# AUTOLOGOUS RADIOIODINATED FIBRINOGEN, SIMPLIFIED

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***A method for the rapid preparation of radioiodinated autologous fibrinogen has been improved. Plasma is separated from cells by a single centrifugation. Fibrinogen is isolated in two salting-out steps at a 1:4 dilution of plasma and 22.5% saturated ammonium sulfate. Iodination is accomplished with chloramine T at a chloramine T-to-fibrinogen molar ratio of 12:1. Free iodide is removed by a single dilution and salting-out step at 29% saturated ammonium sulfate. Preparation time is about 1 hr; clottable radioactivity is 90–95%. Denaturation due to storage is undetectable at 6 weeks. Advantages of this procedure are (A) a short preparation time and (B) a purified autologous radioiodinated fibrinogen preparation.***

The use of radioactive fibrinogen has been found to be a sensitive method for external detection of active intravascular thrombosis (*1,2*). Leg scanning after intravenous administration of radioactive fibrinogen has provided information concerning the incidence of venous thrombosis in postoperative patients (*1,2*). The effect of prophylactic regimens on fibrin deposition has been demonstrated with the same technique (*3,4*). Radioactive fibrinogen has been used in in vivo survival studies to measure fibrinogen consumption (*5*). However, the risk of transmission of hepatitis viruses by the administration of labeled homologous fibrinogen has limited its use for either investigative or clinical purposes in the United States (*6,7*).

A salting-out procedure making autologous fibrinogen preparations practical has been reported (*8*). This report describes modifications that simplify as well as increase the reliability of that procedure. Advantage is taken of the improvement in purification resulting from (A) diluting the plasma when fractionating and (B) lowering the concentration of the precipitating salt. The procedure is applicable to plasmas containing a wide range of fibrinogen concentrations. After labeling, unbound iodide is removed in a single step. The final product is highly clottable and has a long shelf-life.

## MATERIALS AND METHODS

**Reagents.** Potassium phosphate, trichloroacetic acid (TCAA), and sodium ethylenediaminetetracetic acid (EDTA) were reagent-grade chemicals. The ammonium sulfate used was the ultrapure grade of Schwarz-Mann, Orangeburg, N.Y. Chloramine T, No. 1022, was purchased in 250-gm amounts from the Eastman Kodak Co., Rochester, N.Y. Bovine thrombin is a product of Parke-Davis, Detroit, Mich. Iodine-125-sodium or $^{131}I$, biochemical grade in a minimum volume of 0.1 *N* sodium hydroxide, was obtained from New England Nuclear, Boston, Mass.

**Solutions.** All solutions were made up with sterile, pyrogen-free water. Phosphate buffer was 0.1 *M* pH 7.4 (buffer). EDTA solution was 0.013 *M* EDTA in buffer, adjusted to pH 7.4. Ammonium sulfate, 30.1% saturated (30% SAS), was made by adding 215 ml saturated ammonium sulfate (20–25°C) to 500 ml buffer.

The chloramine T was stored in its original container in a refrigerator. Without mixing the contents, a layer of approximately 2 cm was discarded before use. A weighed amount, 560 mg, was added to each of 20–30 tubes, 12 × 75 mm. The tubes were corked and stored in the refrigerator. When the stock bottle was used next, the top layer was again discarded. Tubes with preweighed amounts of chloramine T are convenient, stable, and result in consistent binding for several weeks. Immediately before use the contents of one tube were dissolved in a volume of

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water equal in milliliters to 600 divided by the amount of fibrinogen to be labeled in mg. When 10 $\mu$l of such a chloramine T solution are added to the fibrinogen solution, the molar ratio of chloramine T to fibrinogen is 12:1. The graduated cylinder used in making up the solution was rinsed with distilled water prior to use.

The radioactive iodide was diluted with 0.001 *M* sodium hydroxide to a concentration of 40–60 $\mu$Ci/$\mu$l. The bovine thrombin was dissolved in buffer to a concentration of 1 unit/$\mu$l, dispersed into approximately 0.1-ml amounts, and stored frozen. Assay tubes, 12 $\times$ 75 mm, containing 1 ml of diluted plasma (1:4 in EDTA buffer) were prepared and stored frozen. The assay tubes can be used for 6 weeks. The TCAA concentration was 30%.

**Equipment.** EDTA Vacutainers, 10 ml, obtained from Becton-Dickinson, Rutherford, N.J., were used for blood collection.

A high-speed centrifuge capable of generating forces of 10,000 *g* can be used for preparing the plasma. A single centrifugation of the blood in a round-bottom polycarbonate tube can then be performed. As an alternate procedure, a lower-speed angle-head centrifuge capable of generating 1,500–2,500 *g* can be used as described in the procedure using 12 ml heavy-wall, conical pyrex centrifuge tubes. For collecting the ammonium sulfate precipitate, an average force of only 300 *g* is required. Some table-top centrifuges are not controllable at the low speed needed.

Transfers of plasma were made with disposable Pasteur capillary pipets using a rubber dropping bulb. Disposable glass serological pipets of 5- and 10-ml capacity, cotton plugged and sterile, were always used with a rubber pipet filler which has flow-control valves for filling and emptying. Polystyrene culture tubes 16 $\times$ 125 mm with screw caps (Catalog number 3033, Falcon Plastics, Oxnard, Calif.) were used for isolation of fibrinogen and 17 $\times$ 100-mm polystyrene tubes with snap caps (Catalog number 2051, Falcon Plastics) were used for the iodination step. Micro-pipets of 1-, 10-, 50-, and 500-$\mu$l capacity (Oxford samplers, Oxford Laboratories, Foster City, Calif.) were used with disposable tips.

One- to two-liter quantities of 30% SAS and buffer were filtered through a 47-mm diam 0.45-micron pore size Millipore filter before being divided into bottles for sterilization. Fifty milliliters of 30% and 10 ml buffer in 100-ml and 10-ml serum bottles obtained from Wheaton Scientific, Millville, N.J. were steam-sterilized with the rubber stopper and aluminum seals clamped on. All other equipment used in the preparation—disposable pipets, Pasteur pipets, gauzes, glass stirring rods, rubber bulbs, centrifuge tubes, etc.—were gas-sterilized and required gas sterilization pouches. Sterilization of the radioactive fibrinogen solutions was achieved by filtration through a Swinnex Sterilization Filter Unit, Type HA pore size 0.45-micron, 13-mm diam filter obtained from Millipore Corp., Bedford, Mass.

**Determination of fibrinogen concentration.** After two ammonium sulfate precipitations, the fibrinogen concentration must be determined in order to adjust the concentration of the chloramine T solution used for labeling. A 50-$\mu$l sample of fibrinogen solution was used. The sample is added to 3.0 ml of buffer and the absorbance determined in a spectrophotometer at 280 nm using a 1-cm light-path cuvette with the reference cell containing buffer. Using the absorbance coefficient for fibrinogen $E^{0.1\%}_{1\,cm} = 1.55$ (*9*) and the dilution factor, the absorbance times 39.4 equals the concentration of fibrinogen in milligrams per milliliter.

If an ultraviolet spectrophotometer is not available, the protein concentration can be determined by any of a number of readily available methods that are fairly rapid and do not require large sample sizes. Duplicates should be performed when using a colorimetric method.

*Assays.* The clottability of the labeled fibrinogen was determined by two methods: spectrophotometric and radioisotopic.

For spectrophotometry, light absorbance was measured at a wavelength of 280 nm in the following order: (A) a solution containing 0.5 ml of the prepared fibrinogen and 2.5 ml of EDTA buffer; and (B) the same solution after fibrin has been wound out as a clot on a glass rod 2 hr after the addition of 1 unit of thrombin. Clottable protein is expressed as a percentage as (A) $-$ (B) $\div$ (A) $\times$ 100%. The presence of EDTA in the buffer prevented brittleness of the fibrin clot and made winding out easier.

With the radioisotopic method, the assay procedure used was that of Regoeczi (*10*). One microliter of labeled fibrinogen followed by 10 $\mu$l of thrombin is added to duplicate assay tubes. The clot is allowed to form for at least 1 hr. The total counts per minute (T) are determined, the clot is wound out on an applicator stick, and the "serum" counts per minute (S) determined. One milliliter of TCA is added slowly with vigorous mixing and the tube centrifuged at 2,000 *g* for 10 min. One milliliter of the supernatant containing TCAA-soluble or free iodide is carefully removed and counted (I). *Percent clottable radioactivity* is defined as the percentage of total radioactivity removed with the clot, i.e.,

$$\frac{T - S}{T} \times 100\%.$$

In contrast, *percent clottable protein* is defined as the percentage of the total protein removed with the clot, i.e.,

$$\frac{T - S}{T - I} \times 100\%.$$

*Percentage TCAA soluble radioactivity* or free iodide is defined as

$$\frac{I}{T} \times 100\%.$$

**Experiments.** Experiments were designed to evaluate variables for the purpose of simplifying the procedure, shortening the preparation time, and increasing labeling efficiency.

1. The separation of plasma from blood and its effect on (A) iodination efficiency and (B) filterability of the final radioiodinated fibrinogen were studied.
2. The purity of the fibrinogen isolated from plasma by ammonium sulfate precipitation depends on the concentration of the plasma proteins and the concentration of the ammonium sulfate (*11,12*). Experiments were conducted to determine the optimum concentration of two variables: pH and temperature.
3. Aspects of fibrinogen radioiodination with chloramine T (*13*)—the concentration of the protein, the molecular ratio of the chloramine T to fibrinogen (CT:F), the method of addition of the chloramine T, the stirring, the rate of the reaction, the presence of ammonium sulfate, and the container used—were studied. The conditions for obtaining maximum iodination of fibrinogen were determined. The conditions for efficient removal of unbound iodide by a third ammonium sulfate precipitation were studied.
4. The in vitro stability at room temperature and at −20°C of radioiodinated fibrinogen was studied.
5. In vivo half-life was studied. In vivo survival studies were conducted in four healthy volunteers—three males and one female aged 29–39—and in two patients with venous thrombosis.* Half-life of radioiodinated fibrinogen was determined by a semilogarithmic plot of clottable radioactivity, the regression curve being drawn by eye. Utilizing a double-isotope method, comparisons of in vivo survival of various fibrinogen preparations were made. Fibrinogen iodinated at CT:F 12:1 was compared with fibrinogen iodinated at CT:F 6:1 and with fibrinogen iodinated with iodine monochloride (*14*). Radioiodinated fibrinogen stored for 6 weeks at −20°C was compared with freshly prepared radioiodinated fibrinogen.

**Preparation of autologous radioiodinated fibrinogen.** Blood is collected in two 10-ml EDTA vacutainers, transferred to polycarbonate tubes, and centrifuged at 10,000 *g* for 5 min after reaching top speed. Alternatively, the blood is centrifuged in the vacutainer at 1,500 *g* for 10 min and the plasma transferred with a Pasteur pipet to a heavy-walled conical glass tube and recentrifuged at 3,000 *g* for 10 min.

Two 3-ml samples of plasma are removed using a 5-ml pipet, avoiding any disturbance of the precipitate, and are delivered to two 16 × 125-mm screw cap plastic tubes. Using a 10-ml pipet, 9 ml of 30% SAS are added to each tube. The tubes are capped, inverted four or five times, and centrifuged at 300 *g* for 3 min. The supernatant is decanted; the tube is inverted on a gauze and allowed to drain for 30 sec. Each precipitate is dissolved in 1.5 ml of buffer. The solution in one tube is transferred to the other with a Pasteur pipet and 9 ml of 30% SAS added. The tube is capped, inverted, and recentrifuged at 300 *g* for 3 min. The supernatant is decanted and the tube allowed to drain. The precipitate is dissolved in 0.5 ml of buffer using a 500-μl micropipet. Fifty microliters are removed for a protein determination. Five-hundred microliters are transferred to a 17 × 100-mm snap cap plastic tube for labeling. Appropriate amounts of $^{125}I$ are added and gently mixed with the fibrinogen solution. Ten microliters of chloramine T solution are added and the reaction mixture gently mixed for 10 sec.

After 6 min, 12 ml of 30% SAS are added and the tube is centrifuged at 300 *g* for 3 min. The supernatant is discarded into a proper receptacle and the tube allowed to drain. The precipitate is dissolved in 2 ml of buffer and duplicate 1-μl samples transferred to assay tubes. The radioactive fibrinogen solution is sterilized by drawing the solution into a syringe and then attaching a Swinnex filter unit in place of the needle. A sterile needle is attached to the filter unit and the tip inserted into another syringe through its needle adaptor. The fibrinogen solution is injected from one syringe through the filter into the second syringe. For the most rapid filtration very

* The in vivo studies were conducted with human volunteers. All subjects gave their informed consent and were treated with 250 mg potassium iodide by mouth daily for 1 week.

**TABLE 1. PREPARATION OF RADIOIODINATED FIBRINOGEN**

| Step | Description | Time required (min) | Clottable protein (%) |
|---|---|---|---|
| 1. Separation of plasma | Centrifugation of blood, 10,000 *g* for 5 min | 15 | |
| 2. Isolation of fibrinogen | 1:4 dilution of plasma, precipitation at 22.5% saturated $(NH_4)_2SO_4$, centrifugation 300 *g* for 3 min | 10 | 40–80 |
| | Repeat precipitation and centrifugation | 10 | |
| 3. Iodination of fibrinogen | Measurement of fibrinogen concentration, addition of radioactive iodide and chloramine T. Iodination 60–90% efficiency | 15 | 85–95 |
| 4. Separation of free iodide | Precipitation at 29% saturated $(NH_4)_2SO_4$, centrifugation 300 *g* for 3 min | 10 | 92–97 |
| 5. Sterilization | Filtration of radioiodinated fibrinogen through 0.45-micron pore size filter | 5 | |
| 6. Assay | Determination of clottable radioactivity, clottable protein, and free iodide (1–4%) | | |

little pressure should be applied to the syringe. If the pressure is increased unnecessarily, not allowing sufficient time for the viscous protein solution to flow through the pores of the filter, the filtration will slow and stop regardless of how much pressure is applied. The procedure is summarized in Table 1. Preparation time is about 1 hr.

Some patients have unusually high plasma fibrinogen concentrations, resulting in bulky ammonium sulfate precipitates and longer solution time. With experience, the operator will be able to judge if the amount of the first precipitate is excessive, i.e., if it will result in a concentration of fibrinogen for labeling of 18 mg/ml or greater. In such cases, using only one tube, without pooling and adding 3 ml of buffer and proceeding as usual, will save time and yield sufficient fibrinogen for labeling.

On occasion, when a precipitate that dissolves slowly is obtained, a stirring rod may be used to hasten the process.

## RESULTS

**Preparation of the plasma.** Plasma from blood centrifuged once at 860 *g* for 5 min was compared with a portion of the same plasma recentrifuged at 2,200 *g* for 10 min. Fibrinogen was isolated under identical conditions from both samples by two ammonium sulfate precipitations. The fibrinogen concentrations were adjusted to 6 mg/ml and labeled with a molar ratio of chloramine T to fibrinogen of 12:1. The rate of the reaction was followed by assaying for the percent clottable radioactivity for 18 min. The results are shown in Fig. 1. The iodinated fibrinogen was isolated from its reaction mixture by a third ammonium sulfate precipitation and dissolved in buffer. The fibrinogen isolated from the plasma centrifuged twice readily passed through a 13-mm diam 0.22-micron pore size Millipore filter whereas the fibrinogen isolated from plasma centrifuged only once would not pass through a 13-mm diam 0.45-micron pore size Millipore filter.

The effect of centrifugation was studied further. Plasma from blood centrifuged once at 1,200 *g* for 10 min was compared with a portion of the same plasma recentrifuged at 2,200 *g* for 10 min. Fibrinogen was isolated under identical conditions from both samples by two ammonium sulfate precipitations. The fibrinogen concentrations were adjusted to 6 mg/ml and labeled with a molar ratio of chloramine T to fibrinogen of 6:1. The fibrinogen isolated from the plasma centrifuged twice had 38% clottable radioactivity in the reaction mixture in con-

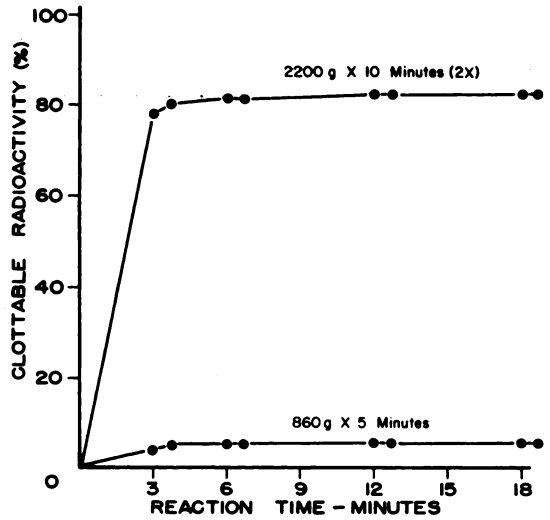


**FIG. 1.** Effect of separation of plasma on radioiodination of fibrinogen is shown. Fibrinogen isolated from single centrifugation of plasma at 860 *g* for 5 min was compared with fibrinogen from double centrifugation of same plasma at 2,200 *g* for 10 min each. Fibrinogen preparations were adjusted to same concentration and iodinated at chloramine T-to-fibrinogen (CT:F) ratio of 12:1.

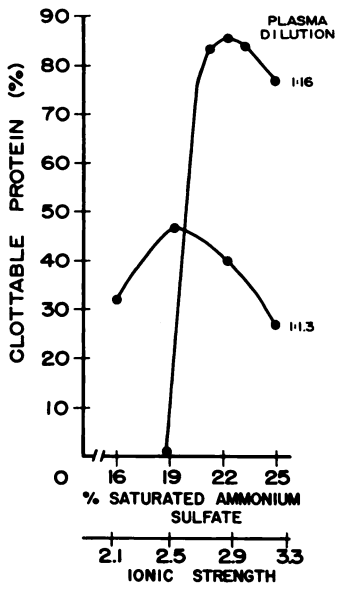


**FIG. 2.** Clottability of precipitated protein as function of ionic strength is indicated by course of each curve. Clottability of precipitated protein as function of plasma dilution is indicated by relation of one curve to other. One milliliter of single donor's plasma was diluted with phosphate 0.1 *M* and saturated ammonium sulfate to produce indicated ionic strength and dilutions of plasma. Precipitates were drained for 15 min and then dissolved in 0.5 ml of water. Water as solvent diluted high salt concentration of precipitate and prevented interference with salt-sensitive clottability assay. This solution was added to 2.5 ml of assay buffer in cuvette for spectrophotometric clottability assay.

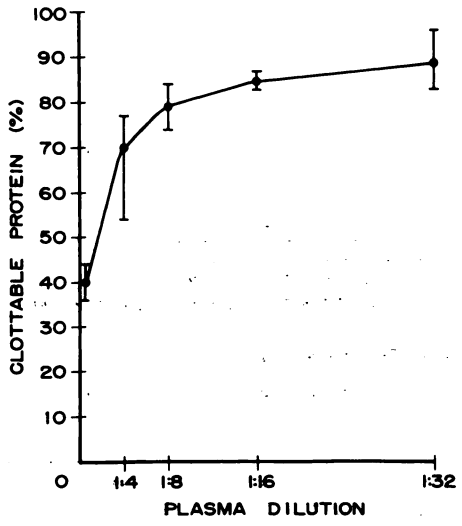


**FIG. 3.** Clottability of precipitated protein is plotted as function of plasma dilution at ionic strength of 2.9. Each point is average of three experiments conducted as described for Fig. 2.

trast to 28% for the fibrinogen isolated from the plasma centrifuged once.

**Purification of fibrinogen.** The effects of ionic strength, pH, and temperature on the purification by salting out with ammonium sulfate were examined in an attempt to increase the efficiency of the separation of fibrinogen from plasma.

*Ionic strength.* The effects of a range of ionic strengths on the purification of fibrinogen from plasma are shown in Fig. 2. The effect of dilution of plasma on fibrinogen purification by salting out is also shown in Fig. 2. Purification at an optimal ionic strength is greatly enhanced by the dilution of plasma. The extent of this effect is shown in Fig. 3. Purification increases with greater dilution although with diminishing increments.

As a compromise between enhancement of purification and convenience in handling, a plasma dilution of 1:4 and a final ammonium sulfate concentration of 22.5% saturated were selected for the routine purification procedure. Table 2 indicates the increase in fibrinogen purity due to differences in ammonium sulfate concentrations and plasma dilution.

*pH.* The effect of pH on purification of fibrinogen by salting out was insignificant between 6.2 and 8.0 (Fig. 4). No attempt was made in routine preparations to modify the final pH of the mixture of ammonium sulfate and diluted plasma.

*Temperature.* The effect of low temperature on purification of fibrinogen by salting out was an adverse one (Fig. 5).

**Radioiodination of fibrinogen.** The Hunter-Green-

**TABLE 2. EFFECT OF AMMONIUM SULFATE CONCENTRATION AND PLASMA DILUTION ON PURITY OF FIBRINOGEN**

| Concentration of $(NH_4)_2SO_4$ in precipitating mixture | Percentage of clottable protein* after two $(NH_4)_2SO_4$ precipitations | |
|---|---|---|
| (% saturation) | Undiluted plasma | Diluted 1:4 |
| 22.5 | 86 | 92 |
| 25.0 | 67 | 86 |

* Clottable protein was determined by radioisotopic assay.

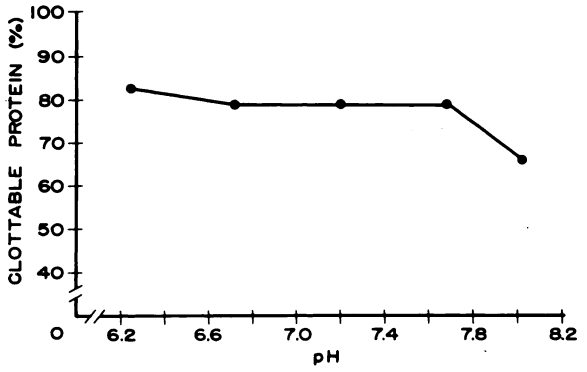


**FIG. 4.** Effect of pH of first ammonium sulfate precipitating mixture on purity of precipitated fibrinogen is indicated. Clottability was determined spectrophotometrically.

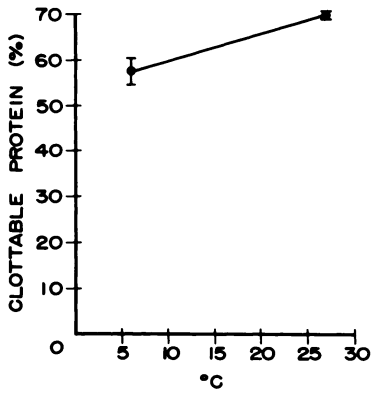


**FIG. 5.** Effect of temperature of first ammonium sulfate precipitating mixture on purity of precipitated fibrinogen is indicated. Each point is average of two experiments on plasma from single donor. Clottability was determined spectrophotometrically.

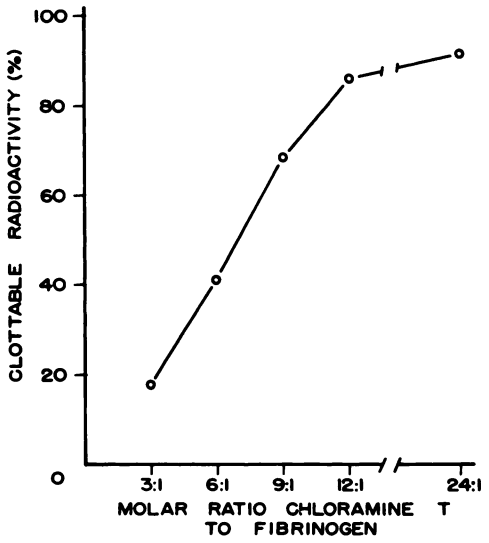


**FIG. 6.** Effect of chloramine T-to-fibrinogen ratio on extent of iodination of fibrinogen is indicated. Aliquots of single fibrinogen solution were used for this experiment. Efficiency of iodination is expressed as clottable radioactivity determined by isotopic assay.

wood method of radioiodination (*13*) was modified by using a chloramine T-to-fibrinogen (CT:F) molar ratio lower than generally used.

The efficiency of iodination of fibrinogen as determined by percent clottable radioactivity of the iodinating mixture was found to be directly proportional to the molar ratio of chloramine T to fibrinogen in the range 3:1 to 12:1. At ratios greater than 12:1 the increase in iodination efficiency was negligible (Fig. 6). At the 12:1 ratio, iodination efficiency was 81 ± 10% (mean ± 1 s.d., n = 12). Iodination efficiency is also affected by the concentration of fibrinogen being iodinated, increasing slightly with increasing concentration of fibrinogen (Fig. 7).

An optimal reaction time is 6 min with little increase in binding beyond this time (see Fig. 1).

The method of adding the chloramine T to the fibrinogen solution containing the radioactive iodide did not affect iodinating efficiency. Injecting the chloramine T directly into the fibrinogen solution and mixing for 5 sec, delivering the chloramine T to the container wall just above the fibrinogen solution and mixing for 10 sec, delivering it to the container wall just above the fibrinogen solution and mixing for 60 sec, and delivering it and waiting 10 sec before mixing for 10 sec all resulted in the same binding within 1%. Continuous mechanical stirring with a micromagnetic stirrer during the entire reaction resulted in less binding.

The removal of unbound iodide and residual chloramine T was accomplished by a third ammonium sulfate precipitation. The larger the volume of the supernatant, the greater the amount of free iodide was removed from the precipitated iodinated fibrinogen. Excessive loss of fibrinogen due to its solubility in the supernatant was reduced by the higher final concentration of ammonium sulfate used, 29% saturated. Free iodide in the final fibrinogen solution was 1–4% of the total after the third precipitation. Nonclottable protein was also reduced, thereby increasing the purity of the final fibrinogen preparation.

**Assay of final radioiodinated fibrinogen.** The degree of purification of fibrinogen as determined by clottable protein with isotopic assay was 94 ± 1.4% (mean ± s.d., n = 12). The final fibrinogen solution contained 92 ± 1.6% clottable radioactivity and 1.8 ± 1.0% free iodide, TCAA-soluble radioactivity.

In vitro stability of radioiodinated fibrinogen as measured by percent clottable radioactivity after storage is shown in Table 3. At room temperature and

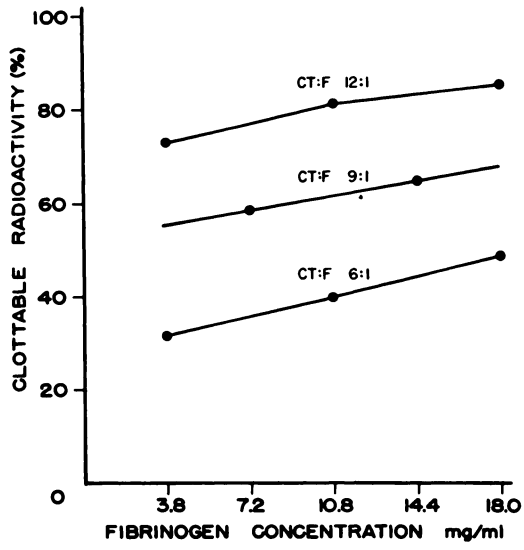


**FIG. 7.** Effect of fibrinogen concentration on efficiency of iodination is shown. Single fibrinogen preparation was diluted to concentrations indicated.

**TABLE 3. STABILITY OF RADIOIODINATED FIBRINOGEN**

| | Time | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 hr | 20 hr Room temperature | | 3 days −20°C | | 10 days −20°C | |
| Chloramine T:fibrinogen | | Percentage of clottable radioactivity | Decrease | Percentage of clottable radioactivity | Decrease | Percentage of clottable radioactivity | Decrease |
| 3:1 | 89.8 | 87.4 | 2.4 | 88.2 | 1.6 | 88.4 | 1.4 |
| 6:1 | 94.1 | 92.8 | 1.3 | 93.3 | 0.8 | 93.6 | 0.5 |
| 9:1 | 95.7 | 94.2 | 1.5 | 95.0 | 0.7 | 95.3 | 0.4 |
| 12:1 | 96.7 | 94.8 | 1.9 | 95.3 | 1.4 | 96.0 | 0.7 |
| 24:1 | 95.0 | 92.4 | 2.9 | 94.1 | 0.9 | 95.4 | +0.4 |

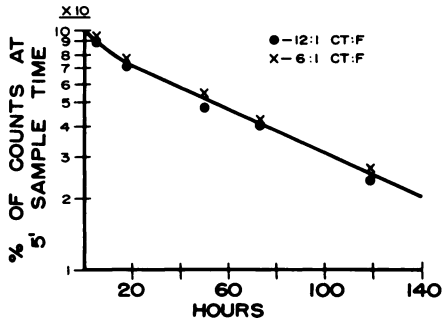


**FIG. 8.** Effect of chloramine T iodination on in vivo survival in normal subject. Purified fibrinogen solution was divided into two parts, one iodinated with $^{125}I$ at CT:F 12:1 (●) and other with $^{131}I$ at CT:F 6:1 (x). These preparations were combined and administered together to donor. Clottable radioactivity for each isotope was determined on plasma samples as indicated. Radioactivity of clots was measured by deep well scintillation counting with correction made for decay and for spillover of $^{131}I$ counts into $^{125}I$ channel.

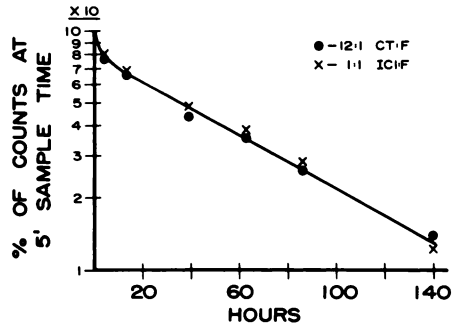


**FIG. 9.** Comparison of chloramine T iodinated and monochloride iodinated fibrinogen in vivo survival in subject with leg vein thrombosis. Fibrinogen solution was divided, one part iodinated with $^{125}I$ and CT:F 12:1 (●) and other with $^{131}I$ and iodine monochloride (ICl), ICl:F 1:1. Final iodide-to-fibrinogen molar ratio was less than 1:2 (x). In vivo comparison was made as described for Fig. 8.

at −20°C the radioiodinated preparation appears stable for a convenient period of time.

Denaturation due to iodination with chloramine T, assessed by comparing the in vivo disappearance rates of fibrinogen labeled at CT:F ratios of 12:1 and 6:1, was not detectable (Fig. 8). Denaturation assessed by comparing the in vivo disappearance rate of fibrinogen labeled by the chloramine T method with fibrinogen labeled with the iodine monochloride method was not detectable (Fig. 9). The abnormally short half-life, 2.0 days, for both the chloramine T and the monochloride radioiodinated fibrinogen preparations shown in Fig. 9 is attributable to an active thrombotic process known to be present in the subject at the time of the study.

It was also possible to estimate denaturation of radioiodinated fibrinogen due to storage in a patient studied for recurrent thrombotic disease (Fig. 10). There was no detectable difference in in vivo disappearance rates between the fresh and the stored (−20°C, 6 weeks) preparations.

The in vivo fibrinogen half-life in four healthy volunteers was 3.9 ± 1.2 days.

## DISCUSSION

Separation of plasma by a single centrifugation of blood instead of two as previously described (*8*) is satisfactory provided high-speed centrifugation is available. If cellular debris remains in the plasma, it remains with the precipitate during salting out and inhibits iodination.

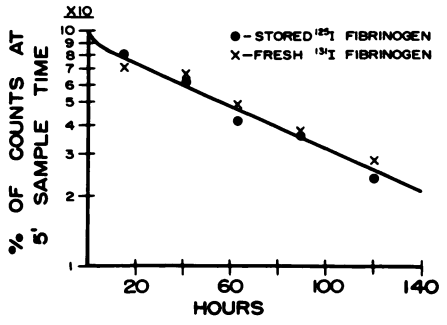


**FIG. 10.** Comparison of fresh and stored radioiodinated fibrinogen in vivo survival in subject with leg vein thrombosis. Fibrinogen solution iodinated with $^{125}I$ and CT:F 12:1 (●) was stored 6 weeks at −20°C, thawed, and mixed with fresh fibrinogen preparation from same donor iodinated with $^{121}I$ and CT:F 6:1 (x). In vivo comparison was made as described for Fig. 8.

An additional reason for an adequate centrifugation of the plasma (a single one at high speed or two at lower speed) is to prevent the clogging of the sterilization filter used in the final step.

Lipids (referring to the turbidity present in some plasmas remaining after centrifugation) do not affect the fractionation or binding.

Isolation of fibrinogen was modified to utilize both optimal ionic strength and dilution of plasma as suggested by Dixon and Webb in a discussion of fractionation of proteins (*12*). Dilution can accentuate differences in solubilities of proteins at a given ionic strength and permit a more effective separation of the protein of interest. Utilizing this principle, a purification of fibrinogen comparable in purity to that of fibrinogen isolated by longer procedures (*14–16*), can be achieved in two steps employing only two solutions.

When fibrinogen is isolated in plastic tubes, the precipitate sticks to the wall and forms a pellet at relatively small *g* forces. The precipitate collected by a gentle centrifugation dissolves readily. A force of 300 *g* at the center of the liquid column was found to be satisfactory while 600 *g* prolonged the time necessary to dissolve the precipitate.

The second precipitate dissolved in 0.5 ml of buffer results in sufficient solution volume for the determination of protein concentration and labeling. If duplicate 50-$\mu$l samples are needed for colorimetric determinations, the precipitate should be dissolved in 0.6 ml. It is unnecessary to adjust the concentration of fibrinogen since it is more convenient to adjust the concentration of the chloramine T to obtain a molar ratio of 12:1. It has been shown that the higher the concentration of fibrinogen in the reaction mixture (Fig. 7), the greater is the iodination efficiency and yield of clottable radioactivity.

The iodination efficiency with this method of preparation was 80% as measured by clottable radioactivity. This is somewhat higher than that reported for other radioiodinated fibrinogen preparations using iodine monochloride (*17*). Denaturation due to iodination using an iodide-to-fibrinogen molar ratio greater than 1:2 with the monochloride method has been reported (*14*). In the present method the iodide-to-fibrinogen molar ratio is 1:300. Denaturation due to high chloramine T-to-protein molar ratios, e.g., 75:1 in the case of fibrinogen, has been reported (*18,19*). In the present method a chloramine T-to-fibrinogen molar ratio of 12:1 was used, thus minimizing damage to protein due to oxidation. High ratios of chloramine T to protein are necessary for efficient iodination of microgram amounts of protein as originally described (*13*). In the present method milligram amounts of protein, e.g., fibrinogen, are involved, which permit high iodination efficiency at a lower CT:F ratio, 12:1. Fibrinogen iodinated by chloramine T and iodine monochloride methods were compared in a patient with a thrombotic process. There was no difference in the half-life of the labeled fibrinogens indicating no quantitative difference between the two iodinating methods. The short half-life of 2.0 days for both was no doubt due to the active thrombosis in this patient (*20*).

The half-life of fibrinogen prepared by this rapid method in four healthy volunteers, 3.9 $\pm$ 1.2 days, is in agreement with a review of reported values, 3.9 $\pm$ 0.7 days (*21*). The affinity of fibrinogen prepared by this method for a thrombus is being evaluated. Data to date suggest that fibrinogen thus prepared is comparable to other methods of preparation reported in the literature. Clinical correlations between radioiodinated fibrinogen, impedance plethysmography, and venography in the detection of venous thrombosis will be reported shortly as will the use of radioiodinated fibrinogen for the detection of intracardiac thrombosis.

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