CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. XIV. APPRAISAL OF ISOHEMAGGLUTININ ACTIVITY [1]

By ELMER L. DeGOWIN

(From the Department of Internal Medicine, College of Medicine, State University of Iowa, Iowa City)

(Received for publication February 17, 1944)

The development of the by-products, from the preparation of human serum albumin for the Armed Forces (1), made available large quantities of globulins containing isohemagglutinins. It was found possible to produce material rich in isohemagglutinins from pools of human group-specific plasma, derived from bloods unselected for agglutinin titer. The method of preparation has been reported by Pillemer and co-workers (2). It became the problem of the Subcommittee on Blood Substitutes of the National Research Council to evaluate the product. A group of consultants,[2] qualified in the field of blood grouping, collaborated in testing various lots of concentrated isohemagglutinin preparations made at the Pilot Plant, Department of Physical Chemistry, Harvard Medical School. Samples from 14 lots of material were distributed to the investigators for study. The first 6 lots were in the liquid form while the remaining 8 were converted to the dry state and sealed *in vacuo*.

The consultants all agreed that the 14 preparations submitted for evaluation were sufficiently potent to be acceptable as grouping sera. These conclusions were based on each man's experience in testing the various lots by his own method and comparing the reactivity with that of other grouping sera which he had previously found satisfactory for routine use.

A secondary result of this project led to some rather surprising conclusions. Many writers on the technic of blood grouping have published minimum titration values for desirable grouping sera. It has been tacitly assumed that these numerical results could be duplicated by other workers without much regard for the details of the method employed. This study afforded the opportunity to compare titration values obtained by 11 experts on the same material. Each consultant reported the maximum dilution of serum (uncorrected for added cell suspension) in which agglutination of test erythrocytes occurred. He also noted the appearance time of macroscopic agglutination in a serum-cell mixture on a manually agitated slide and the time at which agglutination was completed. The results showed the wide ranges indicated in Table I.

It was evident from an analysis of the results of each worker that although his values were not comparable with those of another on the same serum, by employing his own method, fairly consistent agreement could be obtained with the others as to the relative potency of various lots of isohemagglutinins. In a conference of the consultants, the variables in the methods of evaluation of the sera were discussed. Aside from the details of mixing serum and cells, it was agreed that the chief variables were the different criteria of the end-point of agglutination, the variations in sensitivity of the erythrocytes derived from different sources, and the differences in concentrations of the cell suspensions employed in determining the agglutination time. Some methods involved the detection of minimal agglutination with the microscope and others

[1] The products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] Dr. W. L. Boyd, Boston University; Dr. L. W. Diggs, University of Tennessee; Dr. Paul I. Hoxworth, Cincinnati General Hospital; Dr. Karl Landsteiner, Rockefeller Institute (Dr. Landsteiner died before the completion of this work); Dr. Philip Levine, Newark Beth Israel Hospital; Dr. R. O. Muether, St. Louis University; Dr. Max M. Strumia, Bryn Mawr Hospital; Dr. William Thalhimer, Manhattan Convalescent Serum Laboratory; Dr. Alexander S. Wiener, Office of the Chief Medical Examiner of New York City; Dr. Ernest Witebsky, Buffalo General Hospital; Dr. Elmer L. DeGowin, State University of Iowa.

TABLE I

Results obtained by various consultants on titration of isohemagglutinins

Serum lot number	Group of test cells	Titrations in serum dilutions, ranges	Agglutination time in seconds,* ranges
81A	B	1 : 32 to 1 : 1000	10–40 to 15–60
81B	A_1	1 : 64 to 1 : 1000	10–40 to 15–60
	A_2	1 : 20 to unreported	
85A	B	1 : 20 to 1 : 200	10–30 to 35–110
86B	A_1	1 : 40 to 1 : 532	7–40 to 43–98
	A_2	1 : 10 to unreported	
87A	B	1 : 16 to 1 : 800	11–50 to 30–300
88B	A_1	1 : 64 to 1 : 800	7–24 to 15–600
	A_2	1 : 20 to 1 : 80	
89A	B	1 : 30 to 1 : 640	4–19 to 30–120
90B	A_1	1 : 120 to 1 : 640	3–8 to 20–60
	A_2	1 : 30 to 1 : 128	8–53 to 30–180
91A	B	1 : 32 to 1 : 160	7–18 to 25–120
94B	A_1	1 : 120 to 1 : 640	5–15 to 20–60
	A_2	1 : 32 to 1 : 64	15–30 to 60–180
92A	B	1 : 40 to 1 : 320	4–12 to 15–120
9094B	A_1	1 : 120 to 1 : 320	3–14 to 15–60
	A_2	1 : 16 to 1 : 128	8–32 to 40–180
9193A	B	1 : 32 to 1 : 160	8–24 to 17–52
104B	A_1	1 : 16 to 1 : 320	3–30 to 25–45
	A_2	1 : 8 to 1 : 80	16–75 to 50–

* Agglutination time is expressed as: seconds required for appearance of macroscopic agglutination—seconds for agglutination to be completed, e.g., 7–30.

employed the criterion of the appearance of macroscopic clumps of red cells. This factor is readily capable of standardization. Careful attention to the concentration of red cell suspensions in the determination of agglutination time would lead to more nearly reproducible results. Hitherto, there has been no satisfactory method of testing the sensitivity of the erythrocytes employed in the tests. There is agreement that the cells ought to be freshly collected. Each worker must therefore rely on sources of test cells convenient to his laboratory. Ordinarily, this makes difficult the comparison of the results of 2 workers.

The device of employing a "reference serum" was finally adopted. Lots 9193A and 104B were made in quantity at the Harvard pilot plant. These preparations were attested by the chemists as being representative of the chemical methods employed. The dried material was placed in glass ampules and sealed *in vacuo*, so that 5 cc. of the reconstituted material was contained in each package. The lots were judged acceptable as grouping sera by all the consultants who tested them. It was understood that the reference sera represent neither the maximum, minimum, nor optimum potency but that they contain arbitrary concentrations of isohemagglutinins which are acceptable for use as grouping sera. They can be employed in either of 2 ways. The sensitivity of the agglutinogens in the test erythrocytes may be determined by titration against the reference serum. This would seem to remove the last serious obstacle to an accurate standardization of the method of titration of grouping sera. In lieu of this, the relative potency of a grouping serum may be determined with respect to the reference serum by testing both simultaneously against the same suspension of red cells, the same methods being employed for each serum. The investigator may then state the strength of the unknown serum in terms of the potency of the reference serum with respect to the test cells employed. If the unknown is approximately twice the strength of the reference material, it may be expressed as 2R; if half as strong, as 0.5R.

SUMMARY

All 14 preparations containing isohemagglutinins, concentrated from pooled human plasma of appropriate blood group, but unselected for agglutinin titer, made by the method of Pillemer and co-workers, were found acceptable as grouping sera by a panel of consultants, working under the auspices of the Subcommittee on Blood Substitutes of the National Research Council.

When various experienced workers tested the same preparation of isohemagglutinins for titer and agglutination time, it was found that numerical values obtained by one worker could not be compared with those of another without a degree of standardization of technic which does not at present exist in this field. Some of the variation in results could be attributed to different criteria employed in judging the end-point

of agglutination. Another factor was the variation in sensitivity of test red cells, derived from different sources.

A lot of material containing anti-A agglutinins and one containing anti-B activity were accepted by the consultants as reference sera. These were packaged to insure maximum stability. They may be employed to determine the sensitivity of test erythrocytes or in comparison with sera of unknown potency in simultaneous parallel tests.

BIBLIOGRAPHY

1. Cohn, E. J., Oncley, J. L., Strong, L. E., Hughes, W. L., Jr., and Armstrong, S. H., Jr., Chemical, clinical, and immunological studies on the products of human plasma fractionation. I. The characterization of the protein fractions of human plasma. J. Clin. Invest., 1944, **23**, 417.
2. Pillemer, L., Oncley, J. L., Melin, M., Elliott, J., and Hutchinson, M. C., Chemical, clinical, and immunological studies on the products of human plasma fractionation. XIII. The separation and concentration of isohemagglutinins from group-specific human plasma. J. Clin. Invest., 1944, **23**, 550.