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SERUM IMMUNOGLOBULINS IN DESI DUCKS*

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The classes of immunoglobulins (Igs) reported to be present in duck serum are IgM (Grey, 1967a., b., Hadge and Ambrosius, 1984., Higgins and Warr, 1993) and 7.8 S and 5.7 S IgG (Grey, 1967a., b., Zimmerman *et al.*, 1971, Liu and Higgins, 1990, Higgins and Warr, 1993). This low molecular weight Igs are now aligned with IgY (Higgins *et al.*, 1995). Structurally and antigenically the 5.7 S IgY resembles F(ab)2 fragment of 7.8 S IgY (Higgins *et al.*, 1995). A difference in proportion of 7.8 S and 5.7 S Igs have been reported in the serum of White Pekin and Muscovy ducks (Grey, 1967a., Zimmerman, 1971).

The serum Ig profile of Desi ducks which forms about 90% of the duck population in Kerala, was studied and presented.

Materials and methods

Duck serum

Blood collected from 3-4 month old desi ducks by jugular vein section was used for separation of sera. The separated serum was clarified by centrifugation at 3000 rpm for 10 minutes and stored at -20°C after adding merthiolate (1 in 10,000) as preservative.

Separation of globulins

Globulins from the duck serum were precipitated with 45% ammonium sulphate following the procedure described by Parry and Aitken (1975). The protein concentration of the precipitated globulin was estimated by Biuret method (Inchiosa, 1964).

The precipitated globulins were further fractionated by gel filtration chromatography using sephadex G-200 in Tris NaCl buffer, pH 8.0. The procedure described by Talwar (1983) was followed. This was done at room temperature, manually, adjusting the flow rate at 14 ml/hr; 2 ml per fraction. The absorbance of each fraction was determined at 280 nm in a UV-Vis spectrophotometer and quantitated by comparing with known protein concentration and its absorbance. The peaks obtained were concentrated separately and passed again through sepahdex G-200 till a single line of precipitation was obtained with each peak when tested against antiduck globulins by agar gel diffusion test (AGDT).

Rabbit antiduck globulins (RADSIg)

Newzealand White rabbits required for this purpose were purchased from the Small Animal Breeding Unit of Kerala Agricultural University. The following schedule was used for immunization of rabbits.

Two ml of 1 in 5 dilution of duck serum in phosphate buffered saline was mixed with equal volume of Freund's complete adjuvant and inoculated into rabbits intramuscularly at the rate of 2 ml per animal. This was followed by three booster doses of one ml each without adjuvant at seven days intervals by the same

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route. A week later, sera from these rabbits were collected, globulins separated as above and stored at -20°C in small aliquotes for further use.

Antisera to various peaks obtained in gel filtration chromatography were also prepared in rabbits for which the protein concentration was adjusted to 2 mg/ml. The schedule of immunization was the same used for RADSIg.

Agar gel diffusion test (AGDT) and Immunoelectrophoresis (IE)

These tests were done in 0.8% agarose in PBS and Tris barbiturate buffer (pH 8.6, 0.05M) respectively for AGDT and IE.

Results

The globulins separated from the duck serum had a concentration of 12.28 mg/ml.

On agar gel diffusion test between duck serum and RADSIg, three lines of precipitation were seen. The two lines on either side were faint but the middle one was very distinct (Fig. 1).

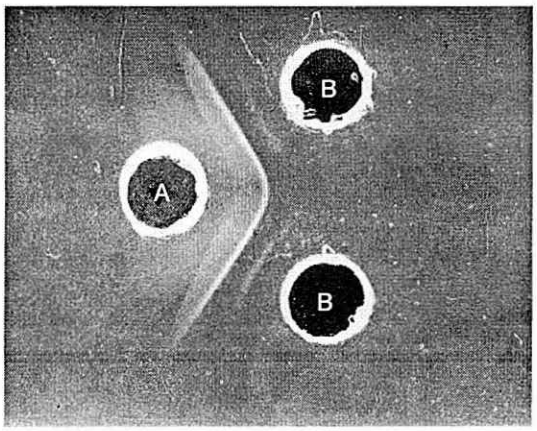
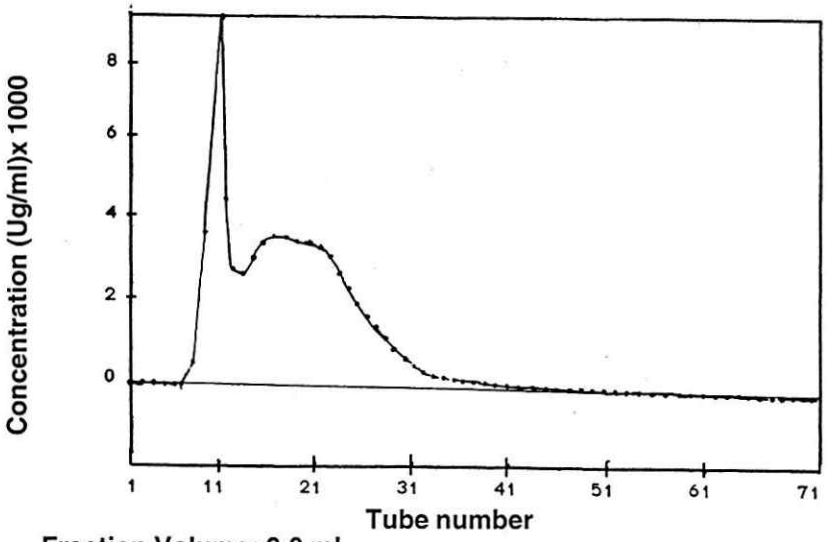


Fig. 1 - Agar gel diffusion test of duck serum against Rabit anti duck immuno globulin. A- RADSIg B- Duck Serum

The separated globulins when subjected to gel filtration chromatography through sephadex G-200, two peaks were seen, one between the 8th and 12th fraction and the second between the 15th and 27th fraction (Fig. 2). Pooled and concentrated first and

Fig. 2. Sephadex G-200 Chromatogram of serum Ig



Fraction Volume: 2.0 ml

second peak fractions against RADSIg gave a distinct line of precipitation in AGDT for the first peak with a spur over the line given by the 2nd peak which was sightly diffuse in nature (Fig. 3).

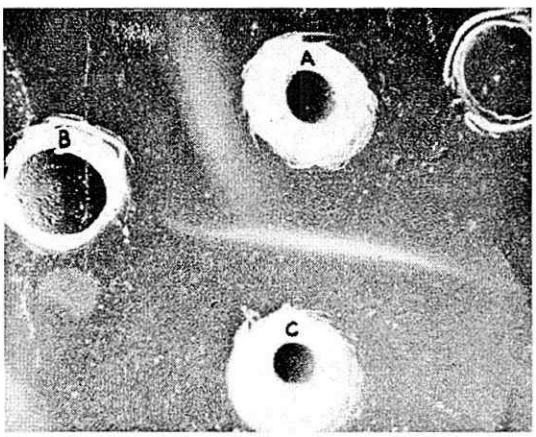


Fig. 3. AGDT of 1st and 2nd peaks of serum Ig obtained by chromatography against RADSIg.

A- RADSIg B- Second peak C- First peak

The pooled and concentrated first and second peak fractions when subjected to a second sephadex G-200 gel filtration revealed single elution peak each, indicating their purity.

Immunoelectrophoresis of fractions from 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 between the well and trough but closer to the latter (Fig. 4).

Discussion

Separation of globulins from pooled duck serum has been tried with 33-40% ammonium sulphate (Nair, 1990) and sodium sulphate 33-35% (Toth and Narcose, 1981). Nair (1990) reported more of non globulin proteins when precipitated with $(NH_4)_2 SO_4$ in concentrations higher than 33%. Higgins *et al.* (1995) opined that precipitation of gamma globulins from duck serum with Na_2SO_4 can reduce the non Ig fraction in the separated globulins.

The three lines of precipitation formed in AGDT between RADSIg and duck serum indicated the presence of three Igs in the serum (Grey, 1967a., b). The faint line formed closer to the serum (Ag) well might be the IgM which could not be resolved by gel filtration. The second line which was very distinct and the hazy third line may be representing the 7.8 S and 5.7 S Igs respectively.

Sephadex G-200 gel filtration of duck serum Ig revealed only two peaks of elution. These two peaks revealed proteins having partially identical antigneic configuration as evinced by AGDT against RADSIg. Moreover, the position of the precipitation arcs produced by these two peaks in IE also suggested that they could be IgG fractions. As against our observations, Grey (1967a) reported three elution peaks on sephadex G-200 gel filtration of duck serum Ig, the first

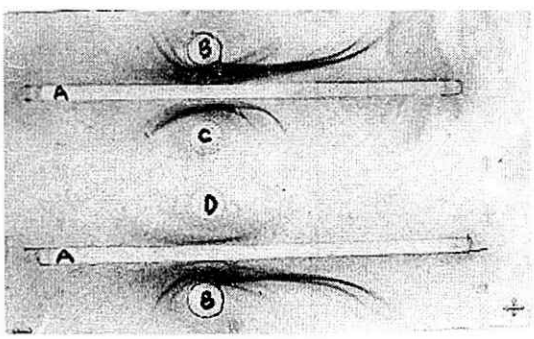


Fig. 4. Immunoelectrophoretogram of seum Ig and its 1st and 2nd peak obtained by chromatography

A- RADSIg B-SIg C 1st Peak D- 2nd Peak

corresponding to IgM along with varying amounts of lipid and aggregated material and two incompletely resolved peaks representing the 7.8 S and 5.7 S IgG. The failure to recover the IgM fraction in the present study might be due to the inefficiency of gel filtration technique to separate IgM or the quantity of IgM in the duck serum might be below the level that can be detected by AGDT or IEP. The quantitative differences of serum IgM might also be a contributory factor for the failure of chromatographic separation of IgM as experienced by Saif and Dohms (1976), while fractionating turkey serum by sephadex G-200 gel filtration.

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, 7.8 S IgG in the second peak and 5.7 S IgG in the third. They obtained pure samples of 7.8 S IgG by pooling the leading edge of the second peak and 5.7 S IgG from centre of the third peak. In the present study, though the void volume was pooled and concentrated from several sephadex G-200 filtration fractions, Ig could not be identified either by AGDT or IE against RADSIg. This could be due to the low concentration of IgM.

Toth and Norcross (1981) reported duck IgM as 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, thus indicating the presence of both 7.8 S and 5.7 S IgG.

In conclusion the present study shows that the blood serum of desi ducks of Kerala contains three types of Igs namely IgM, 7.8 S IgG (IgY) and 5.7 S IgG (IgY) and confirms the fact that the Ig profile of ducks are quite distinct from that of other avian Igs, lacking IgA and thus, ducks occupy a unique position in the ontogeny chain.

Summary

Globulins from the serum of desi ducks aged 3-4 months were precipitated with 45% ammonium sulphate and fractionated by sephadex G-200 gel filtration chromatography. The two peaks resolved were analysed by agar gel diffusion and immunoelectrophoresis and identified as 7.8 S and 5.7 S IgY respectively. Though the IgM peak could not be resolved, nor could it be demonstrated in the void volume, it was possible to get evidences that IgM is also present in Desi duck, but in minute quantity.

Acknowledgement

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