# CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

## II. ELECTROPHORETIC AND ULTRACENTRIFUGAL STUDIES OF SOLUTIONS OF HUMAN SERUM ALBUMIN AND IMMUNE SERUM GLOBULINS [1,2]

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The importance of the standardization of new products of therapeutic value need hardly be stressed in this place. In the fractionation of plasma proteins on a large scale, it is necessary to have means of determining the uniformity of the products for the purpose of insuring predictable clinical responses. In the case of the products from human plasma, it seemed most appropriate to employ not only clinical but also physical chemical methods of control—procedures which had been of value in the development of these products.

### NORMAL HUMAN SERUM ALBUMIN

*Electrophoretic analyses* of plasma and of the products derived from plasma have been considered in Paper I of this series (1). Of the 6 readily separable electrophoretic components of plasma, the albumin has the most rapid electrophoretic mobility, and is thus readily distinguishable from all but the most rapidly moving globulin components. The earlier electrophoretic analyses of human serum albumin solutions were carried out with a phosphate buffer of pH 7.7, ionic strength 0.2. The results obtained by the analysis of 113 solutions of serial preparations of human albumin, by 7 laboratories, are recorded in Table I. The data are reported in terms of the total percentage of globulin, this being entirely $\alpha$-globulin in almost every case. The sensitivity of this method for the estimation of the total globulin is of the order of 1 per cent. It was found that 100 of these samples, analyzed by a uniform technic, contained less than this amount of globulin.[4]

In order to effect a more nearly quantitative separation between the albumin and the most rapidly moving of the globulins, $\alpha_1$-globulin,

TABLE I

*Electrophoretic analyses of normal human serum albumin solutions. Number of separate analyses*

(Potassium phosphate buffer pH 7.7, ionic strength 0.2)

| Processing plant | Less than 1 per cent globulin | 1 to 1.5 per cent globulin | 1.5 to 2 per cent globulin |
|---|---|---|---|
| A | 14 | 6 | 1 |
| B | 14 | 2 | 0 |
| C | 11 | 0 | 0 |
| D | 26 | 1 | 0 |
| E | 10 | 2 | 0 |
| F | 22 | 1 | 0 |
| G | 3 | 0 | 0 |
| Total | 100 | 12 | 1 |

[1] This work has been carried out under contracts, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Wisconsin and between the Office of Scientific Research and Development and Harvard University.

[2] This is paper No. 14 in the series, "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

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[4] Other investigators, using a somewhat different electrophoretic analytical procedure, have reported the presence of slightly over 2 per cent globulin in standard albumin preparations. Such findings do not necessarily reflect a systematic error in analysis but are indicative of the fact that in using a method of sensitivity as low as 1 per cent, departure from the technic that we have routinely employed may consistently lead to slightly different values.

more recent electrophoretic studies have been carried out at a more alkaline pH and a lower ionic strength. A diethyl barbiturate buffer, first introduced by Longsworth (2), of pH 8.5 and 0.1 ionic strength has been used. This buffer gives a more complete separation of $\alpha$-globulin and reveals somewhat more total globulin, since much of the $\alpha$-globulin is not distinguished from albumin when studied in the phosphate buffer at pH 7.7. Such analyses are summarized in Table II. It will be seen from these results that only small amounts of $\alpha$-globulin are detected in these serum albumin solutions (average of 1.5 per cent) and that fibrinogen, $\beta$-, and $\gamma$-globulin are all absent within the limits of sensitivity of the test.

*Ultracentrifugal analyses* of serum albumin solutions have not been routinely carried out, since the sedimentation diagrams depend to a considerable extent upon the ionic strength and pH of the solvent. Preparations of serum albumin thus far studied reveal slightly asymmetrical sedimentation diagrams in the ultracentrifuge. Whatever the interpretation of this asymmetry, it is clear that it must be due to a small amount of material with a sedimentation constant very nearly that of normal serum albumin and too small to be mistaken for that characteristic of normal serum globulin. Such diagrams have indicated, however, that materials of a sedimentation constant, very different from normal albumin, are not present within the accuracy of this method of analysis. Since it has been demonstrated that faster sedimenting components are sometimes formed as a result of drastic conditions introduced in the fractionation of certain unstable albumin preparations, it is of considerable importance to have demonstrated the absence of such material in all other preparations of albumin that have been studied. As a routine test, such unstable preparations have been more readily detected by nephelometric and viscometric stability studies, reported by Scatchard and coworkers, in the fourth paper of this series (3).

TABLE II

*Electrophoretic analysis of normal human serum albumin preparations. Average distribution of components*

(Barbiturate or veronal buffers pH 8.5, ionic strength 0.1)

| Processing plant | Number of analyses | $\alpha_1$ Globulin | $\alpha_2$ Globulin | $\beta$ Globulin | Albumin |
|---|---|---|---|---|---|
| B | 12 | 0.7 | 0.7 | 0 | 98.6 |
| C | 8 | 1.2 | 0.9 | 0 | 97.9 |
| D | 9 | 0.9 | 0.8 | 0 | 98.3 |
| E | 4 | 0.5 | 0.6 | 0 | 98.9 |
| F | 9 | 0.7 | 0.6 | 0 | 98.7 |
| G | 20 | 1.4 | 0.8 | 0 | 97.8 |
| Total | 62 | | | | |
| Average | | 0.9 | 0.7 | 0 | 98.4 |

## HUMAN IMMUNE SERUM GLOBULIN

*Electrophoretic analyses* of nearly all of the preparations of immune globulin have been carried out by using diethyl barbiturate buffer, pH 8.5, ionic strength 0.1. The separation of $\beta$-globulin from both $\alpha$- and $\gamma$-globulin is not complete under these conditions, and the anlyses therefore are somewhat unsatisfactory. They have, however, been carried out in the same way for all of these solutions, and the last 4 columns of Table III record the values obtained by a standardized procedure. It will be seen that some albumin and $\beta$-globulin are present in these preparations. If the results obtained with all preparations are averaged, we obtain values of about 2 per cent albumin and about 11 per cent $\beta$-globulin. The preparations fractionated by later methods (3A and 3B) average about 2 per cent albumin and 4 per cent $\beta$-globulin, whereas those fractionated by the more recent method (see Table III, Paper I) (1) consist of over 98 per cent $\gamma$ globulin.

*Ultracentrifugal analyses* of all these solutions were carried out with 0.15 molar sodium chloride as solvent. The sedimentation studies were made at the pH of the immune globulin preparation, usually between pH 6.8 and 7.4. The ultracentrifuge components have been designated as slow moving, normal, and fast moving. The sedimentation constants of the slow moving components were of the order of 4 to 5 Svedberg units, and presumably represented albumin at least in large part.[5] The fast moving components had sedimentation constants varying from 8 to 18 Svedberg units, and this high molecular weight material must be largely $\gamma$-globulin since

[5] See the discussion of sedimentation (1).

TABLE III

*Chemical and physicochemical assay of various preparations of Fraction II*

| Preparation number | Method of fractionation | Cholesterol concentration | Ultracentrifugal analysis | | | Electrophoretic analysis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Slow | Normal | Fast | Alb. | α-Glob. | β-Glob. | γ-Glob. |
| | | *mgm. per ml.* | | | | | | | |
| A48 | 2 | 0.2 | 9 | 78 | 13 | 8 | 0 | 4 | 88 |
| A54R | 2 | 0.3 | 14 | 77 | 9 | 8 | 0 | 8 | 84 |
| A54K | 2 | 0.2 | 13 | 78 | 9 | 8 | 1 | 9 | 82 |
| A58 | 2 | 0.1 | 10 | 81 | 9 | 9 | 0 | 12 | 79 |
| A29 | 3* | 0.6 | | | | 4 | 1 | 6 | 89 |
| D26 | 3* | 0.6 | 8 | 78 | 14 | 4 | 0 | 13 | 83 |
| A35 | 3* | 1.2 | 4 | 80 | 16 | 2 | 1 | 8 | 89 |
| A74B | 3* | 0.9 | 4 | 75 | 21 | 1 | 0 | 14 | 85 |
| C36 | 3 | 0.7 | 4 | 78 | 18 | 2 | 0 | 13 | 85 |
| A66 | 3 | 0.9 | 6 | 80 | 14 | 2 | 1 | 4 | 93 |
| A72 | 3 | 0.5 | 4 | 82 | 14 | 2 | 0 | 16 | 82 |
| C51 | 3 | 0.7 | 7 | 80 | 13 | 1 | 0 | 9 | 90 |
| C70 | 3 | 0.7 | 5 | 78 | 17 | 2 | 0 | 17 | 80 |
| C80 | 3 | 1.3 | 4 | 79 | 17 | 1 | 0 | 14 | 85 |
| C97 | 3 | 0.5 | 4 | 77 | 19 | 1 | 0 | 8 | 91 |
| C102 | 3 | 0.5 | 5 | 74 | 21 | 1 | 0 | 13 | 86 |
| C103 | 3 | 1.1 | 4 | 73 | 23 | 1 | 0 | 12 | 87 |
| C104 | 3 | 1.5 | 3 | 65 | 32 | 0 | 0 | 21 | 79 |
| C105 | 3 | 2.2 | 3 | 74 | 23 | 0 | 0 | 17 | 83 |
| C106 | 3 | 1.7 | 3 | 74 | 23 | 0 | 0 | 15 | 85 |
| C107 | 3 | 1.9 | 2 | 74 | 24 | 1 | 0 | 11 | 88 |
| C108 | 3 | 1.3 | 4 | 74 | 22 | 1 | 0 | 16 | 83 |
| C109 | 3 | 1.3 | 3 | 78 | 19 | 1 | 1 | 13 | 85 |
| D36 | 3 | 1.3 | 2 | 78 | 20 | 1 | 0 | 17 | 82 |
| A80 | 3 | 1.3 | 3 | 75 | 22 | 1 | 0 | 21 | 78 |
| A84 | 3 | 0.3 | 4 | 74 | 22 | 1 | 0 | 11 | 88 |
| A109 | 3 | 0.9 | 3 | 68 | 29 | 2 | 0 | 13 | 85 |
| A97 | 3A | 1.0 | 5 | 70 | 25 | 3 | 1 | 6 | 90 |
| B1 | 3A | 0.2 | 2 | 78 | 20 | 2 | 0 | 2 | 96 |
| B2 | 3A | 0.2 | 5 | 71 | 24 | 3 | 0 | 2 | 95 |
| A74A | 3A* | 0.2 | 3 | 72 | 25 | 1 | 0 | 4 | 95 |
| AS84 | 3A* | 0.2 | 6 | 75 | 19 | 4 | 1 | 4 | 91 |
| A269 | 3B* | 0.7 | 4 | 72 | 24 | 2 | 0 | 6 | 92 |
| A291 | 3B* | 0.5 | 6 | 69 | 25 | 1 | 0 | 5 | 94 |
| A111 | 3B | 0.4 | 5 | 74 | 21 | 1 | 0 | 4 | 95 |
| Grand average | | 0.8 | 5 | 75 | 20 | 2 | 0.2 | 11 | 87 |
| Average methods 3A and 3B | | 0.4 | 5 | 73 | 23 | 2 | 0.2 | 4 | 94 |

* These preparations were derived from Fraction II + Fraction III which had been frozen.

in amount it is often in excess of the amount of electrophoretically determined α- and β-globulins. Of this faster moving material, only a small percentage is material of sedimentation constant 18; the bulk of it represents material moving only slightly faster than normal globulin.

*Cholesterol analyses* are also reported in Table III.[6] The values recorded here are in milligrams per milliliter, and should be divided by 2 in order to express the percentage of cholesterol in this material, since these solutions all contain about 200 mgm. of protein per ml. It will be observed that these values have a considerable range, higher values usually being observed for preparations high in β-globulin by electrophoretic analysis. An average value of about 0.8 mgm. cholesterol per ml. is obtained from all preparations, the more recent methods (3A and 3B) yielding a lower average of about 0.5 and often as low as 0.2 mgm. per ml.

[6] These analyses have been carried out by Paul Gross at the Department of Physical Chemistry, Harvard Medical School, following the method of Bloor, Pelkan, and Allen (4).

SUMMARY

Results of electrophoretic and ultracentrifugal analyses on serum albumin solutions have indicated that fibrinogen, $\beta$-, and $\gamma$-globulin, and components of molecular weight as large or larger than the normal globulins of plasma, are not present within the accuracy of these methods of analysis. The electrophoretic analyses have been carried out on 162 preparations delivered to the armed forces by 7 different laboratories and indicate that the albumin is routinely concentrated by this method of fractionation from a value of 55 or 60 per cent in plasma to a value of 98.5 per cent.

The immune serum globulins of 35 preparations from 4 laboratories have been studied and indicate that the $\gamma$-globulin content of these materials has been increased from about 11 per cent in plasma to about 87 per cent, and, in most of the more recent preparations, to over 95 per cent, the main impurities being $\beta$-globulin and albumin. An average value of very nearly 20 per cent of fast moving material, in large part $\gamma$-globulin, has been observed in the ultracentrifuge, the amount of this material being quite uniform in nearly all preparations.

These studies have given evidence of the reproducibility of these materials, and have provided the chemical specification of purity used in setting up minimum requirements for the acceptance for the armed forces of these products of plasma fractionation.

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