TABLE II. Reversibility of the Protective Action of Sodium Chloride.

Mixture†	Relative viscosity*			
	Before heating	After heating	After dialysis	After heating dialyzed sol.
Nucleate in H_2O	27.3	—	6.4	3.4
"	27.3	5	3.8‡	3.3
Nucleate in salt	12.8	—	10.2	3.9
"	12.8	12	10.2‡	3.6

* Viscosities measured at 30° and 6 cm H_2O external pressure.

† Final concentrations of nucleate and sodium chloride 0.5% and 1.0 M, respectively.

‡ Refers to the viscosity of the heated solutions after dialysis.

obtained with potassium chloride and magnesium chloride.

Fig. 1 describes the effect of heating at various temperatures on the viscosity of aqueous and of salt-containing mixtures with nucleate. Final concentrations of nucleate and of sodium chloride were 0.5 and 1 M, respectively. The period of heating was 20 minutes. At temperatures over 85°, mixtures of nucleate and salt sometimes yielded a small amount of white precipitate.

The structural character of the viscosity of the nucleate heated in the presence of salt is revealed in Fig. 2.

The reversibility of the salt protection was demonstrated by dialysis. Aqueous and salt-containing solutions of nucleate were heated, dialyzed, made up with water to equal concentrations of nucleate as measured spectrophotometrically in the ultraviolet, and heated again. Several such experiments revealed without exception that after loss of salt by dialysis the nucleate solution suffered a loss in viscosity on heating. A sample experiment is described in Table II. An interesting finding is that not only does salt protect against the effect of heat, but also apparently against the equally marked effect of dialysis on reducing the viscosity of the nucleate solution.

In another series of experiments, water and sodium chloride solutions (2M) were added to equal volumes of heated 1.0% nucleate solutions in water. Only a marked decrease in viscosity resulted after addition of the salt solution. The salt must be present while heating the nucleate solution in order to prevent the viscosity drop.

Summary. The viscosity of aqueous solutions of sodium thymonucleate is reduced both by heating and by addition of sodium chloride and other salts. If, however, the nucleate solution is heated in the presence of a sufficiently high concentration of salt, there is no further decrease in viscosity beyond that induced by the salt. The protective effect of the salt is reversible, for if the salt is removed by dialysis from the heated nucleate-salt mixture, the residual aqueous solution of nucleate again suffers a marked reduction in viscosity on heating.

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Human Plasma as a Source of Prothrombin in the Ac-Globulin Assay.* (18183)

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The approach to the problems of blood coagulation from the point of view of studying single factors received a major stimulus with the development of the 2-stage prothrombin assay by Warner, Brinkhous and Smith (1). This provided for the measure of pro-

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1. Warner, E. D., Brinkhous, K .M., and Smith, H. P., *Am. J. Physiol.*, 1936, v114, 667.

thrombin, thrombin and antithrombin with a minimum of interference from other factors. Two decades later, Ware and Seegers(2) based their Ac-globulin assay on the 2-stage method and provided the investigator and clinician with another tool in the study of coagulation problems. By using relatively pure prothrombin, thromboplastin and fibrinogen and, by dilution, rendering inactive the various anticoagulation factors, they created readily reproducible Ac-globulin "curves." Because the preparation of prothrombin in relatively pure form is a difficult process requiring considerable experience, the assay of Ac-globulin has been limited to relatively few laboratories.

Owren's(3) treatise on the discovery and assay of Factor V (Ac-globulin) describes the use of oxalated human plasma as a source of prothrombin in the one-stage assay of the prothrombin accelerator. It is the purpose of this paper to describe the adaptation of this source of prothrombin to the 2-stage assay for Ac-globulin. Fahey, Ware and Seegers(4) have shown that both prothrombin and Ac-globulin are relatively stable in citrated, stored plasma, the former being more stable than Ac-globulin. In oxalated plasma, however, Ac-globulin disappears rapidly, being unmeasurable by the 2-stage assay in most stored human plasma samples in 10 to 15 days when kept at 4°C. The prothrombin during this time diminishes by 10 to 30% of its original measurable potency. Inasmuch as the required concentration of prothrombin in the last step of the 2-stage Ac-globulin assay is 1.67 Iowa units, this remaining quantity provides more than an adequate amount of prothrombin. It allows also for the dilution feature of the 2-stage test which is important in the neutralization of certain anticlotting factors.

Collection and processing of human plasma. A healthy human blood donor whose prothrombin level is at least 80% of normal is selected and his blood is drawn by clean puncture and aseptic technic into a flask containing 25 ml of 2.5% potassium oxalate. Ordinary blood bank equipment is satisfactory for this procedure. The flask is gently agitated during collection to insure uniform mixing of blood and oxalate. When the combined blood and oxalate measure 250 cc the needle is withdrawn. Proportionately larger amounts may be collected if desired. The blood is stored overnight at 4°C and the following day the plasma is siphoned off aseptically. It is then stored in a glass container stoppered with sterile gauze or cotton. The loss of Ac-globulin is apparently an oxidation process because its loss is materially retarded if the storage flask is hermetically sealed. The plasma is assayed at 3-day intervals for Ac-globulin activity. When it becomes unmeasurable by the 2-stage assay or when the one-stage prothrombin assay shows a timing of over 100 seconds (normal 11 to 12 seconds) the plasma is divided into accurately measured 0.5 ml samples and stored at −35°C until used. It will remain uniformly stable and reactive for several months.

The assay for Ac-globulin is carried out according to the method of Ware and Seegers (2). 0.5 ml of the prepared plasma is diluted in a graduated cylinder 16 to 20 times with saline. (The exact dilution required can be determined when the curves are later plotted. In estimating the dilution required it is helpful to know the prothrombin content of the stored plasma as measured by the 2-stage method. Inasmuch as the prothrombin contained in the solution added to the fibrinogen in the second stage of the assay should be 1.67 Iowa units, the dilution factor can be calculated when the prothrombin unitage is known.) 0.1 ml of the unknown plasma collected by a previously described technic (5) is added and thoroughly mixed with the diluted prothrombin. 1.0 ml of this is next added to 1.0 ml of a thromboplastin solution adjusted to proper activity. (0.1 ml of Soluplastin made by Schiefflin and Company, di-

2. Ware, A. G. and Seegers, W. H., *J. Biol. Chem*, 1948. v172, 699.

3. Owren, P. A., *Acta Med. Scand.*, 1947, Supp. v194, 1.

4. Fahey, J. L., Ware, A. G., and Seegers, W. H., *Am. J. Physiol.*, 1948, v154, 122.

5. Fahey, J. L., Olwin, J. H., and Ware, A. G., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 491.

luted to 15.0 ml has been satisfactory. The dilution will vary with different lots of the material.) 1.0 ml of the mixture of diluted prothrombin, unknown plasma and thromboplastin is added to 2.0 ml of a substrate containing 1 part of a 1% $CaCl_2$ solution, 1 part of imidazole (1.72 g in 90 ml of 0.1 N HCl, this solution then made to 100 ml with water), 2 parts of a 15% acacia solution (calcium-free) and 5 parts of saline. After 10 minutes, 0.4 ml samples of this final mixture are added at accurately timed intervals of 2 minutes to 0.1 ml samples of a 1% fibrinogen solution and the clotting times plotted as described by Ware and Seegers(2). By comparing the curve with those obtained from normal control plasmas the Ac-globulin content of the unknown plasma may be calculated in per cent of normal.

The use of human stored plasma as a source of prothrombin is subject to the same criticism that applies to any process which does not provide for the absolute control of all factors other than the one being measured. Assays in our laboratory for Ac-globulin in the whole stored plasma show less than 0.1% of the normal amount. Antithromboplastin is diminished and in some instances destroyed by freezing(6). Approximately 75% of the normal antithrombin remains, and less than 25% of the physiologic concentration of fibrinogen. Heparin is uncontrolled. All of the above factors including, of course, prothrombin are diluted 20 to 24 times in the final substrate. All of these considerations must be kept in mind but need not interfere with the utility of the method in most instances. A number of comparisons have been made with purified prothrombin† and the crude prothrombin substrate herein described. The Ac-globulin curves are readily reproducible and compare favorably with those obtained with the use of the purified material.

Summary. Oxalated human plasma deficient in Ac-globulin as a result of storage can be used as a source of prothrombin in the 2-stage assay of Ac-globulin.

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† Dr. Walter H. Seegers kindly supplied us with purified prothrombin.

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A Complement-Fixation Test for Poliomyelitis Virus. (18184)

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For several years this laboratory was concerned with a practical serological test for determination of infection with poliomyelitis virus, apart from that of neutralization. Conventional hemagglutination and precipitin tests failed, as did those by means of complement fixation (CF) which occasionally offered incomplete reactions not totally specific and not in any satisfactory way sufficient as a dependable, practical procedure(1). Hitherto Loring *et al.*(2) stated that CF could be observed, but a degree of non-specificity obscured certain results. Roberts(3) brought forward a flocculation test with a complex antigen; its value has not yet been established by other workers. During this investigation the MEFl strain of poliomyelitis virus(4)

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1. Casals, J., and Olitsky, P. K., in *Diagnosis of Viral and Rickettsial Infections,* edited by F. L. Horsfall, Jr., Columbia University Press, New York, 1949, Chapter 6, pp. 57-82.

2. Loring, H. S., Raffel, S., and Anderson, J. C., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 385.

3. Roberts, E. C., *Public Health Rep.*, 1949, v64, 212.