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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. IV. A STUDY OF THE THERMAL STABILITY OF HUMAN SERUM ALBUMIN

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Research Article

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

IV. A STUDY OF THE THERMAL STABILITY OF HUMAN SERUM ALBUMIN[1,2,3]

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Normal human serum albumin, though prepared as a white dry powder, is distributed to the armed forces as a 25 per cent aqueous solution. This is advantageous, since the solution is ready without reconstitution for immediate emergency use, and is possible, because the albumin solution is very stable, and, for a protein, very resistant to heat.

A 25 per cent solution of human serum albumin is a clear, straw-colored liquid. Its viscosity is about the same as that of whole blood with a hematocrit of 50. On standing at very high temperatures, it in time becomes more viscous, and haze with or without visible flocs appears. Later still, gelation occurs. The rate of these changes increases with increase in temperature. The intravenous kit includes a 200 mesh filter to remove flocs, and there is no evidence that the usefulness of the solution is impaired as long as it will flow through the needle. However, haze might be interpreted by the medical officer in the field as bacterial contamination and the development of flocs or of increased viscosity indicates incipient denaturation of protein. Excessively rapid denaturation probably indicates some impurity other than albumin in the original preparation. Therefore, the initial turbidity and its rate of change at 50° C. (122° F.) and 57° C. (135° F.) have been made the criteria of the acceptability of human serum albumin solutions for the armed forces. In addition, we have used the change in viscosity as a measure of the stability of albumin solutions. The two are complementary since turbidity measures the size of individual particles and depends but little on their shape or degree of hydration, while viscosity measures the shape and degree of hydration and depends but little on the size. The stability of albumin is so great that it has been necessary to devise tests which subject the albumin to much more rigorous conditions than it is likely to meet in the field, and measure changes much too small of themselves to be of practical importance.

METHODS

Viscometric studies have been made in an Ostwald type viscometer, modified so that sterility may be maintained and evaporation from the warm surfaces prevented. In this method, the density and time of flow through a capillary are measured. The viscosity is proportional to the product of density and time, and the proportionality constant can be determined by measuring these quantities for a liquid of known viscosity. In a stability study, the density remains constant, so viscosity is proportional to time of flow.

In dilute solutions, the viscosity, η, and its logarithm are both proportional to concentration, but the logarithm remains proportional up to much higher concentrations, and is thus a better measure of the extent of such change. Those reported in this paper are so small that this difference is not important, but we have followed our usual custom and tabulated values of log η.

Turbidity studies have usually been made in a Zeiss-

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

[2] This paper is Number 15 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.

[3] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting views of the Navy Department or the Naval Service at large.

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Pulfrich photometer with nephelometer attachment, in which the scattered beam makes an angle of 45° with the unscattered emergent beam.[5] A very slight visible haze corresponds to 20 or 25 units, and an easily visible haze to 30 or more units. The average well-filtered albumin solution has a nephelometric reading of less than 10 units, even after being heated at 50° C. for 2 weeks. An increment of 20 nephelometric units would thus render the average preparation hazy and has been adopted as a convenient quantitative measure of stability. Some of the solutions have been studied in a special photoelectric tyndallometer [6] which measures the light scattered at any angle. The light source is a high pressure mercury arc. The incident beam can be polarized at any angle and the intensity of its polarized components measured. One unit of this instrument is approximately one-fifth of a Zeiss unit.

In dilute solutions, the scattered light is proportional to the concentration of scattering particles, but at higher concentrations, the effect of the solute on the index of refraction becomes important. In concentrated albumin solutions, the scattering actually decreases as the protein concentration increases. However, there is little change of index of refraction on denaturation, and the increase in turbidity is proportional to the number of scattering particles if the nature of these particles is unchanged.

The light scattering per unit mass depends greatly upon the size of the particles, is at a maximum for particles whose diameter is equal to the wave-length of light (about 5000 Å), and is very small for diameters less than one-tenth or more than ten times this wave-length. For particles smaller than the wave-length of light, the scattering at 45° is approximately twice that at 90°, but larger particles scatter much more through the smaller angle. So the contribution to the scattering of large particles may be determined approximately from the difference in scattering at these two angles. It may be recalled in this connection that the serum albumin molecule is approximately egg-shaped and is estimated to be about 150 Å in length and 38 Å wide (Studies I, Table I) (2).

J. Murray Luck and his associates at Stanford University have devised a turbidity test for the stability at high temperatures by measuring the time necessary to give a visible cloud in a capillary tube illuminated from the side. The turbidity increase at this stage is so rapid that a very approximate measure of turbidity gives an accurate measure of the time. This method is discussed in another paper in this series (1).

Gelation studies have also been undertaken to determine, in quantitative terms, the relation between turbidity and development of structure. This has been estimated by noting the time at which freedom of motion of entrapped air bubbles is lost. The end-point adopted is that the bubbles in a bottle containing 25 per cent albumin, when rotated back and forth, shall exhibit recoil. Whereas gelation limits the conditions to which these very stable albumin solutions can be subjected, incipient turbidity gives the earliest indication of instability, and has indeed formed the basis for the testing and acceptance of albumin for the armed forces.

The effects on stability at different temperatures, of changing the concentrations of albumin, of salt, of hydrogen ion, and of merthiolate, and of storing at moderate temperatures have been determined. Most of these effects were studied first on commercial preparations in which 1 to 2 per cent of the protein was globulin, but many of our more detailed experiments have been carried out on crystallized human albumin of very high purity.[7] Such studies tell us more regarding the albumin itself. The crystallized albumin solutions studied are identified as HA 42, HA 64, and COM 1. The standard albumin preparations from different laboratories are identified by capital letters.

RESULTS

Effect of pH on stability

Early experiments with standard albumin, prepared by the method of Cohn and coworkers (2), showed that there is a maximum stability at approximately 6.8. More careful experiments have now been carried out on 2 samples of crystallized albumin.[8] The stabilities, defined as the time necessary for an increase of 20 units in the nephelometer reading (20 N.U.) and for an increase of 10 per cent in viscosity ($\Delta \log \eta = 0.0414$), are given in Table I and graphically represented in Figure 1, which gives the relation of stability

[5] The instrument is used with a green filter whose maximum transmission is at 5300 Å. Each nephelometric unit is approximately 1 per cent of the turbidity of a standard whose absolute turbidity is given as 0.0193. By removing the filter and focusing on the solution, this instrument can also be used to study visible flocs under standard conditions.

[6] This tyndallometer, designed by Professor Hans Mueller and Dr. George Rado, has been modified by them by omission of the polarization features and limitation to one of three angles, 0°, 45°, and 90° of the scattered light, to yield a simplified instrument for use in the study and acceptance of normal human serum albumin, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Massachusetts Institute of Technology.

[7] These preparations were crystallized by the method of Cohn and Hughes at the Harvard pilot plant.

[8] A concentrated solution of crystalline albumin, HA 42, was prepared and filtered through a Seitz Ser. 3 pad. From this stock solution, other sterile solutions were prepared with different concentrations of protein, salt, and hydrogen ion. The hydrogen ion concentration was varied by adding sodium bicarbonate and then reducing the carbon dioxide concentration approximately to that in equilibrium with air by equilibrating in a tonometer for an hour.

of HA 42 to pH for the solutions of 25 per cent albumin and 0.2 M sodium chloride.

An increase of 20 nephelometric units, although a somewhat arbitrary choice, is a measure of the reading of a solution having a barely visible haze. The choice of 5 per cent or 10 per cent for the increase of viscosity is entirely arbitrary. However, the shapes of the curves are usually so similar that doubling or halving the arbitrary values would make little change in the relative stabilities (Figure 2).

The curves in Figure 1 represent the experimental points satisfactorily except that the point at pH 6.60 appears to show too great nephelometric stability at 57° and too little at 50°. The curves of nephelometric reading versus time for both of these measurements were not of regular form, as shown in Figure 2, so that these points are probably not as certain as the others. Each curve in Figure 1 is symmetrical and of the same form. Each decreases 4 per cent of the maximum value at 0.2 pH unit either side of the maximum and 25 per cent at 0.5 unit from the maximum. The maximum is at 6.75 for viscosity at 55°, 6.92 for nephelometry at 57°, and 7.02 for nephelometry at 50°.

In these experiments, the pH adjustment and CO_2 equilibration followed filtration to avoid variations from filtration. Another set of solutions was therefore prepared from crystalline albumin, HA 64, and each was filtered after equilibration. They were studied nephelometrically at 50° and 57° and viscometrically at 55° and 57°. The composition, pH, and stability of the solutions used are given in Table II. Figure 2 shows curves of nephelometer readings and changes in the logarithm of the viscosity, $\Delta \log \eta$, at 57°. The maxima at 57° are at pH 6.75 for nephelometry and at 6.70 for viscometry. At the lower temperatures, the nephelometric maximum appears to be at a slightly higher pH and the viscometric maximum at a slightly lower one. The maxima are not quite as flat as those for HA 42, but the stability is again greater than 90 per cent of the maximum, within 0.2 pH units of the maximum.

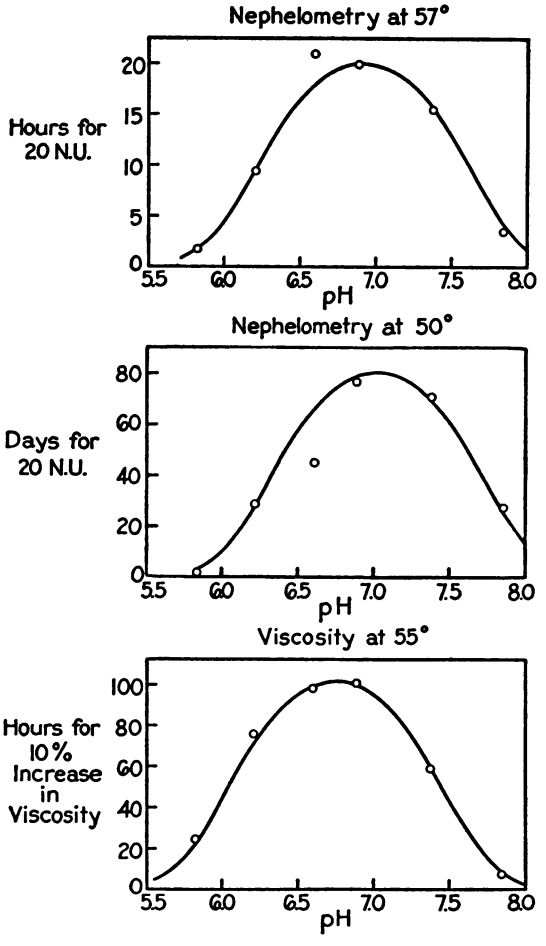

FIG. 1. STABILITY OF CRYSTALLIZED HUMAN ALBUMIN (HA 42)

TABLE I

Stability of crystallized human albumin (HA 42)

pH	Albumin	NaCl	For increment of 10 per cent in viscosity at 54.7°	For increment of 20 N.U. at 57°	For increment of 20 N.U. at 50°
	per cent	*moles per L.*	*hours*	*hours*	*days*
5.83	25.04	0.2045	24	1.7	1.6
6.21	24.72	0.2012	75	9.5	28.5
6.60	24.95	0.2156	98	21.0	45
6.69	24.91	0.1876	98	18.5	68
6.76	27.06	0.2040	96*	16.5*	16*
6.77	20.04	0.1897	110*	27.5*	2.5*
6.79	24.83	0.3075	144	30.0	142
6.88	25.02	0.2020	101	20.0	76.5
7.37	25.27	0.2015	59	15.5	70.5
7.84	25.05	0.2131	7.5	3.5	27.5

* Time for change equivalent to 20 N.U. or 10 per cent in viscosity for 25 per cent albumin.

Effect of salt concentration on stability

Early studies on standard commercial albumin showed that the nephelometric stability is

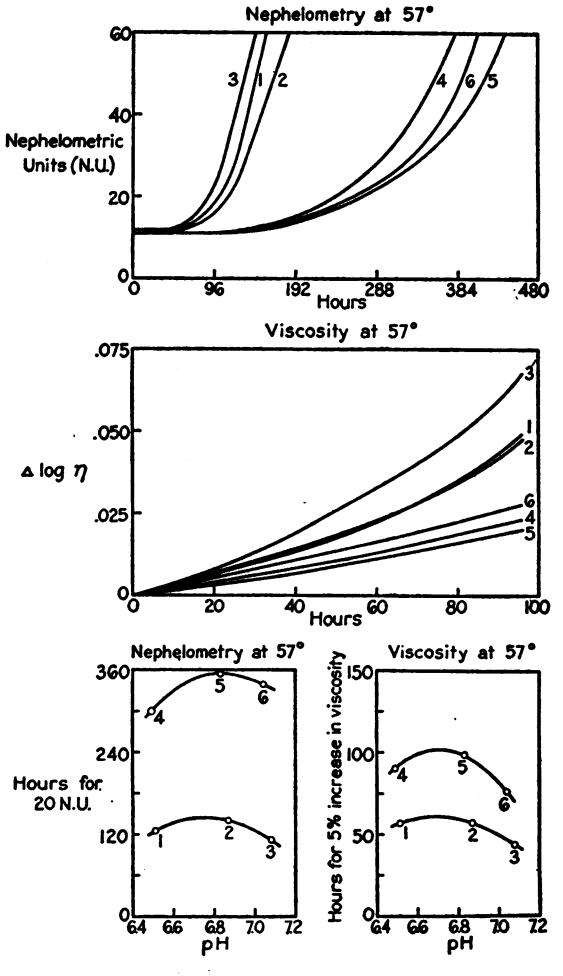

FIG. 2. STABILITY OF CRYSTALLIZED HUMAN ALBUMIN (HA 64)

proportional to the salt concentration from 0.15 M to 0.6 M NaCl, but these results alone could not determine if there was a real decrease in denaturation or if the increased salt merely held in solution the denatured protein. Experiments on HA 42 and HA 64, represented in Tables I and II and Figure 2, indicate approximately the same relationship, with perhaps a larger effect on the nephelometric stability than on the viscometric. The combination of viscometry and nephelometry shows that increase of salt concentration does give an important decrease in denaturation.

J. Murray Luck and his colleagues at Stanford University have undertaken the study of the effect of salts other than sodium chloride on the stability of albumin. Their results appear in another place (1).

Effect of albumin concentration on stability

The effect of albumin concentration on stability is illustrated in the last 2 experiments of Table I. To study this effect further, a 25 per cent solution of HA 42 with 0.2 M NaCl at pH 6.77 was diluted to $\frac{3}{4}$, $\frac{1}{2}$, and $\frac{1}{4}$, and the viscosity of each solution was determined. The solutions were heated together at 57° for 48 hours, and the viscosities redetermined. The most concentrated solution was diluted to the concentrations of the other solutions, and the viscosities of these solutions were also determined.

The results are summarized in Table III, expressed as $1/C \log \eta/\eta_0$. For the unheated solutions, this quantity decreases slightly with decreasing concentration. The change in $1/C \times \log \eta/\eta_0$ on heating at 25 per cent and diluting is almost independent of the concentration, but the change on diluting and heating at C per cent decreases very rapidly. It is, in fact, nearly proportional to the cube of the concentration. So the change in viscosity is much greater for the first process. It is quite possible that the initial denaturation changes quite differently with concentration and that we are measuring merely the chances of a molecule in a state of nascent denaturation colliding in the proper way with another molecule before it adjusts the change intermolecularly.

These stability considerations, presented in

TABLE II

Stability of crystallized human albumin (HA 64)

Preparation	pH	Albumin	NaCl	Time for increment of 5 per cent in viscosity		Time for increment of 20 nephelometric units	
				at 57.2°	at 54.7°	at 57°	at 50°
		per cent	*moles per L.*	*hours*	*hours*	*hours*	*days*
1	6.51	26.88	0.148	58	129	125	116
2	6.87	26.59	0.148	56	101	140	176
3	7.08	26.44	0.149	44	87.5	113	167
4	6.49	26.67	0.293	90	160	300	226
5	6.83	26.58	0.292	100	145	355	(136)*
6	7.04	26.45	0.289	76	112	340	262

* Measurement probably in error, see text.

Table III, would favor reducing the albumin concentration. The importance of the space and weight saved by using concentrated solutions is so great however, that there can be no reduction of the concentration for transport for the armed forces as long as sufficient stability can be attained by other means. There will sometimes be a great advantage in a highly hypertonic solution but sometimes an isotonic solution will be preferred. The stability will have but little influence on the choice of albumin concentration.

Effect of merthiolate concentration on stability

Various mercury-containing chemicals, such as merthiolate or phenyl mercuric borate, are used as preservatives in standard commercial preparations of serum albumin. The preparations of crystallized albumin which we have studied contained no preservative. To determine whether this difference might cause differences in stability, solutions of crystallized albumin, COM 1, were prepared, at a pH of 6.75, containing 25 per cent albumin, 0.15 M NaCl, and 0, 1:10,000, or 1:5,000 merthiolate. The viscometric stabilities at 55° C. were 93, 98, and 97 hours. Our results indicate that merthiolate does not appreciably affect stability, and that the choice of the concentration of merthiolate, and probably of other mercurials, need not be influenced by stability considerations.

Effect of storage at moderate temperatures on stability

Preparations of albumin which have been stored at 0 degrees show no change in stability, either at that temperature or when exposed to high temperature. Those which have been kept at room temperature show no change in viscosity or nephelometry even after very long times. When later exposed to high temperatures, however, they show a somewhat decreased stability. Experiments on a standard commercial preparation and on a crystallized preparation stored at 0°, 25°, and 37° confirm these results. We will give details only of the latter.

TABLE III

Stability of crystallized human albumin (HA 42 at 57°)

Albumin	$\frac{1}{C}\log\frac{\eta}{\eta_0}$ unheated	$\frac{1}{C}\Delta\log\frac{\eta}{\eta_0}$ heated at 25 per cent	$\frac{1}{C}\Delta\log\frac{\eta}{\eta_0}$ heated at C per cent
per cent			
25.0	0.0309	0.0048	0.0048
19.0	0.0265	0.0048	0.0022
14.0	0.0237	0.0047	0.0012
9.0	0.0214	0.0046	0.0001

TABLE IV

Stability of crystallized human albumin (HA 64)

Stability at 0°, 25°, 37° C.
Ratio of viscosity at 100, 200, or 400 days to initial viscosity

NaCl pH		0.15 6.8	0.15 7.0	0.30 6.8	0.30 7.0
days	° C.				
0		1.00	1.00	1.00	1.00
100	0	0.99	1.01	1.01	0.99
200	0	0.99	1.00	1.01	1.00
400	0	0.99	1.00	1.01	1.00
100	25	0.99	1.01	1.01	0.99
200	25	1.00	1.01	1.01	1.00
400	25	1.01	1.02	1.01	1.00
100	37	1.00	1.02	1.03	1.01
200	37	1.02	1.03	1.03	1.02
400	37	1.06	1.04	1.06	1.04

Solutions of HA 64, at pH 6.8 and 7.0 and NaCl concentrations 0.15 M and 0.3 M, were stored in several small bottles at 0°, 25°, and 37° C. At a specified time, a bottle of each was withdrawn, and its stability at 57° determined viscometrically and nephelometrically.[9] The results for 400 days are given in Tables IV and V.

Table IV shows the ratio of the viscosity after storage to that before storage. At 0° and 25°, there is no increase even in 400 days. At 37°, there appears to be a small increase which is, however, greater than the experimental error.

Table V shows the effect on the 57° stability of storage at lower temperatures. At 0°, there is no marked difference. (The nephelometric value at zero time for pH 6.8 and 0.3 M NaCl may be regarded as abnormally high.) At 25° and 37°, there is a marked decrease in the 57° stability. The decrease is larger at 37° than at 25°, larger for 0.15 M NaCl than for 0.3 M, and somewhat larger for pH 6.8 than for pH 7.0.

[9] In order to save material, the light scattering was measured by Dr. George Rado in the tyndallometer of Dr. Hans Mueller.

TABLE V

Stability of crystallized human albumin (HA 64)
Effect of storage at 0°, 25°, and 37° C.
on stability at 57° C.

NaCl pH		0.15 6.8	0.15 7.0	0.30 6.8	0.30 7.0	0.15 6.8	0.15 7.0	0.30 6.8	0.30 7.0
		For increase of 50 Mueller units				For 5 per cent increase in viscosity			
days 0	°C.	*days* 4	3	10	6	*half-days* 4	4	9	7
100	0	4	3	8	7	5	3	10	7
200	0	4	3	8	8	5	4	10	8
400	0					5	3	10	7
100	25	3	2	6	5	3	2	8	6
200	25	2	2	4	5	2	2	6	5
400	25	2	2			2	2	5	6
100	37	3	2	5	5	3	2	7	7
200	37	1	1	3	4	2	2	5	5
400	37	0.5	0.5			0.5	0.5	2	3

These results, as well as those graphically represented in Figure 2, show a marked similarity between the nephelometric reading and the viscosity, in that both begin to change rapidly at the same time. They also show an important difference in that the nephelometric reading remains constant before this rapid change while the viscosity changes from the very start. The relative stabilities could be determined as well from the initial slopes of the viscosity curves as from the later values. This indicates that there is a change in shape before there is any formation of molecules large enough to give much light scattering.

The experiments on storage at moderate temperatures and on filtration, considered in subsequent sections of this paper, indicate that the very first effect is one which affects neither light scattering nor viscosity but only renders the material more susceptible to changes which register by our methods of study. Our results suggest that this first reaction varies less rapidly with the temperature than the subsequent ones, so that it is not negligible at room temperature, and also that the effect of albumin concentration on stability is exerted on the subsequent stages by decreasing the probability of encounters of susceptible molecules. Many careful experiments would be required to establish these points.

Effect of storage at high temperatures on stability

The thermal stability of every commercial preparation has been tested on samples submitted to the Harvard Albumin Control Laboratory. These samples have been incubated routinely at 50° and, during the past 8 months, at 57° C. as well. Nephelometric readings, as previously described, have been made at frequent intervals, and the rate of increase in turbidity

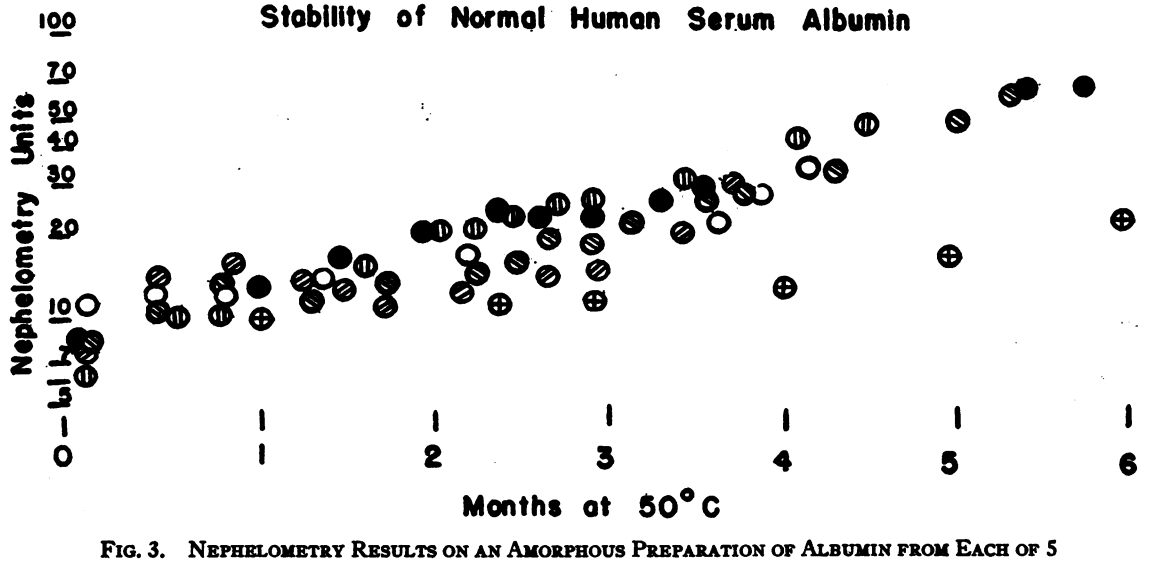

FIG. 3. NEPHELOMETRY RESULTS ON AN AMORPHOUS PREPARATION OF ALBUMIN FROM EACH OF 5 LABORATORIES COMPARED WITH A CRYSTALLIZED PREPARATION (⊕)

determined. Samples both at 50° C. and 57° C. have been kept in the oven until gelation occurred. From these tests, it has been possible to show not only that 25 per cent serum albumin is a remarkably stable protein preparation, but that during the first 2 years of production, there has been a great improvement in the thermal stability of the albumin from all laboratories.

The most marked increases in stability have occurred (*1*) following the adoption of the pH which has been proven optimal for stability, and (*2*) following the adoption of a salt content of 0.3 M (1.7 per cent NaCl) rather than 0.15 M (0.85 per cent NaCl) for the 25 per cent albumin solution. The stability of the standard, commerical, amorphous albumin preparations studied (containing less than 2 per cent globulin) was, however, lower than that obtained with the experimental crystalline lots processed at the Harvard pilot plant. The stability of crystallized lots should represent nearly the maximum attainable under given conditions of salt content, pH, and temperature.

The nephelometric readings from a study of one standard preparation from each commercial laboratory, which has been incubated at 50° C. over a period of months, are graphically represented in Figure 3. Results from the study of crystallized albumin are also charted. Not only the crystallized, but also the standard albumin is quite usable even after 6 months at 50° C. At 57° C., the number of hours necessary to cause an increase of 20 nephelometric units, based on the average of all preparations approved for delivery to the armed forces in the past 6 months, is over 80 hours and for the best preparations over 100 hours.

There is no evidence that the development of the slight haze represented by the highest nephelometric reading recorded in Figure 3 would be injurious clinically. Such preparations have been used without reactions of any kind being noted.

These very careful studies were therefore undertaken to render the preparations (*1*) as nearly uniform as possible, and (*2*) as stable as possible even when exposed to the most adverse conditions likely to be imposed by military medicine.

The relation of turbidity to gelation at high temperatures

Albumin would clearly not be usable in the field if incipient gelation occurred, as flow through the filter and needle would be inhibited. It therefore becomes important to determine the relation between length of time for a standard albumin solution to show haze at a high temperature, *i.e.*, to increase 20 units at 57° and to show incipient gelation. A study of over 150

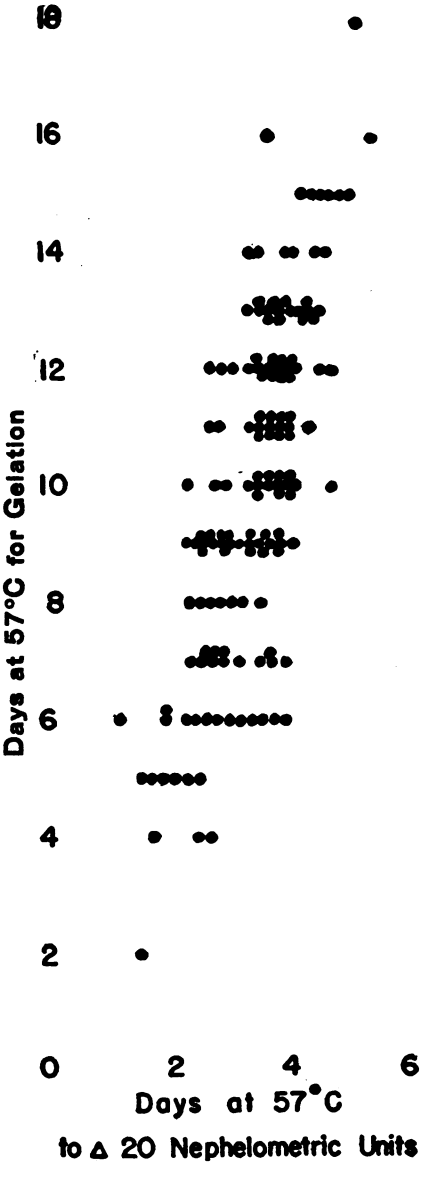

FIG. 4. THE RELATION OF NEPHELOMETRY TO GELATION IN 150 PREPARATIONS OF NORMAL HUMAN SERUM ALBUMIN

TABLE VI

Effect of filtration on heated albumin

HA 35, pH 6.70	Albumin	NaCl	Albumin	Nephelometric reading	Viscosity	Nephelometric stability at 57°	Viscometric stability at 54.7° C.
	per cent	*moles per liter*	*kgm. per mole NaCl*		*at 25 per cent albumin*	*hours*	*hours*
Unheated	25.3	0.161	1.58	6.5	5.66	10	41.5
Heated 11 hours at 57° unfiltered	25.3	0.161	1.58	36.	6.88		
Heated 13.5 hours at 57° unfiltered				65.	7.03		10.5
Heated 16 hours at 57° unfiltered	25.3	0.161	1.58	78.	7.43		
Heated 11 hours at 57° refiltered	25.6	0.160	1.61	13.5	6.37	3.5	24.5
Heated 16 hours at 57° refiltered	25.5	0.162	1.57	21.	6.88		

standard albumin preparations is reported graphically in Figure 4. There is considerable spread in the results, due in part to variations within the permissible pH range and in part to slight differences in practice in the 7 different laboratories which processed the albumin. Only in the case of unsatisfactory preparations which became hazy in less than 2 days did gelation occur soon after the first visible haze. On the average, the later preparations appear to take about three times as long for incipient gelation as for the formation of the first visible haze.

Filtration of partially denatured albumin

It may become important to know whether a part of the material which has become too turbid or too viscous for use can be recovered by filtration. It is also interesting and perhaps important to know what fraction of the protein is altered by denaturation. This can be determined to a reasonable degree of certainty by measuring the protein that can be recovered by filtration. With the small amounts of material available, it is not possible to determine the total amount held in the filter, and the concentration of albumin in the filtrate will depend upon the dryness of the filter pad before the filtration. The ratio of albumin concentration to that of sodium chloride should give the amount of protein held back without the corresponding amount of salt. We may assume that the solution entrapped in the meshes of the filter pad or of the unfilterable residue will contain the same ratio of albumin to salt as the filtrate.

A sample of standard commercial albumin, HA 35, was heated at 57° for 16 hours; the solute concentrations, turbidity, and viscosity were measured; it was filtered through a Seitz Ser. 3 filter; and the measurements were repeated. Another sample was heated 11 hours at 57°; the solute concentrations, turbidity, and viscosity were measured before and after 3 filtrations through Seitz Ser. 3 filters; and the stability of the filtered solution was measured nephelometrically and viscometrically. The results are given in Table VI.

The individual concentrations are changed only slightly and the ratio of albumin to salt indicates that no appreciable fraction of the protein is held back. Yet the nephelometer reading is reduced from 78 to 21 in the first case and from 36 to 13.5 in the second. The latter value is about as low as we achieved with small quantities of material. The filtration reduced the viscosity from 7.43 to 6.15 in the first case and from 6.88 to 6.37 in the second. However, the nephelometric and viscometric stabilities are both much smaller than for the unheated material. These results show that a very large part of the effect on turbidity and viscosity is due to a very small fraction of the material, and also that the stability of the filtered solution is much smaller than that of an unfiltered solution with the same turbidity or with the same viscosity. They give no indication whether this decreased stability is caused by a change in a small fraction of the molecules or by a smaller change in a large fraction of them.

CONCLUSIONS

Many of the results of these experiments have already been reflected in the production of

human serum albumin for the armed forces. The pH is specified at 6.8 ± 0.2 in order that the stability may be within 10 per cent of the maximum. The sodium chloride concentration now used, 0.3 M, lies between the concentration in plasma, 0.15 M, and 0.6 M, the concentration which would make the solution 0.15 M when diluted with water to have the same osmotic pressure as normal plasma. Stability considerations demand that the salt concentrations be as high as physiological requirements permit.

Although it is desirable that albumin solutions be kept in the cold, this does not appear important unless they are to be stored for a long time or are to be subjected at a later time to temperatures well above 100° F. (38° C.).

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