an increased growth or migration. It seems more likely that the stimulating effect of the dilution of the serum resulted from a dilution of some inhibitory substance in the chicken serum.

Summary. 1. The optimal growth in tissue culture of macrophages and the exoerythrocytic forms of *Plasmodium gallinaceum* occurred between 7 and 11 atmospheres of pressure. Growth was reduced to 50% at 5 and 13 atmospheres.

2. High concentrations of serum (over 50%) were inhibitory to both the malarial parasites and the host cell, the macrophage.

Received August 21, 1950. P.S.E.B.M., 1950, v75.

Changes in the Reactivity of Purified Prothrombin After Freeze Drying.* (18162)

ROBERT I. MCCLAUGHRY, EDNA B. ANDREWS AND WALTER H. SEEGERS.

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

In work on the purification of prothrombin it became evident that the protein could not be activated as readily as the prothrombin of native plasma. The question arose whether this might be due to an alteration in the reactivity of the prothrombin or to the removal of essential activators. When evidence for the latter possibility became available in abundance, there followed a general tendency to consider the question closed. However, the reactivity of prothrombin can be changed; for example, under proper conditions, thrombin can produce alterations(1). This altered prothrombin can still be converted to thrombin but not with calcium plus thromboplastin plus Ac-globulin.

We have now found that freeze drying of purified prothrombin preparations produces changes which cause the prothrombin to be refractory to the physiological activators. This makes it desirable to avoid the freeze drying technic when the object is to retain purified prothrombin for long periods of time. The change in reactivity of the purified prothrombin is not noticeable immediately after drying. The loss in activity occurs later, and variable periods of time are required for extreme changes to take place. The sequence of events is approximately as follows: First the prothrombin becomes refractory to the activators such as calcium, thromboplastin, and Ac-globulin. Activation with sodium citrate, thrombin and 3-chloro-4,4′-diaminodiphenyl sulfone is then still possible. Later even this activation procedure is no longer effective and finally the dried prothrombin becomes insoluble in physiological saline solution.

Experimental procedures. Purified prothrombin was obtained from bovine plasma (2). The assay for prothrombin involved the use of the modified 2-stage procedure, which supplies Ac-globulin, calcium, and thromboplastin for the activation of prothrombin(3). Thrombin activity was measured by the method of Seegers and Smith(4). The apparatus used for drying prothrombin from the frozen state was similar to that described by Seegers(5).

Time relationships. The loss in activity of a large number of products has been studied

* This investigation was supported by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

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

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

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

4. Seegers, W. H., and Smith, H. P., *Am. J. Physiol.,* 1942, v137, 348.

5. Seegers, W. H., *Science,* 1945, v101, 284.

TABLE I. Changes in Activity of Dried Purified Prothrombin.

Prothrombin product No.	Initial activity: Total units, × 1000	Initial activity: Specific*	% activity lost	Days after drying
470822	165	925	17	30
480218	105	1260	67	196
480322	231	1305	69	162
480519	167	1240	75	105
480527	180	1350	78	97
480810	215	1335	52	25
490125	300	1270	98	421
491215	465	1370	46	124
470422A	185	720	99	1065
480831	110	1065	98	568

* Specific activity is here expressed in units per mg dry wt.

and representative data are assembled in Table I. All of the preparations had high specific activity, which was completely retained during the drying operation. After drying they were placed in a desiccator containing P_2O_5. The prothrombin activity was periodically measured quantitatively by the modified 2-stage procedure. The amount of activity lost varied from product to product. In one instance 52% of the activity was lost in 25 days and with another product the loss was 17% in 30 days. About a year was required for most of the activity to be lost.

A more detailed study was made of prothrombin preparation No. 491215. Large quantities were made available so that electrophoretic data could be obtained. Analysis of the product immediately after drying indicated that there was no change in activity. A sample was taken for electrophoresis at this time and the boundary patterns were like those obtained with other high quality prothrombin products. After 20 days 12% of the activity was gone, after 50 days 36%, and on the 124th day approximately half of the prothrombin activity was refractory to activation with calcium, thromboplastin and Ac-globulin. Some of the remaining dried material was then examined in the Tiselius apparatus. The boundary patterns were similar to the ones previously obtained with the same prothrombin immediately after it had been dried. The mobility and the percentage composition were essentially the same. By these criteria, therefore, the inactive prothrombin could not be distinguished from the active material.

Activation of altered prothrombin by autocatalysis. If purified prothrombin is dissolved in a 25% solution of sodium citrate, it becomes activated by autocatalysis. Any thrombin added to such a solution initiates or accelerates the process. If, in addition, a small amount of 3-chloro-4,4′-diaminodiphenyl sulfone is added the yield of thrombin is maximal(6). This fact suggested the possibility that one might be able to obtain thrombin from the prothrombin which could not be activated with calcium, thromboplastin, and Ac-globulin. It has been repeatedly possible to do so. The details of one activation experiment are shown in Fig. 1. Prothrombin No. 491215 was used on the 124th day of storage at which time it had lost half its activity.

In the sodium citrate solution, containing 3-chloro-4,4′ diaminodiphenyl sulfone and 400 units of purified thrombin per cc, the prothrombin activity lost during storage in the desiccator was completely recovered as thrombin. From one point of view this can be regarded as a 200% yield of thrombin. In some experiments with other products, yields of more than 1,000% have been obtained by this criterion. This simply means that the sodium citrate solution activated a prothrombin derivative which could not be activated with calcium, thromboplastin and Ac-globulin. It is an interesting fact that the prothrombin which could still be activated with "physiological" substances became refractory to them in the sodium citrate solution very early in the experiment. The activation then involved the production of thrombin almost entirely from inert prothrombin; that is, protein which could not be converted to thrombin with calcium, thromboplastin, and Ac-globulin under the conditions of the modified 2-stage analysis. About half had become inert in the desiccator during a period of 124 days

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

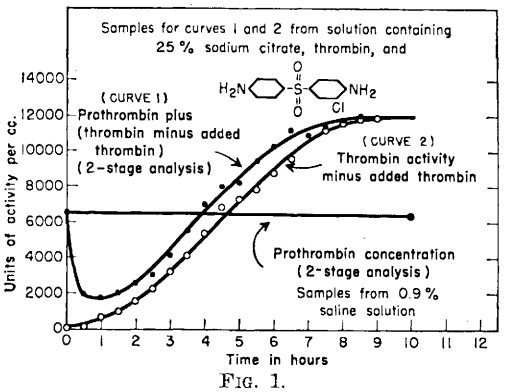

FIG. 1.

The purified prothrombin had lost 50% of its activity. A sample was dissolved in 25% sodium citrate solution. To this was added 400 units of thrombin per cc of solution and enough 3-chloro-4,4'-diaminodiphenyl sulfone to saturate the solution with respect to the sulfone. From this solution samples were taken for 2-stage prothrombin analysis and also for thrombin analysis.

and the other half in the sodium citrate solution within 30 minutes. Presumably the manner in which the prothrombin became inert did not influence the rate of transformation to thrombin in sodium citrate solutions. The question arises whether there were two kinds of altered prothrombin being activated simultaneously; however, it cannot be answered with the data now available.

Discussion. The prothrombin derivative produced by mixing prothrombin and thrombin in aqueous solutions has a lower electrophoretic mobility than the original prothrombin(1). The altered prothrombin produced after freeze drying has electrophoretic properties identical with fully active prothrombin. It must, therefore, be concluded that this paper describes a new derivative of prothrombin which is different from the one produced through the action of thrombin. Both can, however, be activated to thrombin by autocatalysis in sodium citrate solution.

It is evident that one cannot expect to preserve prothrombin samples after they have been dried from the frozen state unless conditions can be found under which the activity will be retained. This is a major practical handicap because the freeze drying technic is convenient not only in the research laboratory but also in industry. Perhaps it is possible to dry prothrombin by using cold acetone. In quite a number of experiments it has been possible to do so without loss of activity. Furthermore, the acetone dried material seems to be stable; however, our experience covers a period of only one half year. It is, therefore, possible to say only that this procedure promises to be satisfactory.

Summary. Purified prothrombin can be dried from the frozen state without immediate loss of activity. Thereafter progressive loss of prothrombin activity occurs, characterized by refractivity to the action of calcium plus thromboplastin plus Ac-globulin. In about one year most of the prothrombin is altered and in addition some is insoluble in aqueous solution. Prothrombin altered as the result of the freeze drying technic can be activated autocatalytically in 25% sodium citrate solution. The altered prothrombin has essentially the same electrophoretic properties as purified prothrombin and represents a derivative of prothrombin not previously described.

Received July 31, 1950. P.S.E.B.M., 1950, v75.

Effect of Dibenamin on the Vascular Response of Rabbits to Typhoid Endotoxin. (18163)

P. BOQUET* AND Y. IZARD. (Introduced by J. D. Aronson.)

From Department of Anatomy, School of Medicine, University of Pennsylvania, and Pasteur Institute, Paris, France.

Menten and Manning(1) observed that injections of *E. coli* and *S. paratyphi* induced hyperglycemia in animals. Zeckwer and Goodell(2) and later Evans and Zeckwer(3) confirmed these results and stated that hyperglycemia was due to the action of adrenalin.