PRESERVATION OF NORMAL HUMAN PLASMA IN THE LIQUID STATE. III. STUDIES ON CHEMICAL AND PHYSICO-CHEMICAL CHANGES DURING THE SECOND YEAR OF STORAGE [1,2]

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The present regulations of the National Institute of Health with regard to the preservation of plasma in the liquid state call for an expiration date of one year. In the first paper of this series (1), 235 administrations of plasma preserved in the liquid state at room temperature for between 8 and 16 months were performed with an untoward reaction rate of 0.9 per cent and with the expected proportion of beneficial therapeutic results. The second paper of this series (2) has indicated that except for an inability to determine fibrinogen in plasma preserved in the liquid state for 6 months, there were no significant chemical changes in the total protein, albumin-globulin ratio, or non-protein nitrogen content of plasma. The clinical innocuousness of plasma preserved for periods longer than 6 months made of interest the investigation of what chemical and physico-chemical changes might be taking place during storage of such duration.

METHODS

Eighteen samples of 11 pools of plasma, prepared by the Blood Plasma Department of the Naval Medical School and preserved in the liquid state at room temperature in Washington, D. C., for between 15 and 24 months, were obtained for chemical and physico-chemical studies. The method of plasma preparation has been described previously (1). Six additional samples of lots, stored for less than a week, served as controls.

Total protein was measured by a modification of the method of Keys (3); albumin-globulin ratio by a modification of Howe's method (4). Fibrinogen determination was attempted unsuccessfully by the recalcification method (5), the salting-out method (6), and the thrombin method (7). Non-protein nitrogen was determined by Keys' method, 3 protein precipitants being employed on aliquots of each sample, 10 per cent trichloroacetic acid, 2.5 per cent trichloroacetic acid, and tungstic acid. The interpretation of the contents of the filtrates was made according to the principles laid down by Hiller and Van Slyke (8). By these principles, the difference between the nitrogen content of the 2.5 per cent trichloroacetic acid filtrate and that of the tungstic acid filtrate is an index of the polypeptide content of the plasma. A wide range of values has been reported for this index in normals (9, 10). The limits of this range are stated in Table II. The residual nitrogen was calculated according to Berglund (11) and the range of normal given in Table II is his. Urea was determined by the urease manometric method of Van Slyke (12). Free amino-acids were measured by determining the α-amino nitrogen by the ninhydrin-carbon dioxide gasometric method of Hamilton and Van Slyke (13 to 15). Osmometric determinations were made by Davis' (16) modification of the Hepp apparatus (17). Electrophoretic patterns were done by Dr. H. Kahler of the National Cancer Institute, using the Longsworth modification (18) of the Tiselius apparatus (19).

RESULTS

The chemical findings are presented in the accompanying tables. All findings are presented corrected for the initial dilution by sodium citrate and glucose.

It will be observed from Table I that no gross changes in the chemical determinations of total

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TABLE I

Nitrogen components of pooled human plasma, preserved in the liquid state at room temperature for from 15 to 24 months

Nitrogen components	Normal	Preserved liquid plasma (18 samples)	
	Range	Range	Average
	grams per 100 ml. of plasma		
Total protein	6.3 to 8.0	6.0 to 7.2	6.5
Albumin	3.7 to 5.3	3.8 to 5.5	4.5
Globulin	1.9 to 3.6	1.6 to 3.4	2.3

protein, albumin, or globulin appear to be taking place during the second year of storage. It was impossible to determine fibrinogen by any of the chemical methods for reasons previously explained (2). There is a vague suspicion that the albumin concentrations were running a little higher than expected, and the globulins, a little lower. This is to be borne in mind in connection with the physico-chemical studies discussed below.

Table II presents the findings with respect to the non-protein nitrogen and certain of its components. All of the urea concentrations fell within normal limits. However, the total non-protein nitrogen and the amino-acid nitrogen both increased slightly on prolonged storage. The former, of course, varied with the protein precipitant used as is discussed below, but in general was 10 to 20 mgm. per 100 ml. higher than normal. The α-amino nitrogens in preserved plasma ran from 5 to 10 mgm. per 100 ml. higher than the controls.

The "residual" nitrogen, that is, the non-protein nitrogen less the α-amino and urea nitrogen, also increases slightly on prolonged storage. The significance of this increase was investigated by means of a determination of the "polypeptide index," the difference in nitrogen content between a tungstic acid filtrate and a 2.5 per cent trichloroacetic acid filtrate. The rationale for this "index" is that the tungstic acid precipitates polypeptides whereas this concentration of trichloroacetic does not (8). The polypeptides, by this method, also appear to increase slightly on storage. They ran from 3 to 10 mgm. per 100 ml. greater than normal. Thus, the increase in non-protein nitrogen would appear to be made up of approximately equal parts of amino-acid and polypeptide nitrogen. Preliminary data utilizing hydrolysis of the protein-free filtrate are confirmatory of this conclusion.

Of these components of the non-protein nitrogen, thus far, only the α-amino nitrogen has been partially correlated with age. It will be observed from Table III that even here the correlation is not perfect.

TABLE II

Non-protein nitrogen components of pooled human plasma, preserved in the liquid state at room temperature for from 15 to 24 months

Non-protein nitrogen components		Normal	Preserved liquid plasma (18 samples)	
		Range	Range	Average
		mgm. per 100 ml. of plasma		
Urea-N		10 to 17	11.3 to 16.5	14.2
α-amino-N (Ninhydrin-Carboxyl)		4 to 6	8.8 to 13.9	10.5
NPN	Filtrates			
	10 per cent CCl_3COOH	18 to 30	32.3 to 49.7	40.1
	2.5 per cent CCl_3COOH	20 to 33	35.7 to 51.1	44.1
	Tungstic Acid	18 to 30	30.9 to 43.7	35.6
"Polypeptide Index" NPN(2.5 per cent CCl_3COOH)-NPN (Tungstic Acid)		3 to 8	3.2 to 14.8	8.4
"Residual" Nitrogen NPN(10 per cent CCl_3COOH)-(Urea-N + Amino-Acid N)		2 to 12	10.5 to 23.2	15.3

TABLE III

Correlation of α-amino nitrogen with age in pooled human plasma, preserved in the liquid state

Age of plasma	α-amino Nitrogen	
	Range	Average
	mgm. per 100 ml. of plasma	
5 days (Control) (3 samples)	4.9 to 5.7	5.2
15 months (12 samples)	8.8 to 11.0	9.9
18 months (1 sample)	10.2	10.2
19 months (1 sample)	11.8	11.8
20 months (3 samples)	11.5 to 14.0	12.5
24 months (1 sample)	12.1	12.1

DISCUSSION

The 6.3 to 8.0 grams of protein in 100 ml. of normal plasma represent from 1000 to 1280 mgm. of nitrogen of which from 700 to 1000 mgm. is α-amino nitrogen (13) upon proper hydrolysis. Thus, the increase of from 5 to 10 mgm. of α-amino nitrogen, such as was observed here upon storage of plasma in the liquid state for 2 years, represents hydrolysis to this degree of only 0.5 to 1.0 per cent of the original protein.

The highest non-protein nitrogen observed, namely 51.1 mgm. per 100 ml., indicates hydrolysis to non-protein size of only 2 per cent of the original protein. Thus, from this chemical point of view, 98 to 99.5 per cent of the original precipitable protein can be said to have remained intact. However, gross cleavages of protein may occur without significant increase in α-amino or non-protein nitrogen as long as the molecules remain large enough to be precipitable by the usual reagents. Chemical methods, such as the albumin-globulin ratio, have failed to demonstrate conclusively the occurrence of such cleavages in this study. So it has been necessary to turn to physico-chemical methods to clarify this point. Thus far, these data are too incomplete to report in detail. Upon completion, they will be reported in a subsequent paper (20). However, the preliminary electrophoretic analyses and osmometric determinations suggest that such cleavages are, to a certain extent, taking place. The electrophoretic mobilities of the globulin fraction are nearer and invade somewhat the albumin component and the plasma as a whole seems somewhat more effective osmotically than fresh plasma. These changes, however, would not detract from the use of such plasma for its colloid, for example, as an anti-shock agent or in hypoproteinemia, in situations where the labile constituents are unimportant. In fact, if subsequent observations confirm the greater osmotic effectiveness of preserved plasma and fewness of untoward reactions, storage in the liquid state may be most desirable for such indications.

It is of interest to speculate upon the nature of the proteolytic enzyme which may be responsible for the changes observed here. An enzyme resembling trypsin has been prepared from plasma treated with chloroform (21, 22). Inasmuch as in preserved plasma, both free amino-acids and polypeptides are increased, the enzyme operating must contain both proteinase and peptidase activity. Therefore, it is not identical with crystalline trypsin of Northrup and Kunitz which does not contain any peptidase activity (15). In its activity at the pH of plasma, 7.4, it does resemble the enzyme of chloroform-treated plasma which has optimal activity at this pH (22).

SUMMARY AND CONCLUSIONS

1. Chemical findings on 18 samples of plasma, preserved in the liquid state for 15 to 24 months at room temperature, are presented.

2. During storage of such duration, the non-protein nitrogen content, the α-amino nitrogen, the "residual" non-protein nitrogen, and the "polypeptide index" all increased slightly.

3. No gross chemical changes can be detected in the total protein, albumin, or globulin content but it appears from the increase in amino-acids and polypeptides that from 0.5 to 2.0 per cent of the original protein is hydrolysed to molecules of these sizes. Therefore, 98 to 99.5 per cent of the original protein remains precipitable.

4. Preliminary electrophoretic and osmometric data indicate that a limited amount of protein cleavage takes place. The osmotic effectiveness of the preserved plasma appears to be slightly increased over fresh plasma.

5. In view of the above findings, it may be concluded that when plasma is prepared by a "closed" system, with scrupulously aseptic technic and careful bacteriologic control, it may be preserved in the liquid state at room

temperature in a moderate climate for periods up to at least two years.

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