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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[2] Pomerat, C.M., and Anigstein, Ludwik, Texas Rep. on Biol. and Med., 3: 1, 122, 1945.

[3] Anigstein, Ludwik, and Pomerat, C.M., Fed. Proc., 4: 1, 111, 1945.

[4] Pomerat, C.M., Fed. Proc., 4: 1, 56, 1945.

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

[1] A. S. Wiener, "Blood Groups and Transfusion," Charles C Thomas Publishing Company, Springfield, Illinois, 3rd ed., 1943.

[2] L. Pillemer, J. L. Oneley, M. Melin, J. Elliott and M. D. Hutchinson, *Jour. Clin. Invest.*, Vol. xxiii, No. 4, p. 550.

[3] W. Thalhimer and S. A. Myron, *Jour. Am. Med. Asn.*, 118: 370, 1912.

[4] E. L. DeGowin, *Jour. Clin. Invest.*, 23: 554, 1944.

that when their sera are pooled after extremely careful selectivity the pool titre is 1–2,048. The selectivity includes three separate tests for activity as well as tests for specificity. In the careful preparation of these serum pools, we know that not every one of the sera used has an agglutinin titre of 1–2,048 individually. In this particular phase of the determination of the strength of the pool the specific cells used were a number of known weak group B and known A_2 subgroup.

Despite this apparently satisfactory titre it has been noted numerous times that the avidity of agglutinins does not always occur in direct relation to the number of agglutinins present in the human serum. Although the titre may show 1–2,048, the serum upon test against specific cells may result only in a moderate agglutination.

During the course of our recent investigations, we found it feasible to dry *in vacuo,* after preliminary deep freeze in dry ice, our pools of anti-A and anti-B serums. These were dried in 0.1 cc quantities, in small vials using a short drying period. We found that upon restoration with either a 1 per cent. or 5 per cent. cell suspension in 0.9 per cent. saline that the avidity of the agglutinins was more than doubled. The reaction equalled that of a serum highly concentrated by drying or a highly concentrated globulin fraction.

Inasmuch as only water is removed during the drying process, it was concluded that the alteration of the sodium chloride content was radically affecting the agglutinin strength. This was shown definitely when upon the addition of distilled water on restoration the activity of the serum was about the same as before drying, *i.e.,* moderate only. The same effect was obtained where solid HaCl was added to liquid serum in order to increase toxicity.

Tests were set up to determine the amount of sodium chloride in cell suspensions which, added to the dry serums, would impart the greatest avidity to the agglutinins. Using 5 per cent. cell suspensions prepared in 0.5 per cent. sodium chloride, 0.6 per cent., 0.7 per cent., 0.75 per cent., 0.8 per cent., 0.85 per cent. and 0.9 per cent. both anti-A and anti-B showed complete agglutination in two seconds and 4+ agglutination at 30 seconds. However, upon varying the amount of sodium chloride from 1.1 per cent. to 2.0 per cent. (by tenth per cents.) in cell suspensions, we noted a gradual lessening of avidity. At 2.0 per cent. the reaction was almost doubtful.

Therefore, at the present time we find that a final salt concentration in a range of 1.45 per cent. to 1.75 per cent. will impart the greatest avidity to the isohemagglutinins.

SUMMARY

(1) The avidity of agglutinins in human anti-A and anti-B serums is influenced by the tonicity of the menstruum.

(2) The addition of sodium chloride in final concentration in a range of 1.45 to 1.75 per cent. will greatly increase the avidity of human serum isohemagglutinins.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CONTROL AND EVALUATION OF BLOOD SERUM ASSAYS FOR PENICILLIN

THE power of the blood of man and animals to inhibit the growth of many bacteria has long been recognized. This inhibitory power varies with species and races, but it manifests itself primarily as an individual difference either in immunity or possibly in metabolism or body chemistry. Blood of normal adults is more inhibitory than that of normal children; disease increases the titre in both. Included among the organisms inhibited by the blood of man are streptococci, staphylococci and certain aerobic spore formers, all of which are employed at present in the assay for the potency of penicillin.

For the past year[1] a serial dilution method of assay employing *Bacillus subtilis* as the test organism has been used. In investigational work on several salts of penicillin,[2] oral penicillin[3] and penicillin "X",[4] several hundred blood samples obtained at varying intervals after the administration of penicillin were assayed. A blood specimen taken from an individual prior to administration of penicillin served as a control. After a number of individuals (laboratory personnel) had volunteered as test subjects several times each, it was observed that the titre of the blood-inhibitory substances for *B. subtilis* was both variable and transitory in a given individual. It was obvious,

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[2] L. E. Putnam, H. Welch and S. Olansky, *Jour. Am. Med. Asn.*, 127: 204, 1945.

[3] H. Welch, C. W. Price and V. L. Chandler, *Jour. Am. Med. Asn.*, 128: 845, 1945.

[4] H. Welch, L. E. Putnam, Wm. A. Randall and R. P. Herwick, *Jour. Am. Med. Asn.*, 126: 1024, 1944.