rather to the elevation in blood pH. Hypocalcemic tetany produced by venoclysis of solutions of phosphate or sodium citrate is indistinguishable from alkalotic tetany but does not eventuate in pulmonary edema. In our experience, the blood pH has to exceed 7.80 and has to be maintained for five minutes or longer before lung edema appears. Artificial respiration with positive pressure does not seem to inhibit this form of lung edema, although it has been reported to inhibit other types of experimental lung edema(6).

The relatively low protein content of the edema fluid indicates that a significant increase in alveolar capillary permeability was not present at the time the fluid was formed. However, the anoxia which follows the obstruction of alveolar spaces and the duct system by edema fluid quickly produces an increase in capillary permeability allowing an appreciable leakage of plasma proteins and often culminating in the rupture of capillary walls with consequent hemorrhage. The high protein content of the edema fluid in animals pretreated with dibenamine may be attributable to a more fulminating onset of lung edema with earlier anoxia. These results indicate that an increase in alveolar capillary permeability is not involved in the initiation of lung edema in alkalosis. The acute lung edema occurring in alkalosis resembles that produced by ammonium salts. Both are preceded by and associated with abnormal neuromuscular phenomena, tetany in the former and tonic and clonic convulsions in the latter (3) and the protein content of the edema fluid is relatively low(4). However, dibenamine, which blocks the excitor effects of sympathetic nerve impulses at the effector organs(7), is exceedingly effective in preventing ammonium lung edema(4), but is quite ineffectual in alkalosis lung edema. An understanding of the pathogenesis of this form of acute pulmonary edema awaits further investigation.

Summary. 1. Venoclysis of solutions of sodium carbonate and sodium bicarbonate in quantities sufficient to produce a severe alkalosis and tetany produced acute pulmonary edema in most cats. 2. This lung edema was not inhibited by artificial respiration nor by pretreatment with dibenamine. 3. The edema fluid contained very small concentrations of proteins, ranging from 0.8% to 1.4%. In two animals that had been pretreated with dibenamine the total protein approached 5%. 4. Severe hypocalcemic tetany produced by venoclysis of a phosphate buffer and citrate solution did not result in lung edema.

1. Koenig, H., *Anat. Rec.,* 1949, v103, 60.

2. Koenig, H., Stahlecker, H., and Koenig, R. S., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 330.

3. Koenig, H., and Koenig, R., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 375.

4. ———, *Am. J. Physiol.,* 1949, v158, 1.

5. Koch, F. C., and Hanke, M. E., *Practical Methods in Biochemistry,* Williams and Wilkins Co., Baltimore, 5th Edition, pp176-177, 1948.

6. Auer, G., and Gates, F. L., *J. Exp. Med.,* 1917, v26, 201.

7. Nickerson, M., and Goodman, L. S., *J. Pharm. and Exp. Therap.,* 1947, v89, 167.

Received May 19, 1952. P.S.E.B.M., 1952, v80.

Nature of an Accelerator of Prothrombin Activation Arising During Storage of Purified Prothrombin.* (19627)

ROBERT I. MCCLAUGHRY AND WALTER H. SEEGERS.

From the Department of Physiology and Pharmacology, Wayne University College of Medicine, Detroit, Mich.

A most fruitful approach to the study of the mechanisms of blood coagulation has been the purification of some of its important components. For example, our laboratory has devoted much effort to the purification of prothrombin and to a study of its conversion to thrombin(1-4). Prothrombin has been separated from bovine plasma in sufficient quantity

* Aided by a grant from the U. S. Public Health Service, National Institutes of Health.

and purity for scientific study. Recent work has shown that the same methods are also applicable to preparation of human prothrombin (5). Autocatalytic activation of prothrombin can be demonstrated by the use of such prothrombin preparations(2). For example, if prothrombin is dissolved in 25% sodium citrate solution and allowed to stand at room temperature, measurable amounts of thrombin are formed after about 5 hours. Thrombin forms thereafter at a rate which corresponds to that of an autocatalytic reaction. The rate of thrombin formation in such an experiment can be accelerated by the addition of small amounts of purified thrombin. In a series of fundamental studies, various derivatives were made. One of these is obtained when thrombin is added to an aqueous solution of prothrombin. The derivative is insensitive to activation with calcium, Ac-globulin, and thromboplastin(6). This derivative may be identified, in part, by electrophoresis, because it has a lower mobility than prothrombin. Another inactive derivative of prothrombin may be formed by adding 3,4,4′-triaminodiphenyl sulfone to purified prothrombin in 25% sodium citrate solution(7). Under similar circumstances, but with 3-chloro-4,4′-diaminodiphenyl sulfone, an intermediate is produced during the conversion of prothrombin to thrombin(7). A derivative of prothrombin can also be formed by drying an aqueous solution of prothrombin from the frozen state and storing it in a desiccator(8). This preparation cannot be activated with biological activators but can be changed to thrombin in 25% sodium citrate solution by autocatalysis.

The present report concerns a previously unrecognized property associated with storage of aqueous solutions of purified prothrombin at approximately —10°C. Such solutions gradually lose prothrombin activity. It will be shown that this loss of prothrombin activity is accompanied by the development of another property; namely, the ability to accelerate the conversion of prothrombin to thrombin.

Methods. Preparation of prothrombin. Purified prothrombin was prepared by the methods of Ware and Seegers(1). It was freed from the last traces of Ac-globulin by heating the prothrombin to 53°C for 2 hours in solution in neutral distilled water. It was then dried by using cold acetone. When needed in the experiments some of the dehydrated prothrombin was dissolved in neutral distilled water so the resulting solution contained approximately 2000 units of prothrombin per ml. *Prothrombin analysis.* Prothrombin activity was measured by the modification of the original 2-stage method of Warner, Brinkhous, and Smith(9) suggested by Ware and Seegers(10). The modification consists of adding Ac-globulin to insure maximum prothrombin activation. *Thrombin analysis.* The method of Seegers and Smith(11) was used for the quantitative determination of thrombin. *Ac-globulin analysis.* Plasma Ac-globulin activity was determined by the method described by Ware and Seegers(12).

TABLE I. Changes Observed in Stored Frozen Purified Prothrombin Solutions.

Storage at −10°, days	Prothrombin, μ/ml	Thrombin, μ/ml	Ac-globulin, in bovine plasma, μ/ml
0	2000	0	120
4	2000	0	120
8	2000	0	120
12	2000	0	120
16	630	Trace	480
32	630	5	720

Experimental. A solution of purified prothrombin which was free from Ac-globulin was prepared by the method above. This solution was shown to be free from thrombin, and when it was used as a prothrombin source for the determination of the Ac-globulin content of the laboratory control bovine plasma, the result was the same as obtained for several years with the standard reagents in use for Ac-globulin analyses. This result was also the same as had been obtained for this plasma for several months with numerous lots of purified prothrombin. The solution of prothrombin was then stored at a temperature of approximately —10°C, and was kept in the frozen state for 32 days. During this time, samples were tested for prothrombin, thrombin, and Ac-globulin every 4 days, for 16 days, and again at 52 days. The analytical results are shown in Table I. This experiment was repeated often during the past 2 years.

The role of the increase in thrombin content in causing the apparent increase in Ac-globulin activity may be questioned. For use in Ac-globulin analyses, the prothrombin solution used is diluted to a final concentration of 1.34 units per ml. In this instance, the prothrombin concentration was 630 units per ml, and the thrombin concentration was 5 units per ml. The dilution employed was, therefore, 630/1.34 or 470 fold, resulting in a final concentration of thrombin only slightly higher than 0.01 unit per ml. During the course of nearly 5 years of experience with Ac-globulin analyses, it has been repeatedly observed that final thrombin concentrations as high as 0.02 unit per ml introduced with the prothrombin have no observable effect on Ac-globulin concentration determinations.

Further exploration of the meaning of the apparent increase in Ac-globulin activity indicated that the stored prothrombin contained an accelerator of prothrombin conversion. Many prothrombin preparations were studied and all of them were found to acquire this unusual property and to a certain extent, this seemed to be independent of the purity of the prothrombin. Further data on a typical preparation from this laboratory are given in Table II. The table shows the values obtained, a) with the standard prothrombin, b) a mixture of 3 parts of standard prothrombin and one part of stored frozen prothrombin, c) equal parts of the 2 prothrombin solutions, and d) the stored frozen prothrombin. In all of these analyses, the actual prothrombin concentration was the same by 2-stage analysis. The bovine plasma used was also the same in all instances so it must be concluded that the quantity of Ac-globulin was the same throughout and that the apparent differences in its concentrations are due to the quality of the prothrombin substrate used.

TABLE II. Effect of Prothrombin Substrate on Quantitative Bovine Plasma Ac-Globulin Analyses.

Prothrombin substrate*	Units Ac-globulin/ml
Stand. purified prothrombin	120
3 parts stand. prothrombin with 1 part stored frozen prothrombin†	250
2 parts stand. prothrombin with 2 parts stored frozen prothrombin†	399
Stored frozen prothrombin†	639

* Total prothrombin concentration was always standard for the procedure; namely, 1.34 units/ml.
† No. 510207.

Discussion. It is considered probable that the property described in the present report is possessed by a derivative of prothrombin. The acceleration of prothrombin conversion described does not occur with the freshly prepared prothrombin solution, nor does it occur as long as the prothrombin concentration, as measured by the use of activators of biological origin, remains unchanged. With the loss of prothrombin activity, however, the accelerator becomes detectable. These circumstances are compatible with the concept that the activity described is due to an alteration of the prothrombin molecule.

One may speculate that the prothrombin molecule is altered in such a way that it does not cause fibrinogen to coagulate, but that it will act in the same way as thrombin in autocatalysis. This would account for the loss of prothrombin activity associated with the gain of prothrombin conversion accelerator activity.

The present evidence, while compatible with the idea of development of a prothrombin derivative, does not exclude other interpretations. It is possible that the activity observed is due to a contaminant in the prothrombin preparations. This view requires that the contaminant becomes activated while the prothrombin loses its activity. That would be an unusual combination. If our estimates of the purity of the prothrombin preparations are correct, then an impurity could only be present in small quantities and its potency would have to be extraordinary.

Summary. Aqueous solutions of purified prothrombin, stored in a freezer at —10°C, lose appreciable activity over a period of a few weeks. Concomitantly, these solutions gain the ability to accelerate the conversion of prothrombin to thrombin, as evidenced by an apparent increase in Ac-globulin activity of plasma analyzed by using the stored solutions as the prothrombin substrate. It is believed that prothrombin itself becomes modified and acquires accelerator properties. The possibility of an impurity acquiring these properties

is not excluded, although that is considered unlikely.

1. Ware, A. G., and Seegers, W. H., *J. Biol. Chem.*, 1948, v174, 565.

2. Seegers, W. H., McClaughry, R. I., and Fahey, J. L., *Blood,* 1950, v5, 421.

3. Seegers, W. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 677.

4. McClaughry, R. I., and Seegers, W. H., *Blood,* 1950, v5, 303.

5. Seegers, W. H., and Andrews, E. B., PROC. SOC. EXP. BIOL. AND MED., in press.

6. Seegers, W. H., and McClaughry, R. I., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 247.

7. Seegers, W. H., Andrews, E. B., and McClaughry, R. I., *Am. J. Physiol.,* 1951, v164, 722.

8. McClaughry, R. I., Andrews, E. B., and Seegers, W. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 252.

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

10. Ware, A. G., and Seegers, W. H., *Am. J. Clin. Path.,* 1949, v19, 471.

11. Seegers, W. H., and Smith, H. P., *Am. J. Physiol.,* 1942, v146, 511.

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

Received May 19, 1952. P.S.E.B.M., 1952, v80.

Effects of Beta Rays upon a Single Nerve Fiber. (19628)

HISAO YAMASHITA AND TOMOHARU MIYASAKA. (Introduced by Kanematsu Sugiura.)

From the Radiological Department, Medical College of Keio University, Tokyo, Japan.

The histological changes of nerve fibers under severe irradiation by x-rays were reported by Janzen and Warren(1), and Davidoff *et al.*(2). Schmitz and Schaefer(3) were unable to demonstrate any changes of action potential of nerve fibers by irradiation. Audiat (4), Paulian(5), Rascanu(6), and others irradiated the nerve trunks and showed some changes of the action potential. It is the purpose of this report to deal with the effect of irradiation on the conduction of nerve impulse physiologically using a single nerve fiber of the sciatic gastrocnemius of the Japanese toad (*Bufo vulgaris*).

Methods. The source of beta rays was radon sealed in a glass tube (30 mg/cm^2 in thickness). The apparatus is shown schematically in Fig. 1. Two glass plates, covered with paraffin, carried parallel mica plates 10 μ thick that were insulated from each other. One of the mica plates had a small shelf, where the radon tube can rest at a distance of 1 mm from the nerve fiber. A single motor nerve-muscle sample of the sciatic gastrocnemius of toad, made by the method of Shimizu and Tasaki(7,8) and immersed in Ringer's solution, rested on the glass plates. Precautions were taken in handling to make a bridge of a single nerve fiber between the pools without exposing Ranvier's rings to air. The action current between insulated poles was measured by an oscillograph and recorded photographically.

Results. 1. *Changes of threshold value of stimulus by irradiation.* The value of threshold stimulation was measured before and after irradiation for short intervals. To properly express the dose received by the nerve fiber, roentgen equivalent physical (rep) should be the unit employed. However, since the con-

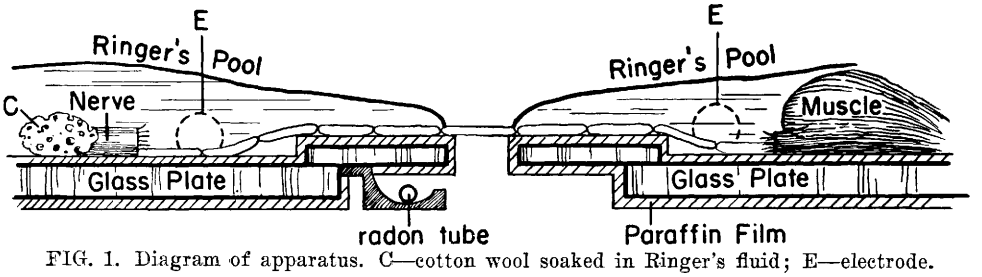

FIG. 1. Diagram of apparatus. C—cotton wool soaked in Ringer's fluid; E—electrode.