ovomucin can be solubilized by reduction with mercaptoethanol (Robinson & Monsey, 1966), it is now possible to determine accurately the composition of a lysozyme–reduced ovomucin complex.

The amount of complex precipitated has been determined from turbidity measurements at 450nm., and its composition calculated from measurements of the amounts of *N*-acetylneuraminic acid and lysozyme in the supernatants after centrifugation. Maximum turbidity was obtained when all the *N*-acetylneuraminic acid of the reduced ovomucin had been precipitated in the presence of excess of lysozyme. In Na_2HPO_4–KH_2PO_4 (*I* 0·05; Long, 1961) containing 0·05M-NaCl at pH 7·40 the ratio of lysozyme to *N*-acetylneuraminic acid present in the precipitated complex was approximately 21:1 (w/w) or 1:2 (mole/mole). In $NaHCO_3$–Na_2CO_3 (*I* 0·05; Long, 1961) containing 0·05M-NaCl at pH 9·85 the ratio was 43:1 (w/w) or 1:1 (mole/mole). Although the total amount of precipitate decreased as the pH value and ionic strength of the test solutions were increased, the ratio of lysozyme to *N*-acetylneuraminic acid present in the precipitates was independent of ionic strength at any given pH value.

Therefore it is suggested that only electrostatic forces are responsible for the interaction of reduced–alkylated ovomucin and lysozyme, whereas the interaction of reduced–alkylated lysozyme with reduced–alkylated ovomucin seems to be due also to hydrogen bonds. The decreased interaction of reduced–alkylated ovomucin and lysozyme at the higher pH value could be compatible with a decrease in the positive charge of the lysozyme molecules.

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Immunoglobulins in the Serum and Mucus of the Plaice (*Pleuronectes platessa*)

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Serum, intestinal and surface mucus of the plaice contained detectable antibody activity 65 days after immunization with human erythrocytes. Similar results were obtained with either limpet haemocyanin or heat-killed *Vibrio ichthyodermis* as antigens. Saline haemagglutinin titres were about 1:512 for serum and about 1:8 for mucus. The mucus contained not more than four of the total macromolecular components of serum so that a specific secretory system must be involved. The occurrence of antibody in the mucus of fish has not been previously reported and has an obvious survival value against infecting organisms.

The haemagglutinin present in both serum and mucus was precipitated by 18% (w/v) Na_2SO_4 and was not significantly retained by gel filtration on Sephadex G-200 or by chromatography on DEAE-Sephadex A-25 with 0·01M-phosphate buffer, pH 7·2, containing 0·1M-NaCl. Electrophoresis or immunoelectrophoresis at pH 8·6 showed the purified material from either serum or mucus to consist of a diffuse band with an anodal mobility corresponding to a 'fast' immunoglobulin of higher vertebrates. The agglutinin isolated from either serum or mucus had similar carbohydrate and amino acid compositions, and contained similar antigenic determinants since the haemagglutinating activity of plaice serum could be completely neutralized by rabbit anti-(plaice mucus).

The serum immunoglobulin has $S_{20,w}$ 12·4s and contained about 4·6% hexose and 4·2% glucosamine. After reduction and alkylation (Prahl & Porter, 1968) the protein was separated into two peaks by gel filtration on Sephadex G-75 with M-propionic acid as dissociating solvent. A direct comparison of the relative retention volumes of each peak showed that one peak corresponded to the alkylated light chain of human immunoglobulin G whereas the other peak was of higher molecular weight than the alkylated heavy chain of immunoglobulin G. Although the structure of the heavy chain of the plaice immunoglobulin has not been studied, Marchalonis & Edelman (1966) have shown that both the 17s and 7s immunoglobulins of the dogfish (*Mustelus canis*) contain heavy chains that most resemble μ-chains of human immunoglobulin M.

In contrast with higher vertebrates, both the plaice and the dogfish appear to synthesize only one class of antibody whose activity remains sensitive to thiol compounds even after prolonged periods of immunization.

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