## IDENTIFICATION OF THE INHIBITORY FACTOR OF RETICULO-ENDOTHELIAL IMMUNE SERUM (REIS) IN A GLOBULIN FRACTION[1]

SUCCESSFUL demonstration of the inhibitory properties of strong concentrations of reticulo-endothelial immune serum (REIS) by *in vitro* (Pomerat and Anigstein[2]) and *in vivo* methods (Anigstein and Pomerat[3]) as well as evidence for its stimulating action at high dilution (Pomerat[4]) have initiated a search for the active principle involved. Isolation of albumin and globulin fractions of REIS have been carried out according to the following procedure.

To 5.0 ml of serum was added, with constant stirring, an equal volume of saturated ammonium sulfate. The suspension was allowed to stand for one hour at 37°, following which the precipitate was separated by centrifugation. The supernatant albumin fraction was removed with a capillary pipette, placed in a Cellophane bag (10 mm diameter Cellophane tubing was found to be satisfactory) and dialyzed against cold Tyrode's solution until the external fluid was sulfate-free. The albumin solution was then passed through a micro-Seitz filter and tested for its REIS effect.

In our first experiments, the precipitated globulins were redissolved in 5 per cent. saline and dialyzed against distilled water until free of chlorides. This procedure apparently resulted in some denaturation, since the precipitated globulins were no longer completely soluble in dilute salt solutions. The procedure finally adopted was to redissolve the precipitate from the original ammonium sulfate treatment in cold Tyrode and then to dialyze the solution against Tyrode for the same length of time as used in the treatment of the albumin fraction. The solution remained completely clear. Sterilization and testing of the globulin fraction were accomplished in the same manner as described for the albumin solution.

REIS fractions were added at various concentrations to tissue culture media in which heart fragments from chicks incubated for 6 days were grown. In some experiments the spleen of 18-day incubated chicks was used. Twelve per cent. embryonic extract and 50 per cent. heparinized rooster plasma served as the basic medium. Several preparations were used for each concentration tested.

Preliminary experiments demonstrated that traces of ammonium sulfate could be detected by the presence of aqueous vacuolization in migrating cells within a few hours of incubation. Tests with ammonium sulfate at 1:800 showed almost complete inhibition. In subsequent fractions, no trace of ammonium sulfate could be detected.

The albumin fraction obtained from homologous (anti-chick) REIS with a complement fixation titer of 1:1200 showed no inhibition of outgrowth from fibroblasts or from splenic fragments.

The globulin fraction, on the other hand, inhibited the outgrowth of fibroblasts and produced clumping of splenic cells to approximately the same degree as controls containing an equal dilution of corresponding REIS.

Globulin fractions of heterologous (anti-rat) REIS with a complement fixation titer of 1:1600 did not produce such inhibitory effects on chick tissues *in vitro*.

Experiments in progress will attempt to develop a method of measuring the potency of globulin fractions of REIS both as to their inhibitory and their possible stimulating action. Further fractionation of the globulins is also under study.

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## THE EFFECT OF SODIUM CHLORIDE BALANCE ON THE AVIDITY OF ISOHEMAGGLUTININS

FACTORS having to do with the relationship of the titre of typing serums used for the determinations of the human blood groups are of importance.

Our own experience has shown, as also stated by others[1,2,3,4] that the agglutinin titres of human serums range from 1–1 to 1–2,048. This is without benefit of concentration or globulin fractionation of the original serum.

However, the individual whose serum shows an extremely high titre is rather rare and scarcely to be depended upon when attempting to prepare human anti-A and anti-B serums. To substantiate this, we have found upon testing the sera of approximately 52,000 bleedings received from the American Red Cross Blood Donor Service from which dried plasma was processed for the Army Medical Corps, that ten per cent. of these bloods show agglutinin activity so

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