somewhat lower than 10 mg %.

The preparation of accelerator globulin is obtained in much lower yield, as might be expected from the greater lability of this substance. The yield may be increased somewhat by omitting the precipitation with citrate, but the product so obtained is not so stable.

The stabilizing effect of citrate during the fractionation procedures is seen with prothrombin as well as Ac-G. The partially purified prothrombin product, Precipitate Pr-II, when dialyzed against saline and stored overnight at 4°C retains only about one-third the activity of a portion of the same preparation kept in 0.02 M citrate.

The stability of the purified prothrombin is also materially influenced by the age of the starting plasma. Preparations made from plasma which had been refrigerated or frozen for a period of several weeks have lower yields and lost one-third to one-half of their activity on overnight storage in the refrigerator. However, even when fresh plasma is used as a starting material, the stability of purified human prothrombin appears to be less than that reported for bovine prothrombin. On storage at —10°C for 3 months, solutions of human prothrombin show a steady decrease to about 20% of their original activity. Stability is not appreciably increased by drying the product from the frozen state followed by storage in the deep freeze.

Summary. A simple and rapid procedure is described for the separation of prothrombin of high specific activity and partially purified accelerator globulin from fresh citrated human plasma. Each product appears to be free from other known clotting factors.

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A One-Stage Method for the Determination of Accelerator Globulin.* (20738)

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An assay designed to measure human accelerator globulin (Ac-G) activity should meet certain criteria if dependable results are to be obtained. Of primary importance, the procedure should be simple and rapid because of the lability of human Ac-G. It should also be specific for Ac-G and unaffected by fluctuations in other clotting components in the test material. If possible, all components supplied to the test should be of human origin in order to avoid difficulty due to species differences.

Such a test system has been achieved by using as a substrate aged human plasma containing an excess of prothrombin and fibrinogen, but nearly devoid of Ac-G. Increased sensitivity to small amounts of Ac-G is obtained by using a low concentration of thromboplastin. To assure reproducible results, and to serve as a constant check on the activity of the thromboplastin, a standardized beef plasma is run with each group of tests.

Preparation of reagents. Substrate. One

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TABLE I. Progressive Deterioration of Ac-G in Aged Human Plasma during Preparation of Substrate.

Day	Clotting time (sec.)		
	Saline	100% stand.	20% stand.
3	75	34	54
4	89	34	60
5	94	42	65
	Dialyzed against 0.02 M citrate		
7	104	44	71

unit of outdated Red Cross plasma (Lilly) is reconstituted, using about two-thirds of the saline solution provided, and allowed to stand until the Ac-G deteriorates. Since deterioration takes place much more rapidly in oxalate than in citrate, the reconstituted plasma is divided into lots of 25 ml and dialyzed at room temperature against 0.02 M potassium oxalate in saline for a period of 2 to 3 days. The contents of the dialysis bags are then pooled, placed in the refrigerator at 4°C, and checked daily for prothrombin and Ac-G.‡ Prothrombin is measured by the one-stage technic of Ware and Stragnell(1). If, as occasionally happens, the prothrombin drops below 60% of normal, the material is discarded. To follow the drop in Ac-G activity, a small aliquot of the plasma (5-10 ml) is adjusted to pH 7.4 with 0.01 N hydrochloric acid, and then tested by using as a substrate in the system described below. The material is considered ready for use when a clotting time of 90-120 seconds is obtained when saline is substituted for the test plasma in the system. On the average, about 6 days are required for the deterioration of Ac-G (Table I). The substrate is then carefully adjusted to pH 7.4 with hydrochloric acid, and dialyzed in the cold against 0.02 M sodium citrate in physiological saline, since the presence of oxalate tends to give difficulty with the end point at slow clotting times. Portions of about 5 ml placed in tightly stoppered tubes and stored at −10°C generally prove satisfactory for a period of several months.

Thromboplastin-calcium solution. A saline-oxalate extract of acetone-dried human brain serves as the stock thromboplastin. Twenty ml of 0.9% sodium chloride containing 0.4 ml of 0.1 M sodium oxalate are warmed to 45°C. To the warm saline-oxalate solution are added 1.2 g acetone-dried human brain powder (prepared from the cerebrum after removing blood vessels). The mixture is inverted and incubated for 30 minutes at 45°C, stirring gently once during the incubation period. Avoid excessive agitation. Remove the larger particles by light centrifugation for a few minutes. The opalescent supernatant is relatively stable for a month or more when stored at −10°C. Avoid thawing and refreezing which often result in a drop in thromboplastin activity. For use in the test, one part of the stock thromboplastin is diluted with 4 parts of saline. The diluted thromboplastin is then mixed with an equal volume of 0.05 M calcium chloride and allowed to stand at 37°C for about 30 minutes before use. The best results are obtained when the clotting times with normal plasma fall between 40 and 50 seconds. It may be necessary to alter the saline dilution of the stock thromboplastin in order to maintain the clotting times in this optimal range.

Standards. Either untreated beef plasma or prothrombin-free beef plasma provides a stable source of Ac-G for use as a standard. Since the Ac-G level of beef plasmas may vary, standardization should be carried out by comparison with 5-10 normal human plasma samples in the test system described below. When kept at −10°C, the undiluted beef plasma is stable for months. It should be divided into small portions to avoid repeated freezing and thawing. Unless there is some reason to suspect deterioration, one standardization against normal human plasma is usually sufficient for a given lot of beef plasma.

Preparation of sample. Plasma. Blood is collected by a clean venipuncture. Nine volumes of blood are mixed immediately with one volume of 3.2% sodium citrate (0.109 M). If much difficulty is experienced with the venipuncture, or if there is the slightest evidence of incipient clotting, the sample should be discarded since the formation of even small amounts of serum Ac-G will tend to give erroneous results. The plasma is separated by centrifugation as soon as possible. If the

‡ As an alternate procedure, add 9 vol. of fresh blood to 1 vol. of 0.1 M oxalate. Separate the plasma and age at 4°C in an open container for 2 to 4 weeks.

assay cannot be done immediately, the plasma should be stored in the refrigerator to avoid deterioration of Ac-G. At 4°C, Ac-G is usually stable in citrated plasma for about 6 hours. For the assay, one volume of the plasma is diluted with nineteen volumes of 0.9% sodium chloride. *Plasma fractions.* If materials other than plasma are to be assayed, suitable dilutions in saline are prepared so that the clotting times in the assay will fall within the range covered by the standard curve. Interference by large amounts of barium, citrate, calcium, or other similar contaminating ions should be avoided by preliminary dialysis of the sample against 0.02 M sodium citrate. The dialyzed sample should then be diluted 1:20 with saline before introducing it into the assay system. *Assay procedure.* The test is carried out in a water bath or a constant temperature block kept at 37°C. The substrate and thromboplastin-calcium mixture are warmed to 37°C before beginning a series of assays, and may be kept at this temperature for several hours for convenience in running a large number of tests. More consistent results are obtained if the diluted plasma sample is also warmed to 37°C just before adding it to the reaction mixture. *Running the test.* 1) In a clean 10 x 75 mm glass tube, place 0.1 ml substrate. 2) Add 0.1 ml plasma (diluted 1:20 with saline). 3) Blow in 0.1 ml thromboplastin-calcium mixture, mix, and note the time for clot formation after addition of this reagent. 4) Determine the Ac-G content of the sample by reference to a standard curve.

Preparation of standard curve. 1) Determine the clotting times of 5-10 fresh, normal human plasma samples (diluted 1:20 with saline) in the assay system described above. The average of these values represents the clotting time of the 100% standard. 2) Make a 1:100 dilution in saline of the same normal plasma samples, and determine the clotting times. Average the values to obtain the clotting time of the 20% standard. 3) Determine the dilutions of the stock beef plasma which will give the same clotting times. This beef plasma may then be used as a reference standard. 4) Plot the results on logarithmic paper, with per cent standard as the horizontal axis and plotting time in seconds as the vertical axis (Fig. 1). Since the values between 10 and 100% fall on a straight line when plotted on logarithmic paper, the use of only two points to construct the curve is adequate. For Ac-G values which lie appreciably above the 100% level, a greater dilution of the test material should be employed in order to bring the clotting times within the range covered by the standard curve.

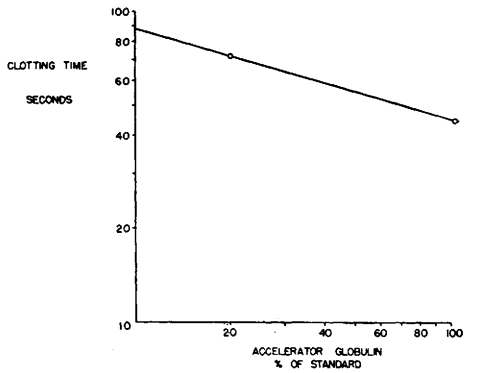

FIG. 1. Standard curve plotted on logarithmic paper.

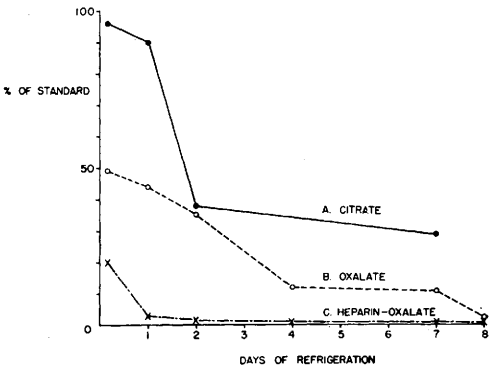

FIG. 2. Relative stability of human plasma Ac-globulin in various anticoagulants. 9 vol freshly drawn blood added to 1 vol of each of following: A. 3.2% sodium citrate. B. 0.1 M potassium oxalate. C. 0.1 M potassium oxalate containing 100 μg heparin/ml.

Stability in various anti-coagulants. The stability of human plasma Ac-G is markedly affected by the type of anticoagulatnt used. As shown in Fig. 2, stability is greatest in citrate, while appreciable deterioration takes place in a matter of hours in oxalate. The presence of the small amount of heparin recommended in the anticoagulant for the modified one-stage prothrombin test(1) hastens de-

terioration. After 24 hours in this mixture, the activity had already dropped too low for accurate quantitative assay, and in a few days the clotting time in the Ac-G assay system was approximately the same as the saline control. Rapid deterioration of Ac-G also takes place when versene is used as the anticoagulant. This finding is in agreement with the report of Olwin(2).

Discussion. The substrate provides an excess of both prothrombin and fibrinogen so that the test is not influenced by reasonable fluctuations in the level of these substances in the material to be tested. Dependable results are obtained on plasma from which the prothrombin has been removed by adsorption, as well as with prothrombin concentrates containing many times the prothrombin content of normal plasma.

In the actual performance of the test, failure to allow a reasonably constant interval of incubation in the constant temperature bath or block before picking up the tube to observe the end point may give rise to erratic results.

When beef plasma is used as the standard, the clotting time of the 20% standard should be determined within a few minutes of making the dilution, since an appreciable drop in activity may occur. This is particularly true of the more dilute standard, although an occasional lot of beef plasma has been encountered which seems to be labile even at a dilution employed for the 100% standard. The cause for this occasional instability has not been determined.

Summary. A simple one-stage procedure has been devised for the determination of accelerator globulin activity in human plasma. The components of the assay system are of human origin. The test is specific for accelerator globulin activity, and is unaffected by large fluctuations in prothrombin or fibrinogen content of the material to be tested.

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Action of Shellfish Poison on Peripheral Nerve and Skeletal Muscle. (20739)

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Shellfish poisoning, the paralytic type of poisoning which follows the ingestion of shellfish which have ingested poisonous plankton, has been adequately described by Sommer and Meyer(1). The causative substance of the paralysis has been characterized as a neurotoxin by Kellaway(2), who briefly described the effects of this toxic principle on peripheral nerve and muscle. He noted that this poison would render a skeletal muscle unresponsive to stimulation via its nerve just as does curare and that motor nerve endings were very susceptible to and readily blocked by this material.

The present investigation was initiated in an effort to characterize further the effects of shellfish poison on peripheral nerve and skeletal muscle.

Materials and methods. The preparations used were the sartorious muscle with its nerve and the gastrocnemius muscle-sciatic nerve of the frog, *Rana pipiens.* The apparatus and experimental protocol for recording action potentials are the same as those described previously in detail by Stover, Fingerman, and Forester(3) with the sole exception that in the present experiments the potentials were

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