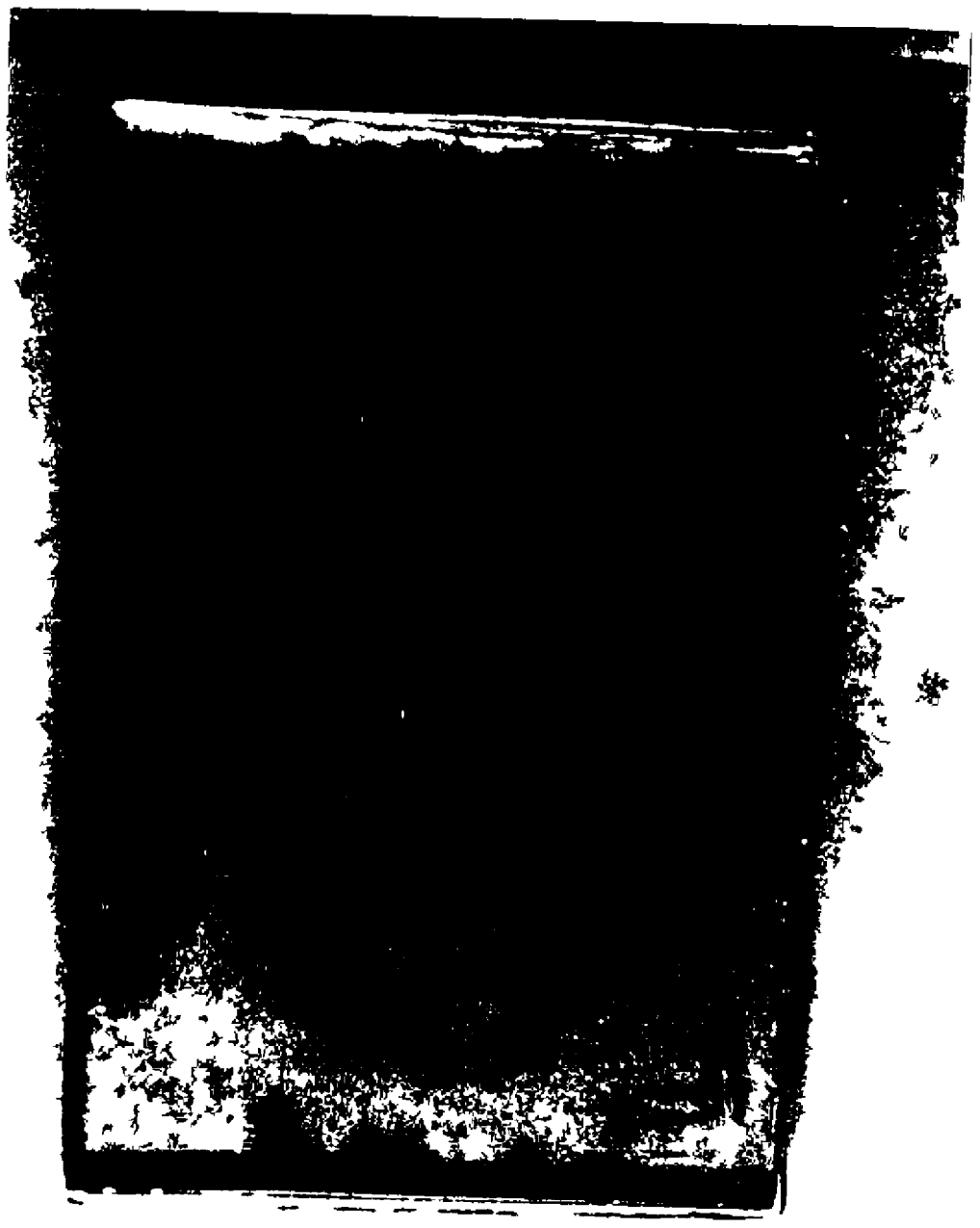


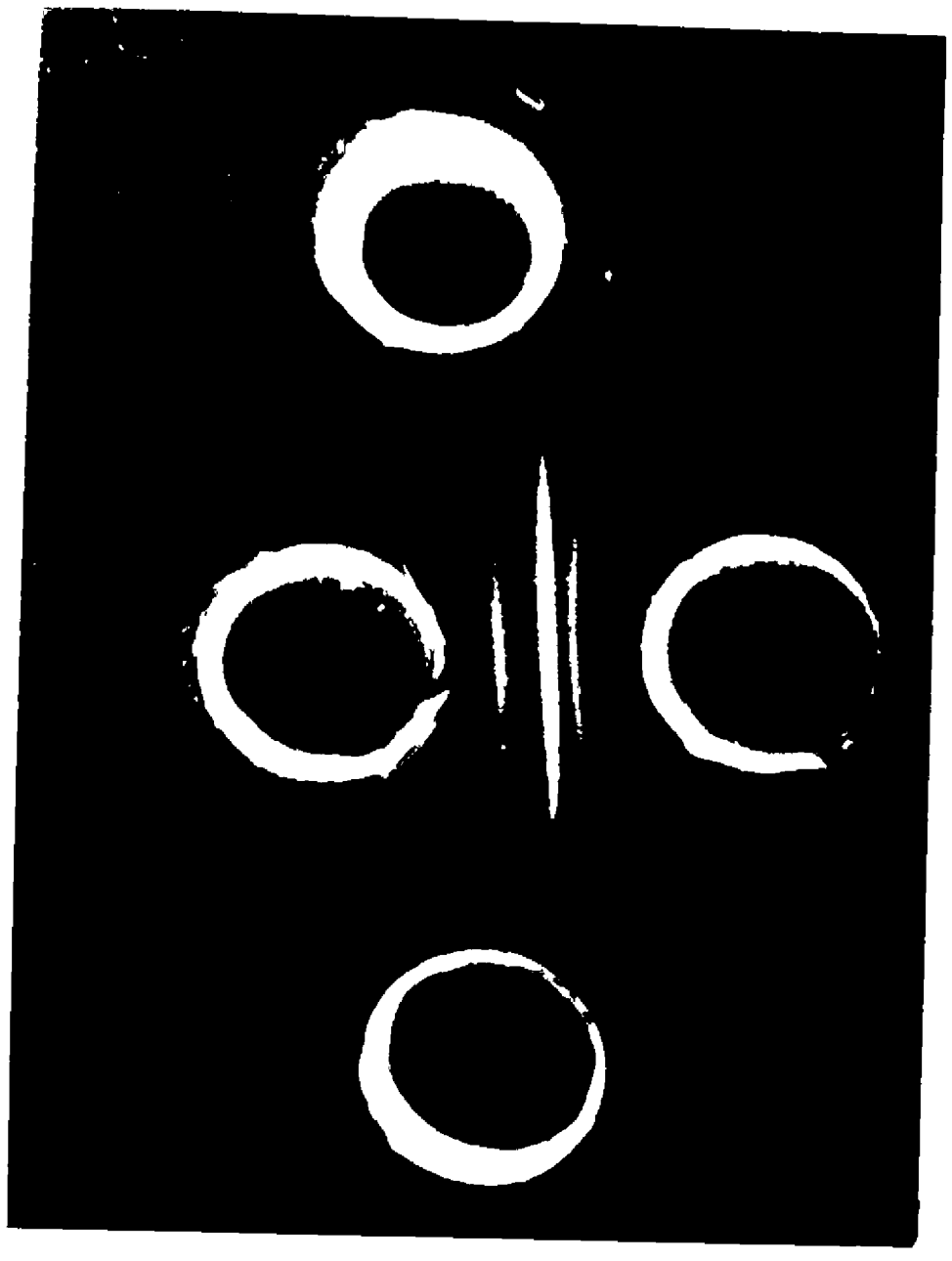
Frame 7 AGPT of first and second peak obtained by chromatography of tracheal Ig against RADSIg

A RADSIg
B 1st peak of TIg obtained by chromatography
C 2nd peak of TIg obtained by chromatography
D TIg

Frame 8 AGPT of first peak fractions (10-19) obtained by chromatography of tracheal Ig against RADSIg

A RADSIg



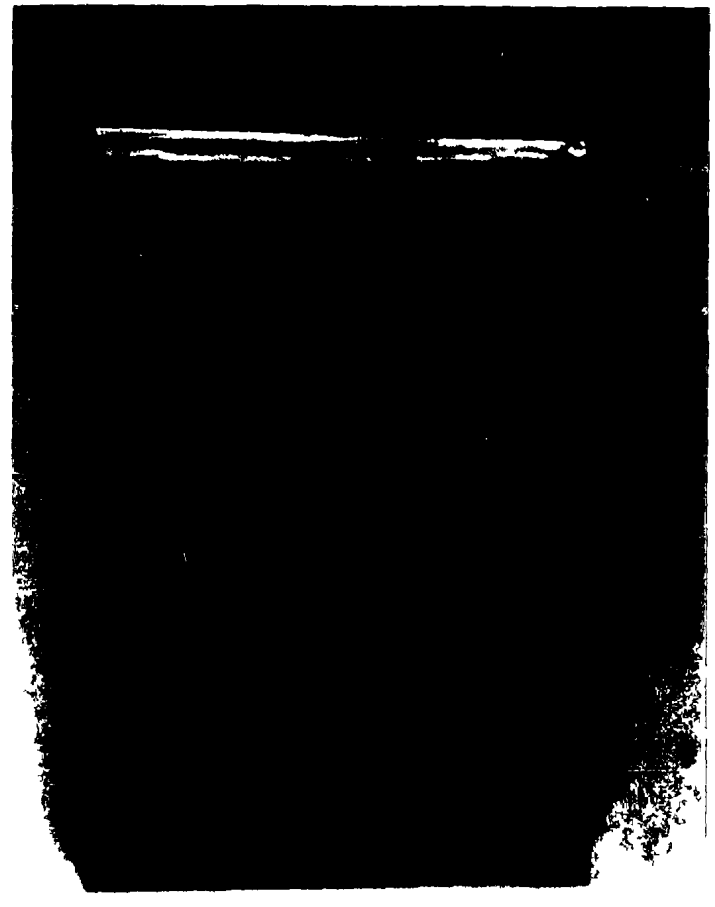


Frame 9 AGPT of second peak fractions (20-29) obtained by chromatography of tracheal Ig against RADSIg

A RADSIg

Frame 10 AGPT of fractions (10-19) obtained by chromatography of intestinal Ig against RADSIg

A RADSIg

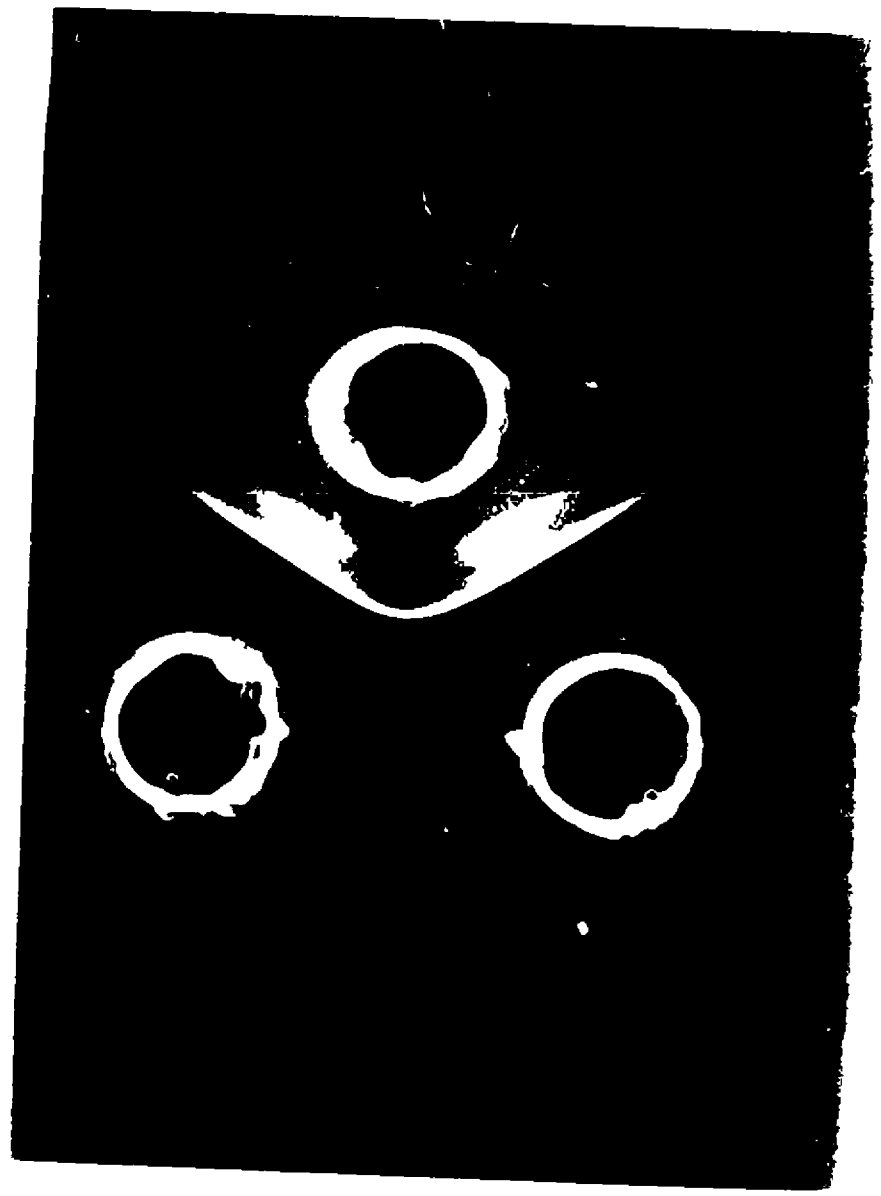


Frame 11 AGPT of duck serum against RADSIg

A RADSIg
B Duck Serum

Frame 12 AGPT of tracheal Ig against RADSIg

A RADSIg
B TIg

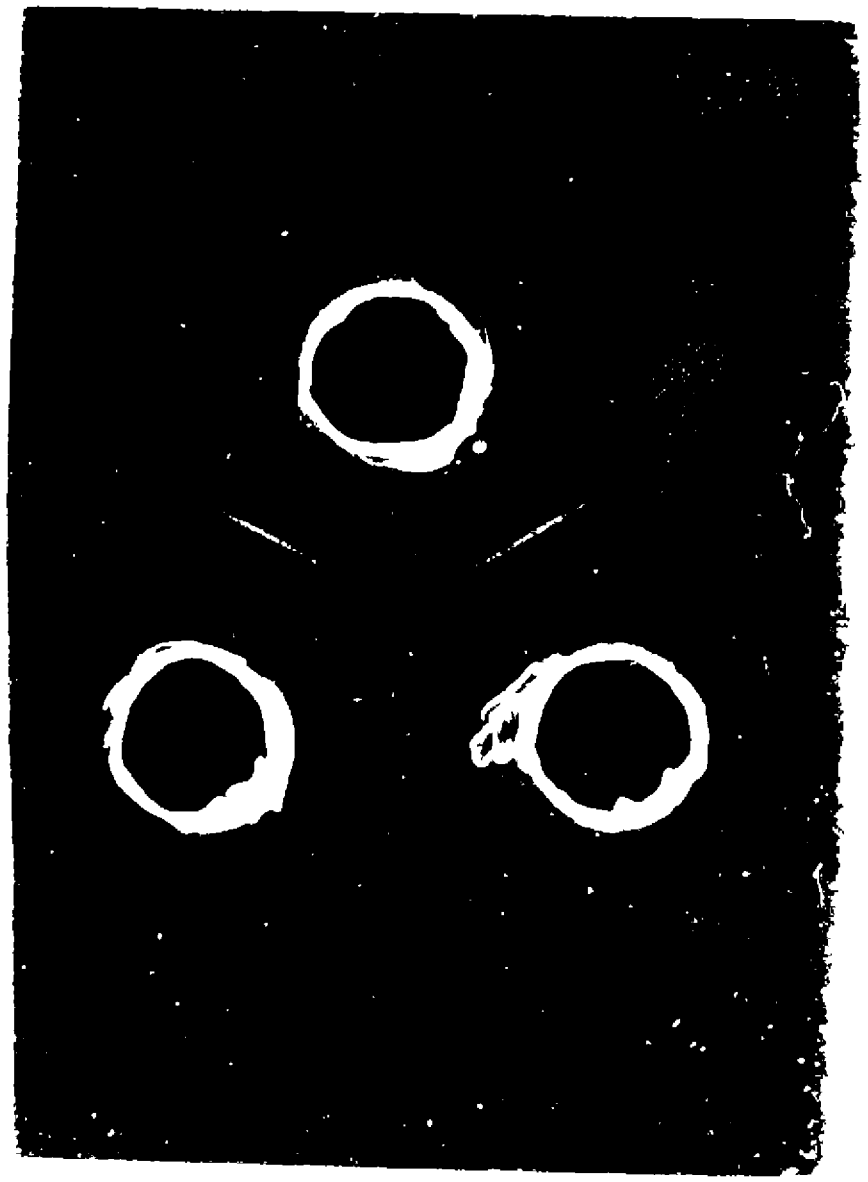


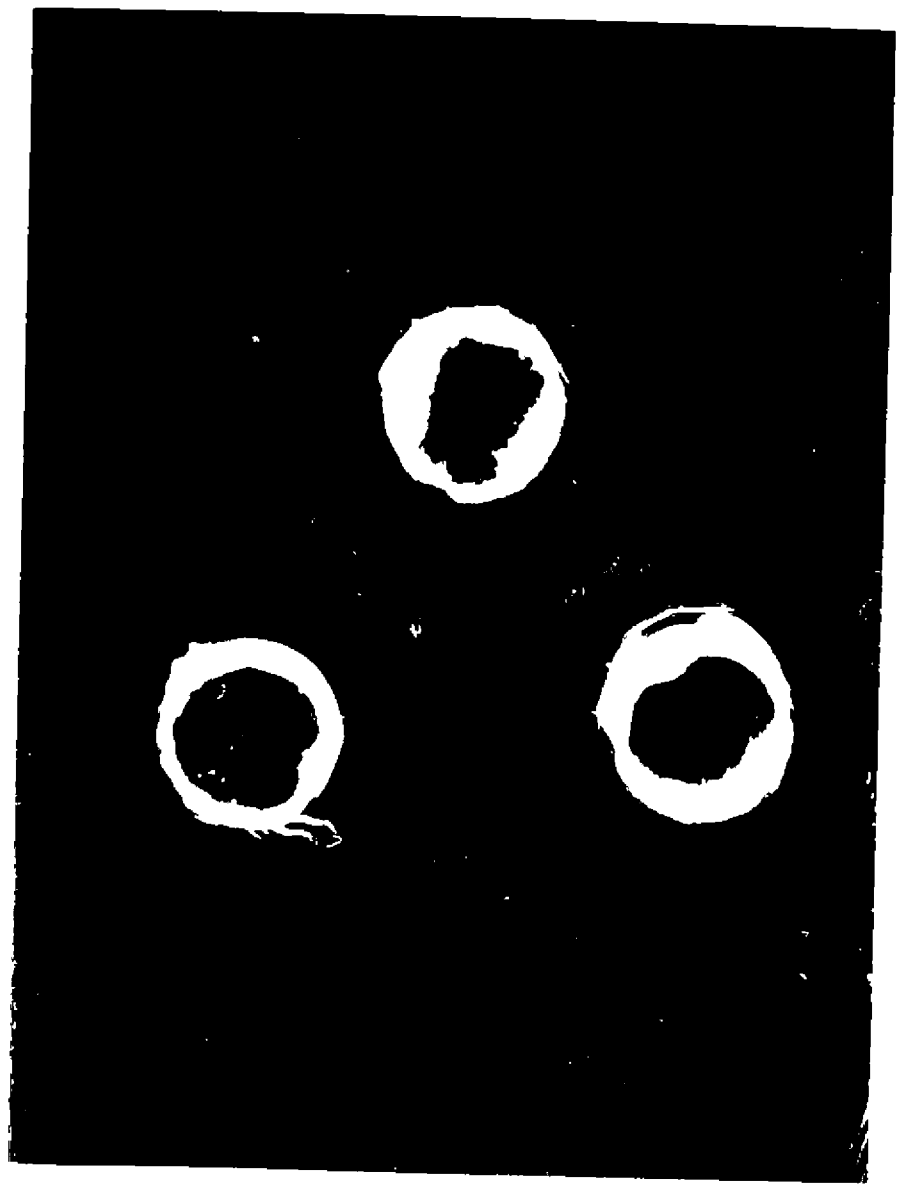
Frame 13 AGPT of bıle Ig agaınst RADSIg

A RADSIg
B BIg

Frame 14 AGPT of ıntestınal Ig agaınst RADSIg

A RADSIg
B IIg



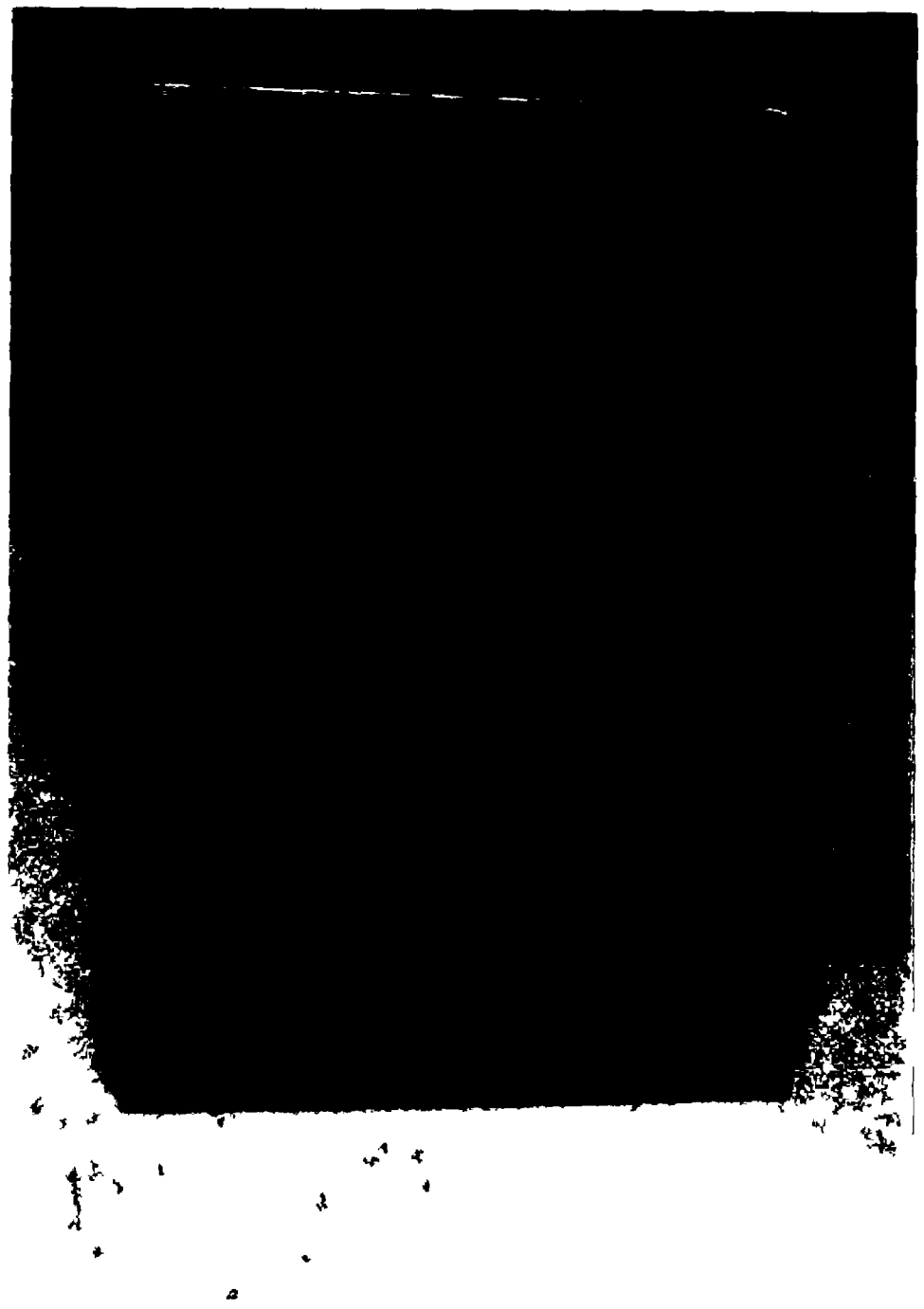


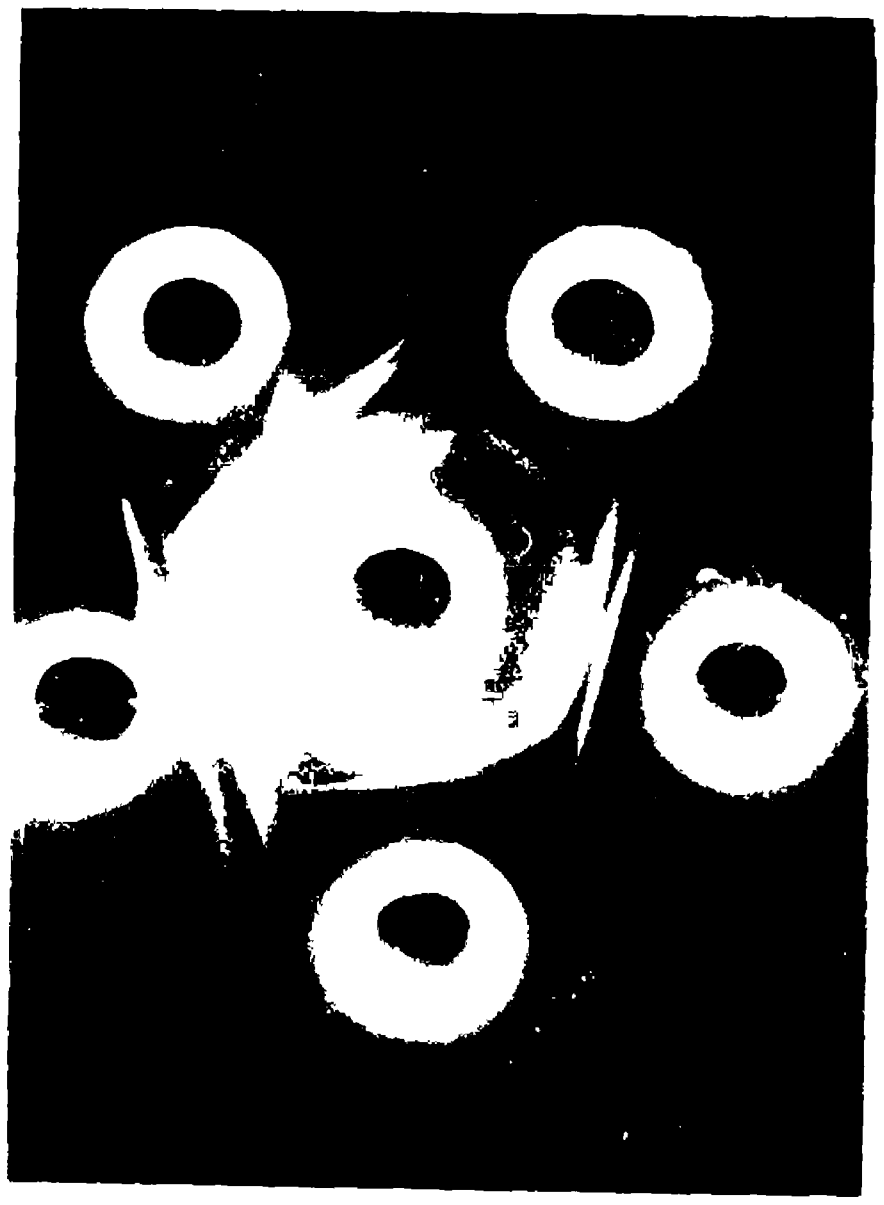
Frame 15 Antigenic comparison of serum on Igs from other sources against RADSIg

A RADSIg
B TIg
C BIg
D Whole duck serum
E SIg
F IIg

Frame 16 Antigenic comparison of serum and Igs from other, sources against RADSIg

A RADSIg
B TIg
C SIg
D - BIg
E Test Ig
F OIg
G IIg



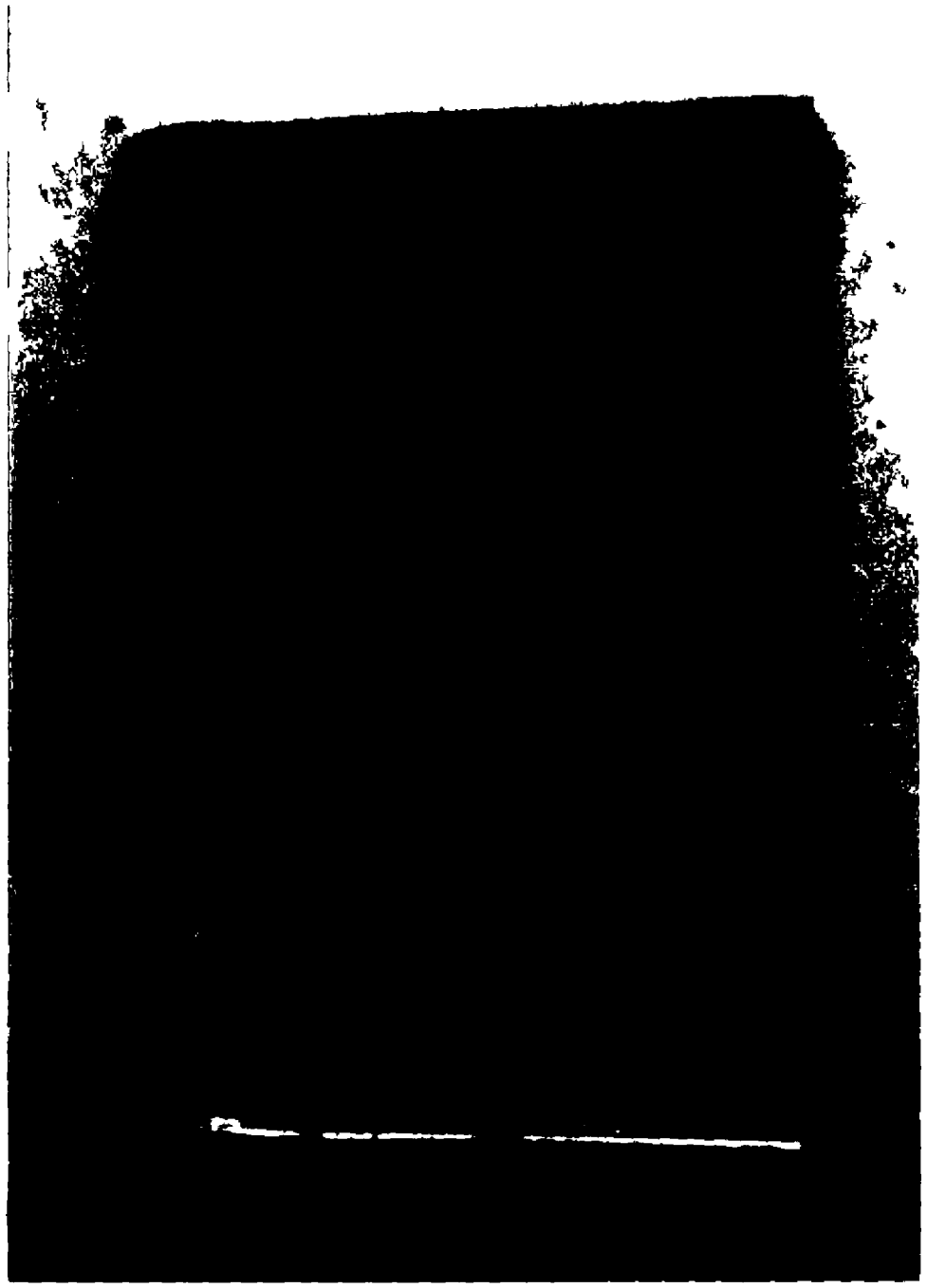


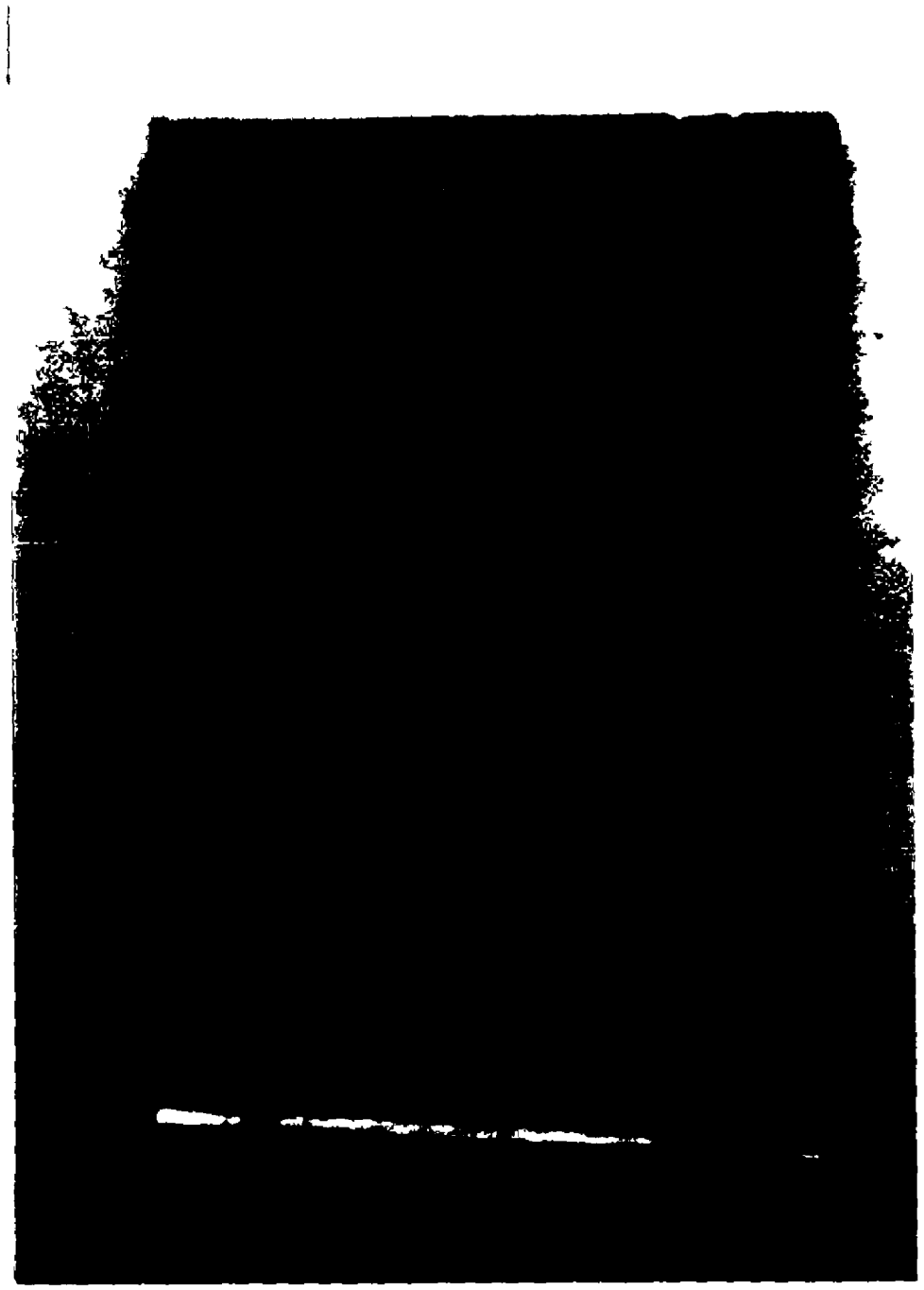
Frame 17 AGPT of Igs from serum and other sources against RA1

A RA1
B TIg
C IIg
D OIg
E Test Ig
F SIg
G - BIg

Frame 18 Antigenic comparison of Igs separated from serum and other sources against RA2

A RA2
B OIg
C BIg
D IIg
E SIg
F Test Ig
G TIg



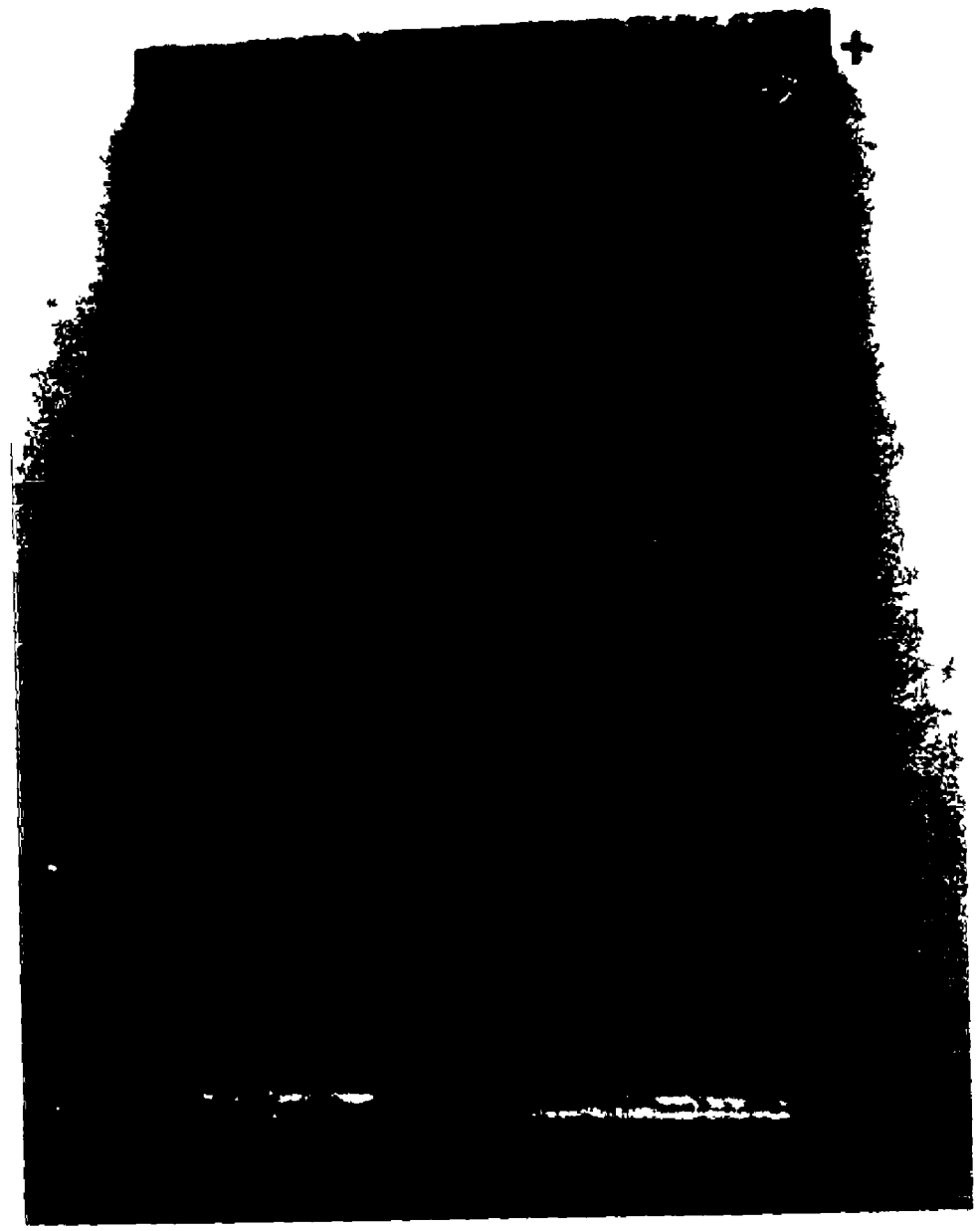


Frame 19 Immunoelectrophorogram of serum Ig and its first and second peak obtained by chromatography

A RADSIg
B SIg
C 1st peak
D 2nd peak

Frame 20 Immunoelectrophorogram of tracheal Ig and its first and second peak obtained by chromatography

A RADSIg
B TIg
C 1st peak
D Dest ending limb of 1st peak



Frame 21 1 Immunoelectrophorogram of second peak of tracheal Ig obtained by chromatography

A RADSIg
B SIg
C 2nd peak

2 Immunoelectrophorogram of first peak of tracheal Ig obtained by chromatography

A RADSIg
B SIg
C 1st peak

3 Immunoelectrophorogram of peak of intestinal Ig obtained by chromatography

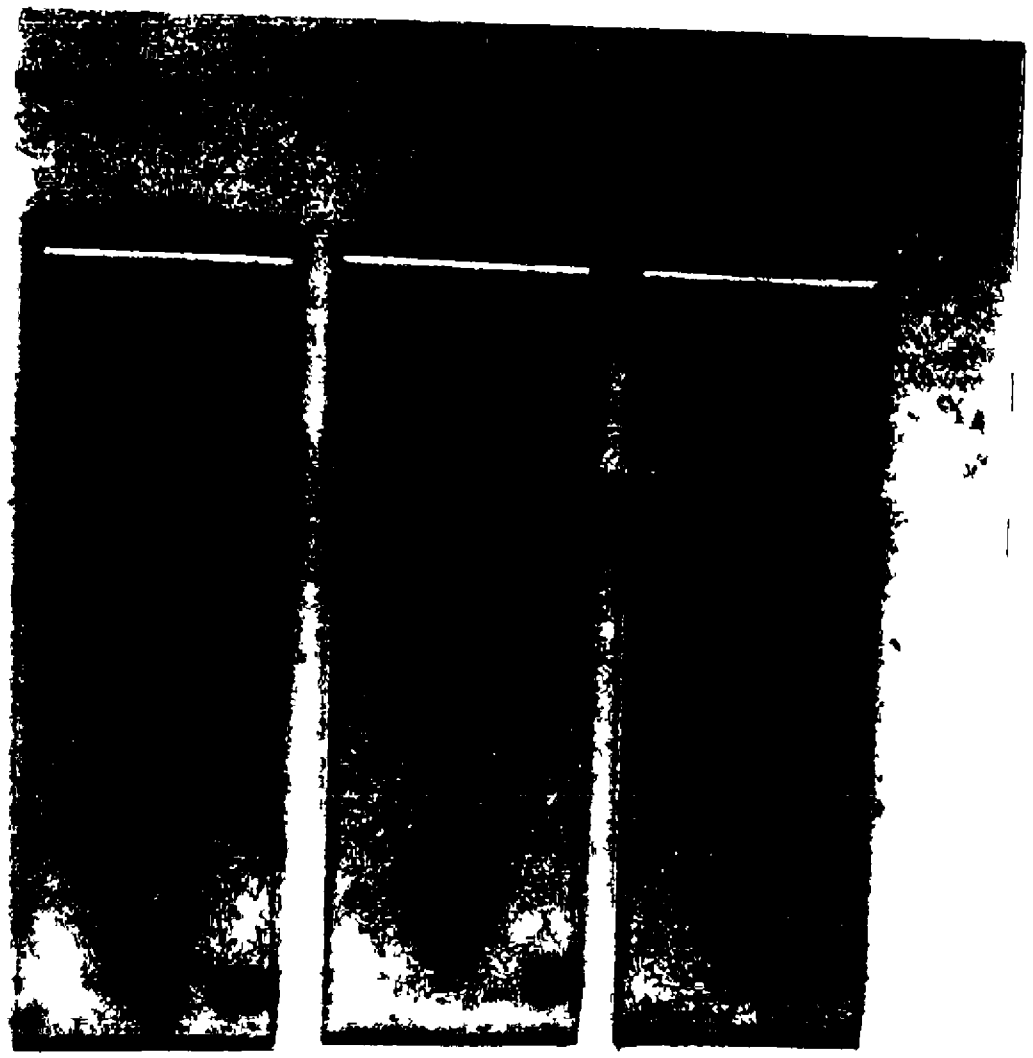
A RADSIg
B SIg
C - IIg

Frame 22 1 Immunoelectrophorogram of oviduct Ig

A RADSIg
B - SIg
C OIg

2 Immunoelectrophorogram of peak obtained by chromatography of bile Ig

A RADSIg
B SIg
C BIg



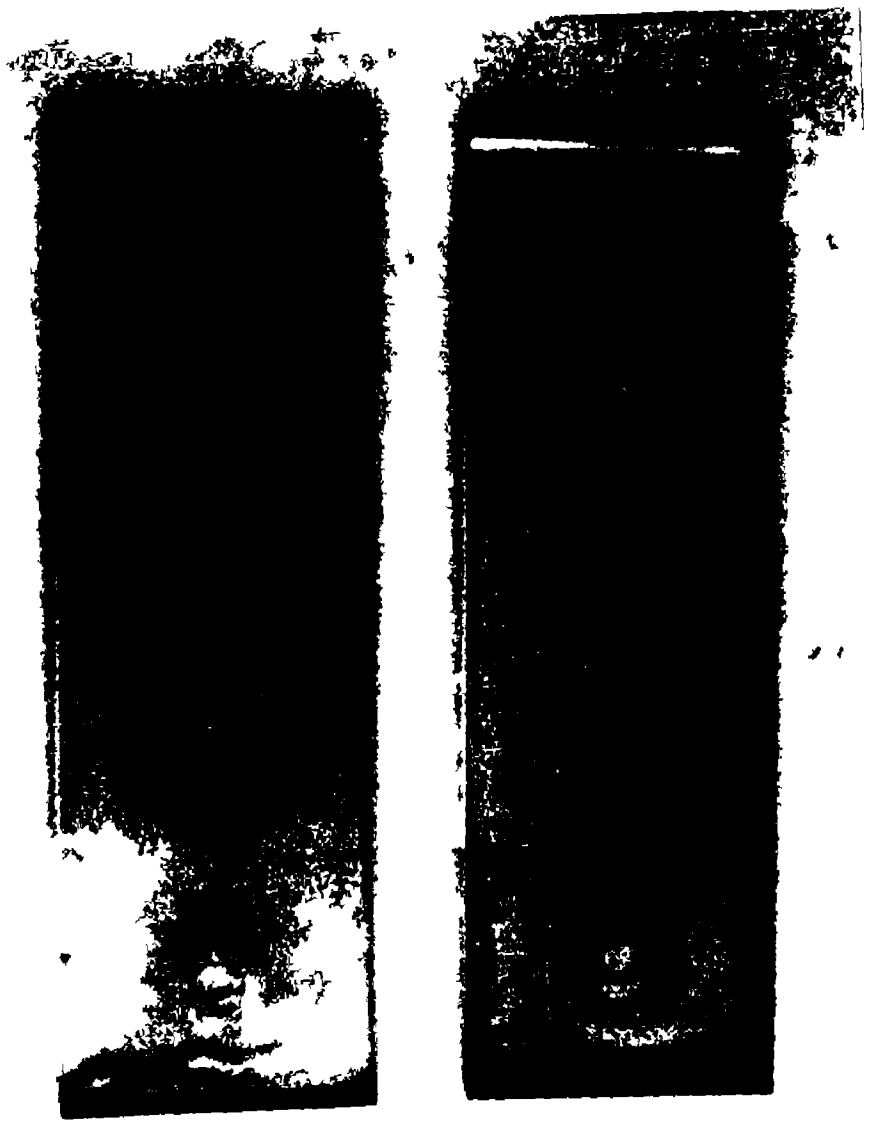
Frame 23 Mancını's standard for estımatıon of fırst fractıon

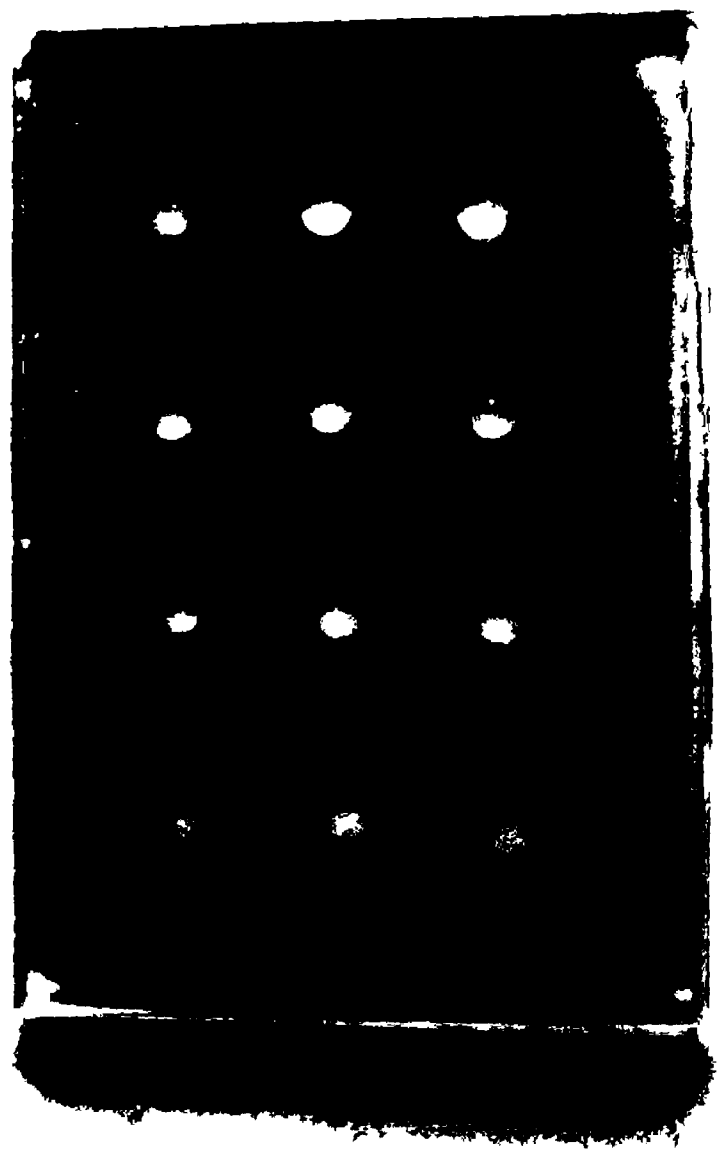
Frame 24 Mancını's test for fractıon 1 from treatment groups gıven *S typhımurıum*

A 0 5 ml IM
B 1 ml IM

Wells from left to rıght

1 Serum
2 Tracheal washıngs
3 - Ovıduct washıngs
4 Intestınal washıngs
5 - Bıle





Frame 25 Mancini's test for fraction 1 in treatment groups given *S typhimurium*

A 1 ml SC
B 0 5 ml SC

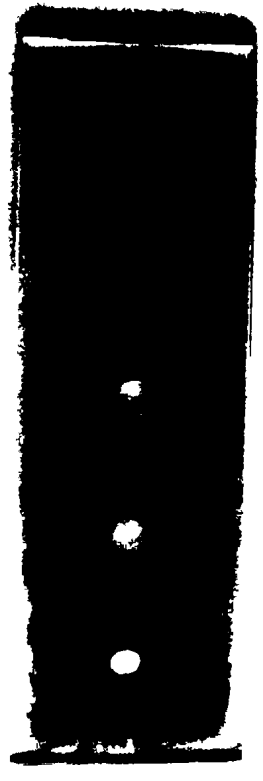
Wells from left to right

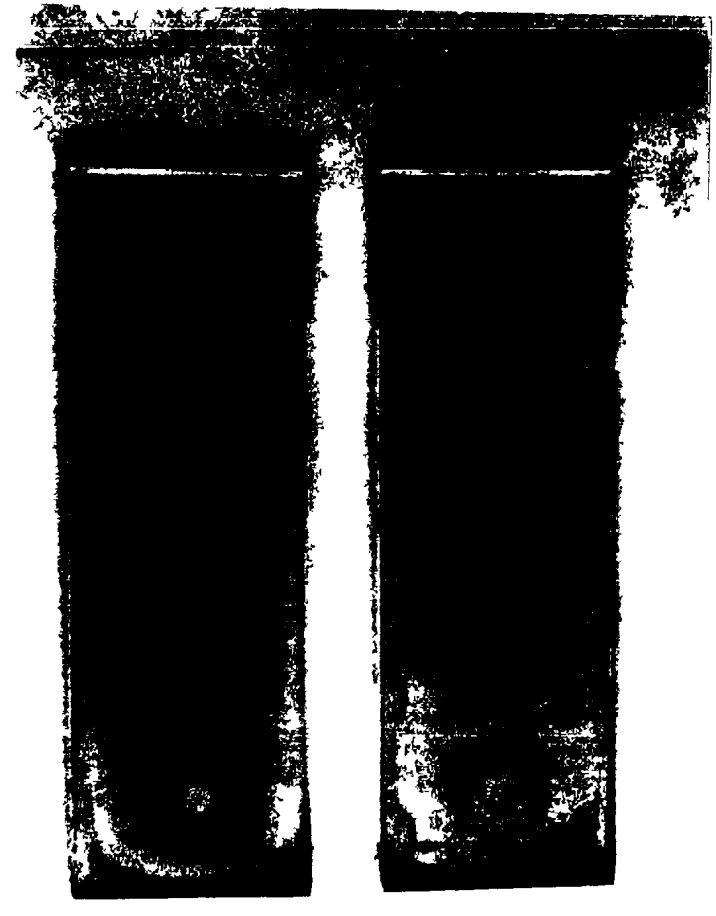
1 Serum
2 Intestinal washings
3 Testicular homogenate
4 Tracheal washings
5 Bile

Frame 26 Mancini's test for fraction 1 in treatment group given 0 5 ml of NDV SC

Wells from left to right

1 Serum
2 - Intestinal washings
3 - Testicular extract
4 - Tracheal washings
5 Bile





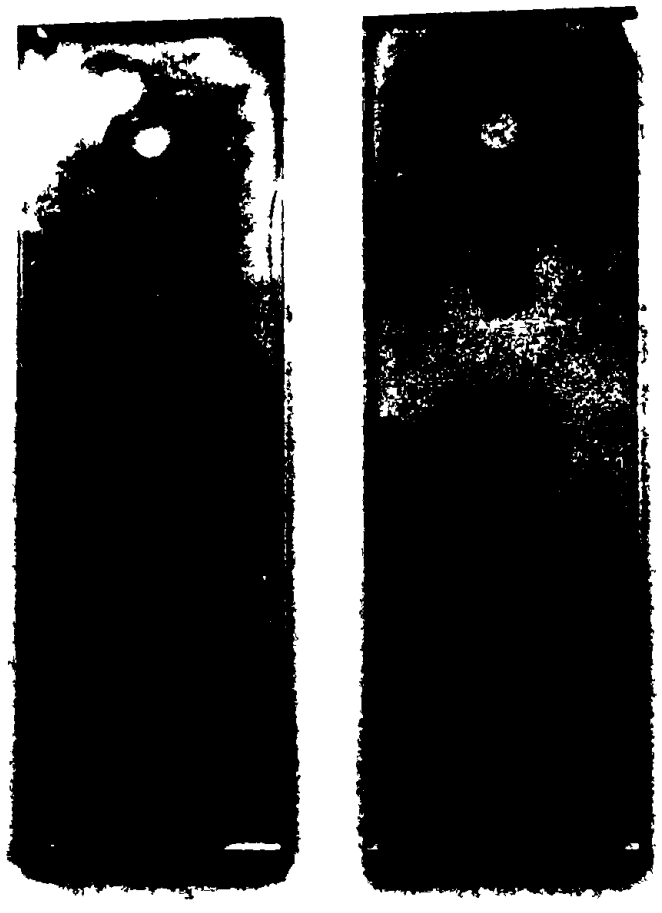
Frame 27 Mancini's test for fraction 1 in treatment groups given NDV

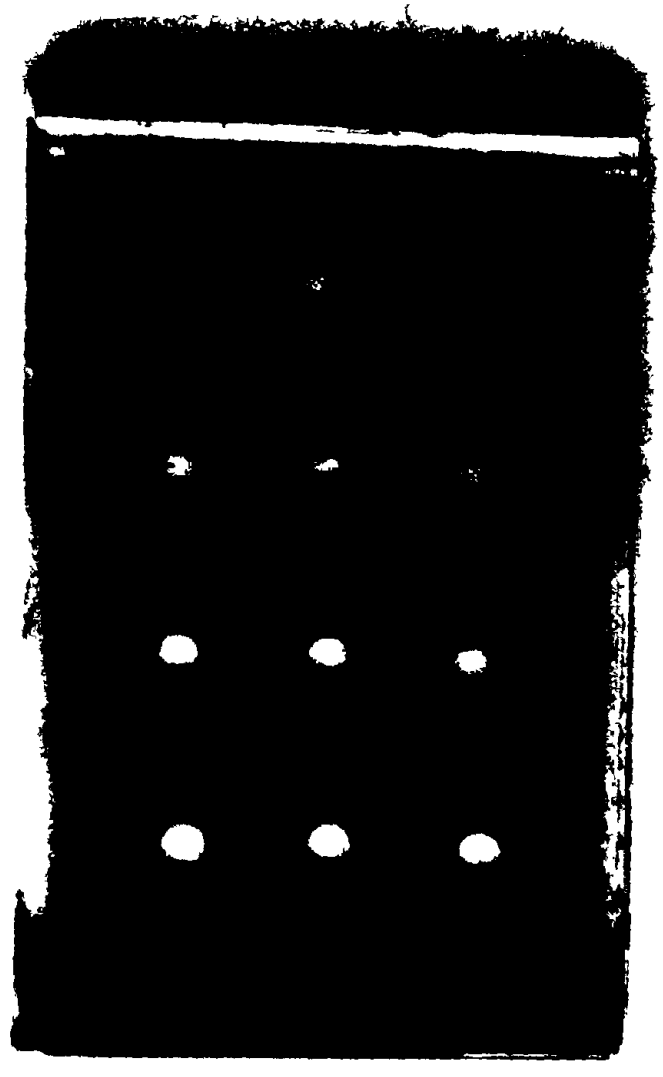
A 0 5 ml IM
B 0 5 ml IN

Wells from left to right

1 Serum
2 tracheal washings
3 Testicular washings (Slide B)
4 Oviduct washings (Slide A)
5 Intestinal washings 6 Bile

Frame 28 Mancini's standard for fraction 2



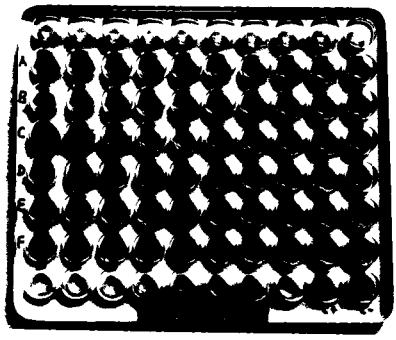


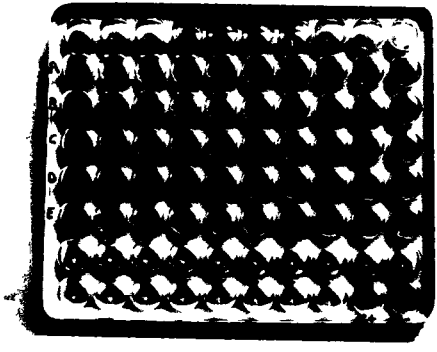
Frame 29 Haemagglutination (HI) test of pooled serum and other samples of control ducks against NDV

A Serum
B Oviduct washings
C Bile
D Tracheal washings
E Intestinal washings
F Testicular homogenate
C+ Positive control
C Negative control

Frame 30 HI test of pooled serum and other samples of ducks given 0 5 ml of NDV IM against NDV

A - Oviduct washings
B - Bile
C Tracheal washings
D - Intestinal washings
E Serum
C+ Positive control
C- - Negative control


A
B
C
D
E
F

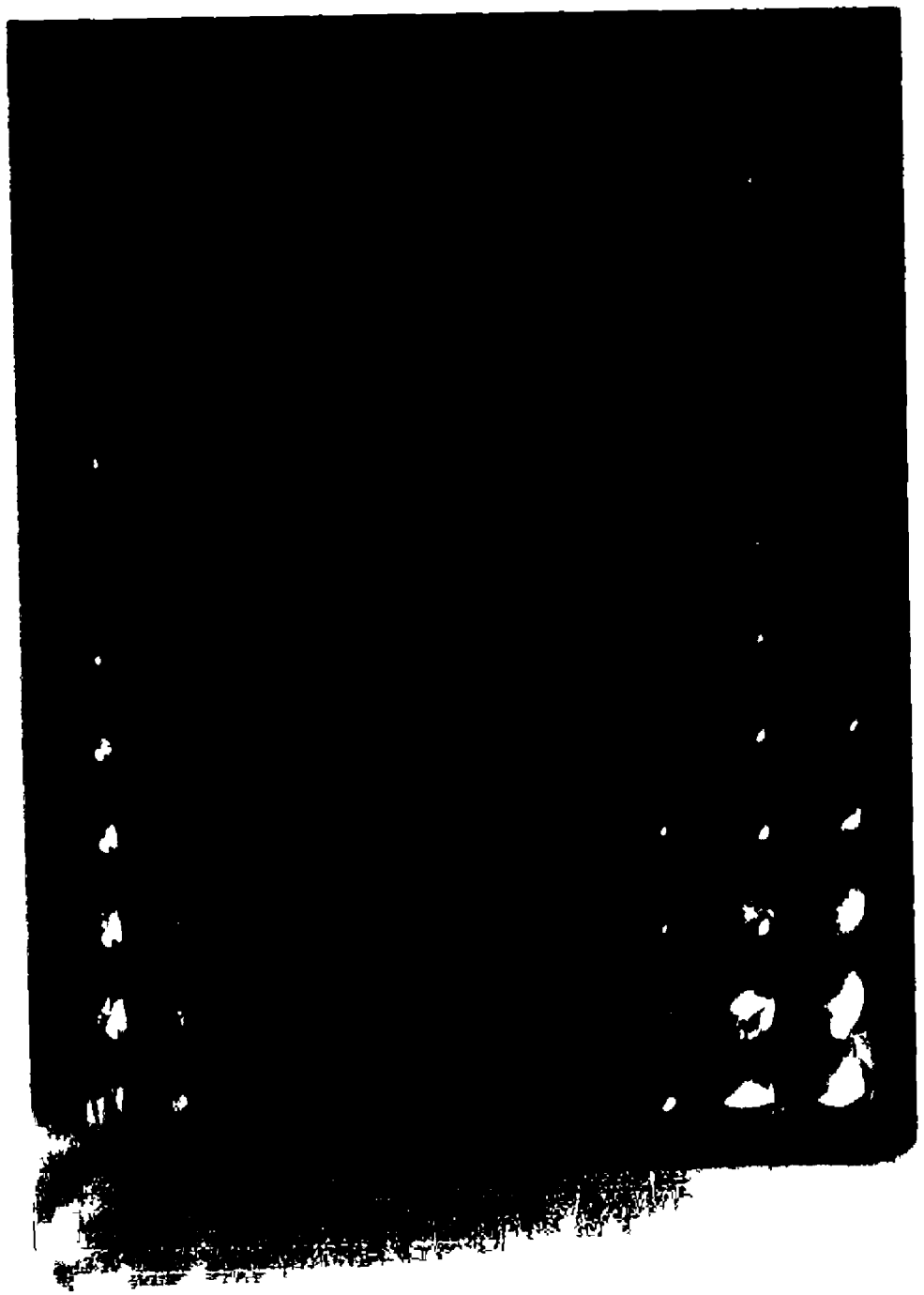

A
B
C
D
E

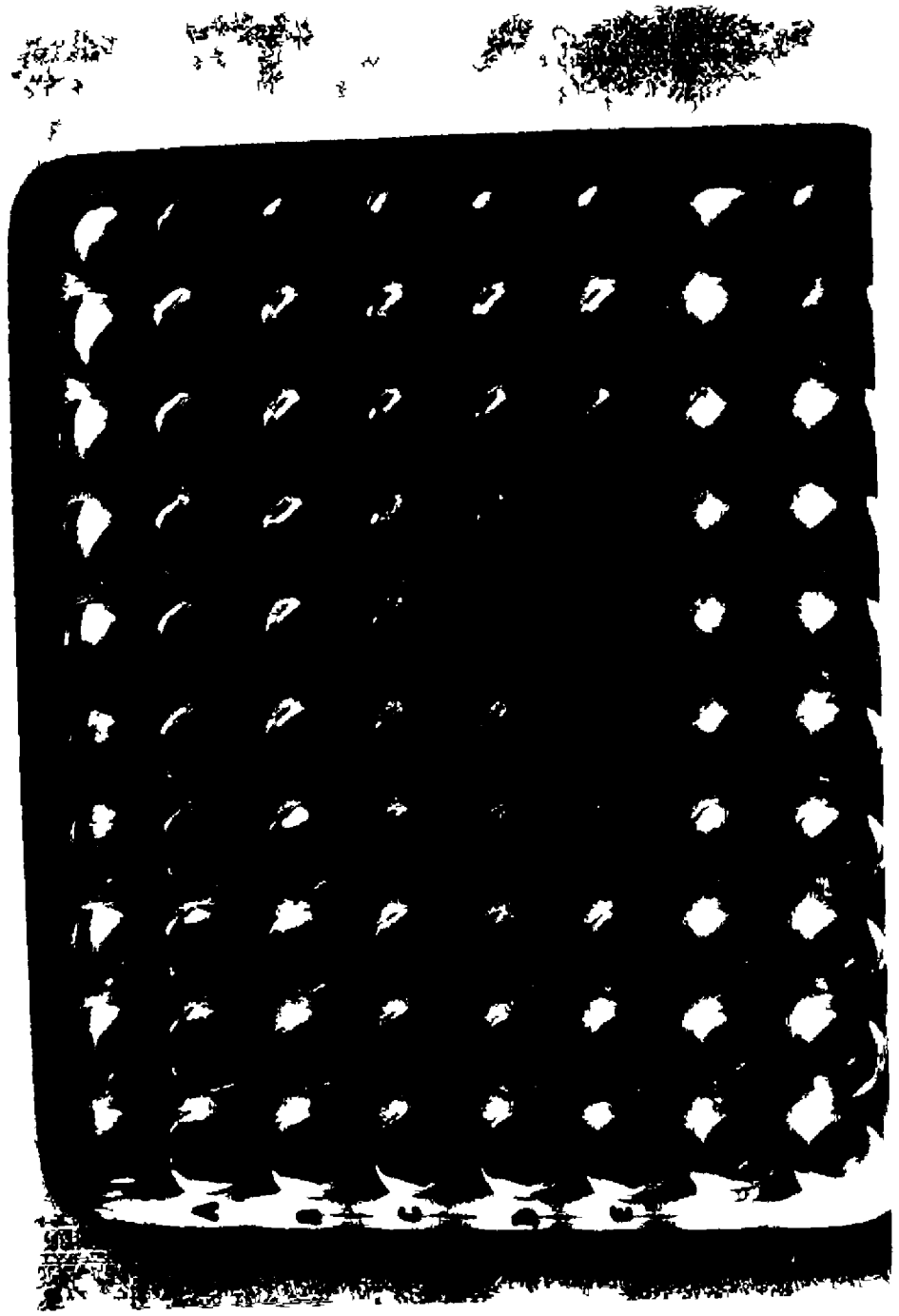
Frame 31 HI test of pooled serum and other samples of ducks given 0 5 ml of NDV SC, against NDV

A	Serum
B	Bile
C	Tracheal washings
D	Intestinal washings
E	Testicular homogenate
C+	Positive control
C	Negative control

Frame 32 HI test of pooled serum and other samples of ducks given 0 5 ml of NDV IN, against NDV

A	Serum
B	Bile
C	Tracheal washings
D	Intestinal washings
E	Testicular extract
C+	Positive control
C	Negative control





Discussion

DISCUSSION

The immunoglobulin profile of anseriforms are quite distinct from that of other avian Igs and thus the ducks as a species occupies a unique position in the ontogenic chain

Interest in duck Igs arose when researchers found that duck Igs were cognizantly deficient in secondary functional activities e g inability to fix compliment, weak or rare precipitation and agglutination etc In spite of those immunological drawbacks, it is a paradox that ducks are generally more resistant to common avian diseases

In this study an attempt was made to elucidate the immunoglobulin profile of serum, bile, mucosa of trachea and intestine of ducks and explore its functional and biological properties by employing a bacterial *(S typhimurium)* and a viral (NDV) antigen

Chromatography of serum Ig

Two main peaks were observed on fractionation of chicken serum Ig on Sephadex G 200 column by earlier workers The first peak being IgM and alpha 2 immunoglobulin and the second IgG (Higgins, 1976, Chhabra *et al* 1980 Nandapalan *et al* 1983)

Based on immunodiffusion and electrophoretic studies the first and second peaks obtained in this study might be representing the second and third peaks obtained by Grey (1967a) whose sedimentation coefficients were 7 8s and 5 7s respectively

Zimmerman *et al* (1971) fractionated duck serum globulins on an upward flow Sephadex G-200 gel after preliminary starch block electrophoresis They observed IgM to appear in the void volume, 7 8s IgG in the second peak and 5 7s IgG in the third They obtained pure samples of 7 8s IgG by pooling the leading edge of the second peak and 5 7s IgG from centre of the third peak

In the present study, though the void volume was pooled and concentrated from several Sephadex G 200 gel chromatography fractions, presence of any Ig could not be identified by AGPT against RADSIg This could be probably due to very low concentrations of IgM

Chromatography of bile Ig

Only a single peak was obtained between the 12th and 20th fraction with the highest concentration in the 16th eluate Though a second minor peak was obtained between 38th and 41st elution tubes (Fig 2), presence of any Ig in this could not be detected by AGPT

The elution paradigm of bile Ig coincided with that of the first peak obtained for serum Ig which suggested that it was in all probability, the 7 8s Ig

Chromatography of tracheal Ig

The first peak was obtained between the 10th and 17th fractions with the highest concentration in the 14th fraction tube (Fig 3) Chromatography repeated with another samples of TIg also gave a first peak between the same range with the highest concentration again in the 14th fraction The second peak was a wider one obtained between 22nd and 31st fraction tubes The elution patterns of the first and second peak of TIg did correspond to elution paradigms of first and second peak of serum Ig leading to a surmise that the first peak was 7 8s IgG, and the second, 5 7 Ig Further evidence in this direction comes from AGPT of the two peaks against RADSIg which found them to be different

Though IgM has been reported to be present in duck serum by Grey (1967a, b) and Ng and Higgins (1986) reports of the presence of IgM in tracheal washings are not available at present In the present study a precipitation line was obtained on immuno-electrophoresis of tracheal Ig which could be IgM by its migration pattern and position of arc But during chromatography, an elution pattern corresponding to that of the IgM was not obtained

Zimmerman *et al* (1971) had observed that the duck IgM eluted in the void volume during chromatography In this study also the same could have occurred, though no IgM could be detected in the void volume of serum eluates

Chromatography of intestinal washings

Single peak was obtained between the 10th and 15th fraction (Fig 4) with the maximum concentration in the 13th tube This coincided with that obtained for the first peak of serum Ig indicating that it to be most probably 7 8s Ig

AGPT against RADSIg

Serum (Frame 11)

Three lines of precipitation indicated the presence of three different Igs in the serum Ig which were by chromatography found to be probably 7 8s IgG and 5 7s IgG The fainter line which indicated a lower concentration (probably IgM) could not be separated by chromatography The distinct line and the hazy line may be probably representing the 7 8s Ig and 5 7s which by chromatography resolved into two peaks and on AGPT showed the formation of a spur as described earlier, indicating partial antigen identity between the two

Trachea washings (Frame 12)

Three distinct lines of precipitation were observed Further evidence towards this was obtained by chromatographic and electrophoretic studies of tracheal Ig But on chromatography, only two peaks were present Hence the additional line might be representing another Ig which could not be resolved by chromatography Chhabra and Geol (1980) while studying the concentration and distribution of Igs in chicken sera and tracheal washings described the presence of IgM IgG and IgA

Bile (Frame 13)

Though two distinct lines of precipitation were produced on AGPT, only one peak containing Ig was obtained during chromatography When analysed by immuno electrophoresis, only a single line was obtained The presence of two lines by AGPT of whole bile reveals the probable presence of a fraction which was probably lost during precipitation process

Intestinal washings (Frame 14)

A single line of precipitation indicated the presence of a single Ig which could have been the 7Ss Ig going by the chromatographic elution pattern

Antigenic comparison of Igs from different sources by AGPT against RADSIg (Frame 15 and 16)

The line of identity connecting all the wells showed the presence of a common Ig in the samples (bile testicular extract, serum, tracheal, intestinal and oviduct washings) which could most probably be the 7 8s IgG Leslie *et al* (1971) described IgY in chicken secretions which is homologous to duck 7 8ss IgG Saif and Dohms (1976) described presence of IgG in turkey secretions

AGPT of serum and other samples against RA 1 (Frame 17)

AGPT of serum and other samples (bile, testicular extract, intestinal, tracheal and oviduct washings) against RA 1 produced a faint line of identity from all the wells which was more distinct for the serum Ig indicating that the 7 8s IgG was present in all the samples Leslie *et al* (1971) traced in chicken the presence of IgY which is analogous to 7 8s IgG in ducks, in tracheo-bronchial washes and faeces and showed that they did not contain any additional antigenic determinant to that of serum Ig In a similar study conducted by Saif and Dohms (1976) in turkey all secretions (oviduct scrapings, bile, tracheal washings intestinal washings) contained IgG and IgM, while IgM and IgG were not found in large intestinal washings

AGPT of serum and other samples against RA 2 (Frame 18)

AGPT of serum and various secretions (bile, testicular extract, tracheal, intestinal and oviduct washings) produced a diffuse line of identity from all wells except for those of bile and intestinal Ig The absence of precipitation lines indicated that the Ig present in the second fraction (most likely 5 7s IgG) was absent in bile and intestinal secretions

Immuno-electrophoresis

1 Serum fractions (Frame 19)

Toth and Norcross (1981a) observed duck IgM to be an electrophoretically heterogenous protein with components migrating slower than IgM of other species, with the cathodal tip of the duck IgM lines extending into the gamma-2 migration zone They also detected that besides IgM and the major IgG arc, there occurred another arc also within the curve of the major IgG arc, merging with it towards the cathodal end which they presumed to be a minor IgG arc

In the present study, the fraction taken from the ascending limb of the first peak gave two arcs which merged towards the cathodal end and bifurcated towards the anode It could be due to the contamination with 5 7s IgG For

elucidation of this aspect a more detailed study is required

The immunoelectrophoresis of the fraction taken from middle of the second peak gave an arc in between the well and the trough, but closer to the latter Grey (1967b) also got similar type of arc formation closer to the trough He also mentioned that this could be 5 7s IgG Hence the second peak in this study might be containing 5 7s IgG

Tracheal Ig (Frame 20)

Chhabra and Goel (1980) described the distribution and concentration of IgA, IgM and IgG in chicken sera and tracheal washings But the existence of IgA in ducks is obscure (Toth and Norcross, 1981a)

In this study, immunodiffusion of tracheal Ig against RADSIg gave three lines of precipitation Immuno electrophoresis against RADSIg revealed three arcs between the well and the trough The bigger arc could be that of 7 8s IgG and the smaller arc which appeared within the bigger arc might be representing the smaller 5 7s IgG The third line which formed away from the well and extended as a haze toward the anode, in all probability might be IgM The second thin line above this haze could be probably be some minor contaminant such as lipoprotein or aggregated IgG These results were in accordance with that observed by

Toth and Norcross (1981a), while conducting electrophoretic studies of duck serum

Fraction from ascending limb of the first peak from chromatography gave a single arc between the well and the trough which could be the 7 8s IgG (Frame 20), whereas pooled and concentrated first peak fractions showed a bifurcation anodally which may indicate a minor contamination with 5 7s IgG (Frame 21 1)

Pooled and concentrated second peak fractions did give only a single arc in between the well and trough which in all probability might be 5 7s IgG Toth and Norcross (1981a) also described two arcs, one for 7 8s IgG and the other for 5 7s IgG (Frame 21 2)

Oviduct Ig (Frame 22 1)

Saif and Dohms (1976) detected IgG and IgM in oviduct washings and many other secretions of turkey In this study two arcs obtained could be most probably, the 7 8s IgG and 5 7s IgG by comparing to the two arcs obtained for SIg Line corresponding to IgM was not observed This could be due to the absence or very low concentration of IgM in oviduct washings

Biliary Ig (Frame 22 2)

Only a single arc was produced against RADSIg which when compared to the arcs produced by serum Igs corresponded to the 7 8s IgG No arcs indicating the presence of IgM was seen This observation contradicted the report by Ng and Higgins (1986) who stated duck bile Igs to resemble serum IgM but possessed additional determinants

A more detailed study is required to reach a definite conclusion

Intestinal Ig (Frame 21 3)

Grey (1967b) found that duck 5 7s protein formed a precipitation band very close to the antiserum trough, while the more antigenically complete and more slowly diffusing 7 8s protein formed a band behind it In this study, only a single arc was seen between the well and the trough It was difficult to determine the type of IgG by the position of the arc but comparing the arc with that obtained for SIg, it could most probably be 7 8s IgG

Quantification of immunoglobulins by Mancini's method

Controls

With respect to fraction 1 all the samples except intestinal washings and testicular extract produced rings of

precipitation, the largest being for sera, indicating it to have the highest concentration of fraction 1 Oviductal washings had the next highest concentration, followed by bile The lowest was in the tracheal washings The absence of precipitation rings for intestinal washings and testicular extract could be due to very low concentration of the Ig Fraction 2 could be detected in serum and tracheal washing Other samples were found to be negative The highest level of this Ig was present in the serum

Effect of immunization with *S typhimurium*

Bile

The concentration of fraction 1 in bile increased in treatment groups indicating that immunization increases the concentration of this Ig in bile

The bile samples from treatment groups were found to be negative for fraction 2 by Mancini's method This finding reveals that only fraction 1 is present in bile and not the fraction 2

Tracheal washings

The concentration of fraction 1 in the tracheal washings did not show appreciable difference between the controls and the treatment groups indicating that

ımmunızatıon had no effect ın ıncreasıng the fractıon 1 ın tracheal mucosa

There was a slıght ıncrease ın the levels of fractıon 2 ın the treatment groups The hıghest levels were detected ın the treatment group gıven 1 ml of the antıgen ıntramuscularly, followed by the group gıven 1 ml subcutaneously The level of thıs fractıon ın treatment group gıven 0 5 ml IM was also hıgher than the group gıven 0 5 ml subcutaneously Thıs poınts out to the fact that the IM route appears to be superıor to the SC route ın achıevıng hıgher levels of thıs ımmunoglobulın

Testıcular extract

Compared to the controls, ın the testıcular extracts of the treatment groups from whıch samples were collected an ıncrease of fractıon 1 could be detected Thıs ındıcates that even ın testıcular tıssue, the level of fractıon 1 rıses consequent to varıatıon

In treatment groups were ıt was sampled, the levels of fractıon 2 ın the group gıven 1 ml of the antıgen subcutaneously showed a slıghtly hıgher level of thıs fractıon to that of the group gıven 0 5 ml of the antıgen subcutaneously, whıch ındıcated some effect of the dose of the antıgen ın ıncreasıng the levels of fractıon 2 ın testıcular extracts

Oviduct washings

The concentration of fraction 1 in oviduct washings remained the same both in experimental groups and control

In treatment groups were it was sampled, the level of Fraction 2 in the group given 1 ml of the antigen intramuscularly showed a slightly higher level of this fraction to that of the group given 0 5 ml of antigen subcutaneously, which indicated some effect of the dose of antigen in increasing the levels of fraction 2 in oviduct washings

Intestinal washings

The level of first fraction in intestinal secretions of the treatment groups showed an increase to that of the control, with the highest in the groups treated with 1 ml of culture intramuscularly

The intestinal washings from treatment groups were found to be negative for fraction 2 by Mancini's method This finding reveals that only fraction 1 is present in intestine and not the fraction 2

Serum

Level of fraction 1 in serum of treatment groups were higher than obtained in the control group with the highest

level in the group treated with 1 ml of culture intramuscularly

The above findings indicate that I/M route is superior to attain higher level of antibodies

There was a slight increase in the levels of fraction 2 compared to the control The treatment group given 1 ml IM of the antigen showed highest level to that of the group given 1 ml SC The level of this fraction in the treatment groups given 0 5 ml of culture IM was also higher than in the group given the same dose SC, but was lower than the group given a dose of 1 ml of the culture This indicated that the dose and route had ssome effect in increasing the levels of this fraction in the serum These findings were in accordance with that reported by Grey (1967a) who observed that upon hyperimmunization, Pekin, Mallard and Muscovy ducks produced relatively higher levels of 5 7s IgG The fact that there was no drastic increase in the levels of this fraction may be because the ducks used in the present study were Desi breeds and not the ones used by Grey

Effect of immunization with NDV

Bile

The concentration of fraction 1 in bile did show a definite increase in all the treatment groups, compared to

the control This indicated that immunization increased the level of antibodies in bile

The bile samples of treatment groups were found to be negative for fraction 2 by Mancini's method This finding reveals that only fraction 1 is present in bile and not the fraction 2

Tracheal washings

There was no significant increase in the concentration of Igs in the treatment groups compared to the control From the above findings it can be summarized that immunization with NDV by various routes did not increase the level of fraction 1 in the tracheal washings

The Fraction 2 was highest in the treatment group immunized intranasally with 0 5 ml of NDV The levels of fraction 2 in the other treatment groups showed slight increase over the control group with the group given 0 5 ml of the antigen intramuscularly showing a slightly higher level than the group given 0 5 ml subcutaneously A higher level of fraction 2 could be observed in the treatment group given NDV intranasally This finding suggests that to attain higher level of this Ig in tracheal mucosa, the preferential route is intranasal This also indicated that the IM route could initiate a better Ab response than the SC route, but was inferior to the intranasal route

Testicular extract

In the samples collected from treatment groups in which they were sampled fraction 1 could be detected in almost the same concentrations indicating an effect of immunization

None of the samples from the treatment group showed the presence of fraction 2 which could be due to the undetectable levels or absence of the said fraction in the above sample

Oviduct washings

The concentration of first fraction in the treatment group in which it was sampled (0 5 ml IM) showed a definite increase in its concentration, compared to that of control group showing that immunization with viral antigen did increase the level of fraction 1 in the oviduct washings

Measurable levels of the fraction was seen in the treatment group (0 5 ml IM) in which it was sampled compared to that of control which did not show detectable levels

Intestinal washings

None of the intestinal washings, either from the control groups or the treatment groups, showed the presence of fraction 1, though its presence was identified by

chromatography and immunoelectrophoresis of IIg This could be due to the very low concentration of the said fraction in the same which might have got diluted further during sampling

The intestinal washings sampled from treatment groups were found to be negative for fraction 2 by Mancini's method This finding reveals that only fraction 1 is present in intestinal washings and not the fraction 2

Serum

The concentration of fraction 1 was more or less the same in all treatment groups except in the group given 0 5 ml of antigen intranasally where it was slightly lower but higher than that in the control groups This indicates that immunization through parental route was more superior than intranasal route in increasing the level of fraction 1 in serum This finding was in accordance with the observation of Zakay Rones *et al* (1971) who reported a higher level of HI antibodies in chicken immunized with NDV by intramuscular route than intranasal route

In contrast to the first fraction, there was no marked increase in the levels of fraction 2 in any of the treatment groups, compared to that of the control The level of this

fraction is the treatment groups receiving NDV paienterally was slightly higher than the intranasal group

As stated earlier, since tracheal washings from the treatment group given NDV intranasally showed a higher level of fraction 2, it can be surmised that administration of NDV intranasally did not cause production of this Ig in serum on par with the other treatment groups, but it did have an effect in increasing the local levels of Ab

STAT of serum and other samples against *S typhimurium*

Controls were found to be negative for Salmonella antibodies

Titres of treatment groups given 1 ml of the *S typhimurium* culture through IM and SC routes were the same (1 160) A lower dose and route of administration of 0 5 ml through SC route gave a lower titre (1 20) than when given through IM route (1 80), indicating that the dose and route of administration might have an effect on Ab tiire

There was significant difference in the ti're of bile in birds in different treatments The highest titre of 1 80 was obtained from bile of ducks given 1 ml IM of the culture and 1 20 for bile from ducks given 0 5 ml intramuscularly Bile from ducks given SC 1 ml of the culture gave a lower

titre of 1 20 and the lowest was seen in treatment group given 0 5 ml of culture subcutaneously (1 10) This indicated that the dose and route of administration were influencing the level of antibodies in bile

No agglutination titres were observed for intestinal washings and testicular extract, whereas pooled oviduct washings from ducks given 1 ml of culture through IM route did produce discernible agglutination in the first agglutination tube

Haemagglutination inhibition test

Control

Serum and other samples except bile from the control birds did not produce any HI A titre of 1 64 was obtained for bile This could be due to the fact that since the birds were purchased locally, some birds might have already been exposed to NDV This was in accordance with the observations of Higgins *et al* (1988) who found antibody activity in bile Ig against influenza A virus at times when no activity was detected in serum Ig

The HI titre of serum samples from ducks given 0 5 ml of NDV by IM route (1 128) was higher than that produced by serum from ducks given 0 5 ml of NDV subcutaneously (1 64) The lowest HI titre for serum was obtained for serum of

ducks given 0 5 ml NDV intranasally (1 32) It can be surmised that the route of administration of the virus does influence the Ab levels when immunization is done parenternally Studies done by Zakay Rones *et al* (1971) in chicken found serum HI antibodies to be higher in the group immunized by intramuscular route than by intranasal route

Bile from groups of ducks immunized by 0 5 ml of NDV by IM and SC routes did give a HI titre of 1 64 in each case Bile in ducks given 0 5 ml NDV intranasally gave a lower HI titre of 1 32 This fact points to the superiority of the parenteral administration of the antigen to confer higher antibody titre in bile

Intestinal washings, tracheal and testicular extract from the treatment group did not produce any HI This could be due to the low concentrations of HI antibodies in these samples

Oviduct washings from ducks immunized with 0 5 ml IM of NDV gave a titre of 1 16 It could be generalised that HI activity does appear in the oviduct when immunized parenterally with NDV This observation is corroborative to the concept of transovarian transfer of antibodies conferring passive protection to the newly hatched

Summary

SUMMARY

An attempt was made to elucidate the immunoglobulin profile of serum, bile, mucosa of trachea and intestine of ducks and to study the functional and biological properties of these immunoglobulins Immunoglobulins from serum, bile mucosa of trachea and intestine of ducks were separated by salting out with 50 per cent saturation of ammonium sulphate

Immunoglobulins obtained from serum and various secretions were fractionated on a Sephadex G-200 column Two peaks were obtained for serum Ig and tracheal Ig Only a single peak was obtained for bile Ig and intestinal Ig On comparing the graphs, the elution pattern obtained for bile Ig and intestinal Ig corresponded with that of the first peak obtained for serum Ig The two peaks obtained for tracheal Ig also gave similar elution patterns of serum Ig

On AGPT of serum and other samples against RADSIg serum formed three lines of precipitation Bile gave two lines of precipitation Tracheal washings produced three precipitation lines Only a single line of precipitation was obtained for intestinal washings

Antigenic comparison of Igs from serum and other samples was done by AGPT against RADSIg which showed the presence of a common Ig in the serum and other samples including testicular extract and oviduct washing

AGPT of Igs against BA1 showed the presence of the first peak in serum as well as all other samples AGPT against RA2 revealed the presence of the second peak in the samples except bile Ig and intestinal Ig

Immunoelectrophoresis of the first major peak obtained from Sephadex G 200 filtration of serum gave two arcs and the sample taken from the middle of the second major peak gave a single arc In all probability the first and the second peak might be representing the 7 8s IgG and the 5 7s IgG respectively going by the migratory zones

Though two precipitation lines were obtained on AGPT of bile Ig against RADSIg, only a single major peak was obtained on chromatography, which on electrophoresis migrated to the 7 8s IgG zone

Immunoelectrophoresis of tracheal Ig gave three arcs in zones occupied by IgM, 7 8s IgG and 5 7s IgG On subsequent chromatography, most of the IgM was lost, but the first major peak and the second major peak produced arcs in the zones of migration of 7 8s IgG and 5 7s IgG respectively

The two arcs obtained on electrophoresis of oviduct washings were in the migratory zones of 7 8s IgG and 5 7s IgG respectively

The antibody response of ducks to a bacterial and a viral antigen was assessed by using an anaculture of *S typhimurium* and R_2B strain of NDV respectively

On conducting STAT of serum and various secretions of ducks treated with an anaculture of *S typhimurium* (AS), there was a significant increase in the antibody titres in serum bile and oviduct washings (controls did not produce any agglutination) No agglutination was observed for tracheal washings, intestinal washings and testicular homogenate of the treated birds

Haemagglutination inhibition test conducted on serum and secretions of ducks given NDV through various routes and doses did indicate that there was a significant effect of the dose of the virus, when given parenterally in increasing antibody levels in the serum of treated ducks The lower titre obtained for serum when NDV was administered intra-nasally indicated the influence of the route of inoculation in increasing the antibody titres

Though titres were produced by bile and oviduct washings there was no significant effect of the route or dose of the virus in increasing the antibody titres

Intestinal washings, tracheal washings and testicular homogenate did not reveal any HI activity

Mancini's test was performed to quantitate the levels of first peak (supposedly 7 8s IgG) and second peak (supposedly 5 7s IgG)

The birds immunized with AS exhibited appreciable increase in the level of first peak serum bile and intestinal washings No significant increase in the level of first peak was observed for tracheal washings and oviduct washings, compared to the control Detectable levels of the first peak was observed in testicular homogenate of the treatment groups were they were sampled The levels of the second peak also showed slight increase in serum tracheal washing, testicular homogenate and oviduct washings

There was considerable increase in the level of first peak to that of control, in serum, bile, oviduct washings and testicular homogenate when immunized with NDV No significant increase of the first peak was seen in tracheal washings to that of the control No precipitation rings were obtained for this fraction in intestinal washing There was slight increase in the levels of the second peak

in serum and tracheal washing treatment groups of ducks immunized with NDV, compared to the controls

The second peak, if present, was below detectable levels in testicular homogenate both in control and treatment groups

Succinctly, the following were the conclusions obtained from the present study

1 Sephadex G 200 filtration alone was not a suitable method for separation of duck IgG and IgM

2 Fractionation of IgM of ducks was tedious by Sephadex G-200 filtration

3 Serum contained both 7 8s and 5 7s IgG in concentrations higher than in any other secretions as evidenced by chromatography electrophoretic analysis and quantitation

4 Bile contained only a single Ig which was similar in chromatographic elution pattern and electrophoretic migration to the 7 8 S IgG in serum

5 Tracheal washings contained 7 8s IgG and 5 7s IgG as evidenced by chromatography and immuno electrophoretic studies Though the presence of IgM could be established by immunoelectrophoresis, it could not be resolved by gel filtration using Sephadex G 200

6 Intestinal washings contained only one Ig, which could be the 7 8s IgG, going by the chromatographic and electrophoretic patterns

7 The immunoglobulin of the first peak (supposedly 7 8s IgG) was present in serum and all the secretions (bile tracheal washings, intestinal washings, oviduct washings and testicular homogenate) of ducks

8 The immunoglobulin of the second peak (supposedly 5 7s IgG) from Sephadex G-200 filtration of serum was also present in all secretions except bile and intestinal washings

9 Oviduct washings contained both first and second peak as evidenced by electrophoretic studies

10 Immunization with AS and NDV did increase the levels of 7 8s IgG and 5 7s IgG in serum and most secretions

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SECRETORY IMMUNOGLOBULINS OF THE DUCK *(Anas platyrrhynchos domesticus)*

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ABSTRACT OF A THESIS

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ABSTRACT

The profile and functional properties of the immunoglobulins of serum, bile, mucosa of trachea and intestine of ducks were studied

The immunoglobulins were separated by salting out using ammonium sulphate The various fractions of immunoglobulins were further resolved by Sephadex G-200 gel filtration which gave two peaks for serum and tracheal immunoglobulins and a single peak each for bile and intestinal immunoglobulins The purity of these fractions were checked by immunodiffusion and immunoelectrophoresis The different fractions obtained were quantified by single radial immunodiffusion The level of fraction 1 in the bile ranged between 1718 µg/ml and 1959 µg/ml and that of serum, 1718 06 µg/ml to 2442 µg/ml, in the *S typhimurium* treated groups The level of fraction 1 in the NDV treated groups ranged from 1115 µg/ml to 1597 35 µg/ml in bile, and 1597 35 µg/ml to 1959 34 µg/ml in serum The level of fraction 2 in serum ranged from 1797 µg/ml to 2591 µg/ml in *S typhimurium* treated group and 1400 µg/ml to 1797 µg/ml in the NDV treated group Fraction 2 was not detectable in bile

The antibody response of ducks to a bacterial and viral antigen (anaculture of *S typhimurium* and R_2B strain of New Castle Disease virus respectively) was assessed

On conducting standard tube agglutination test the serum, bile and oviduct washings revealed antibody tires against *S typhimurium* in inoculated birds ranging between 1 20 and 1 160 in the case of serum, 1 10 and 1 80 in the case of bile and tire less than 1 10 for oviduct washings No antibody titre could be detected for tracheal and intestinal washings and testicular extracts

The HI titre ranging from 1 32 to 1 128 could be observed for serum, 1 32 to 1 64 for bile and a titre of 1 16 was observed for oviduct washings of ducks parenterally administered with NDV The tire was relatively low for serum when NDV was administered intranasally Intestinal and tracheal washings and testicular extract failed to reveal any HI antibodies