was clear cut. The tissues of the animals receiving B_{12} always showed more PNA than those of the controls (Fig. 1-8). Liver, anterior horn cells and autonomic ganglion cells all showed an increase in PNA. Definitive DNA changes in tissues stained by this method could not be observed although the localization, by color, was obvious. The presence of nuclear PNA was too distracting for an objective analysis of a cell's DNA content. Staining of these same tissues by the Einarsson method confirmed the results obtained through the Turchini procedure (Fig. 9-16). Since there is no color differentiation between PNA and DNA, attention was focused primarily upon the cytoplasm. Here one noted clear cut differences between preparations from B_{12} treated and those from control animals. Tissues of B_{12} treated rats always showed more PNA than the controls. The Feulgen stain indicated the presence of DNA. The same histological picture was seen, both quantitatively and qualitatively, for spinal cord and autonomic ganglia of all the animals. Preparations of liver showed a consistent, although slight, rise in DNA content in B_{12} treated rats. The results of the second experiment confirmed those of the first.

Summary. These preliminary results support the contention that vit. B_{12} is related to the genesis of ribonucleoproteins in rat tissues. Weanling and immature rats were placed in 2 dietary groups, chow and basal, the latter dietary group deficient in vit. B_{12}, folic acid and PABA. One-half of each group received parenteral B_{12} every other day. Liver, spinal cord and cervical sympathetic ganglia were examined histologically for changes in pentose and desoxypentose nucleic acids. These 3 tissues from animals which received vit. B_{12} showed more PNA than the controls. No consistent observable difference was seen in spinal cord and cervical sympathetic ganglia for the DNA as visualized by the Feulgen stain. There was a slight increase in the intensity of the Feulgen stain in hepatic cell nuclei from rats which had received vit. B_{12} over the controls.

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Studies on a Proteolytic Enzyme System of the Blood. IV. Activation of Profibrinolysin by Serum Fibrinolysokinase.* (19014)

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Recent studies in this laboratory concerning the activation of profibrinolysin (plasminogen) by fibrinolysokinases of bacterial(1,2) and tissue(3) origin have included observations which suggest that a kinase is also involved in the so-called "spontaneous" activation of serum profibrinolysin. Active fibrinolysin (plasmin) although not present in normal whole serum, has long been known to appear in certain serum fractions(4,5) and in whole serum after treatment with chloroform (6). Our working theory of the interactions of the various components of the fibrinolytic enzyme system of dog serum is:

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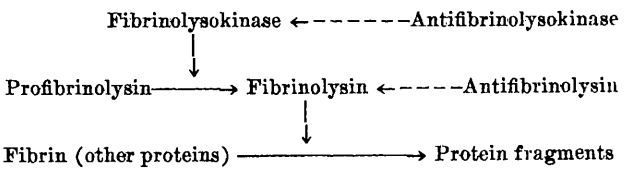

Profibrinolysin, the inactive enzyme precursor, is present in normal serum and may be converted to the active enzyme, fibrinolysin, by the action of a fibrinolysokinase. Fibrinolysokinase activity has been found in certain bacterial products(7-11) and certain tissues (3,12-16). Antifibrinolysin has been widely studied(17-20) and shown to be a normal serum component which inhibits the action of fibrinolysin. Antifibrinolysokinase has not been isolated and studied, but indirect evidence suggests that one or more such inhibitors (*e.g.* antistreptokinase)(7,21) may be present in serum and seem to be distinct from antifibrinolysin.

The experiments presented in this paper were designed to demonstrate the existence of a fibrinolysokinase in normal *serum* and to show that it is independent of cellular elements. All experiments concern dog serum or its fractions.

Methods. Previous studies have described the preparation of borate buffer, pH = 7.7 (20), staphylokinase(2) and our standard serum profibrinolysin preparation, Prolysin (3) (see below, Exp. III), and the determination of fibrinolysin and antifibrinolysin concentrations(20). Profibrinolysin content(2) of a serum fraction, free from antifibrinolysin, was determined by incubating it with 2% staphylokinase at 5°C for 2 weeks and titering the fibrinolysin content at intervals until it is stable.

Results. I. *Is a fibrinolysokinase, released from the cellular elements of the blood, responsible for the "spontaneous" activation of profibrinolysin?* The development of fibrinolytic activity in chloroformed whole serum was used as a test system.

Two lots of arterial blood, obtained from the same dog through a siliconed cannula were each mixed with one-tenth volume of 0.1 M sodium oxalate. Lot I was mixed in a glass container and subsequently centrifuged at 320 g for 15 minutes. Smear of this slightly turbid plasma showed many platelets and occasional erythrocytes and leucocytes. The second lot of blood was collected into a chilled siliconed container, centrifuged for 30 minutes at 2000 g, the supernatant plasma transferred to a cellulose nitrate tube with stainless steel collar and centrifuged an additional 60 minutes at 42,000 g. Smear of this clear plasma showed no cellular elements. Recalcification times were respectively: Lot I, 50 seconds; Lot II, 275 seconds. Sera were prepared from each lot of plasma in two ways: (a) by recalcification: IA, IIA and (b) by addition of thrombin (Upjohn): IB, IIB. The sera were treated with chloroform by shaking vigorously for 2 minutes with ½ volume of chloroform and centrifuging 15 minutes at 720 g to remove excess chloroform and part of the denatured protein. The chloroformed sera and control untreated sera were incubated over a 3-weeks' period at 4°-6°C, and fibrinolysin

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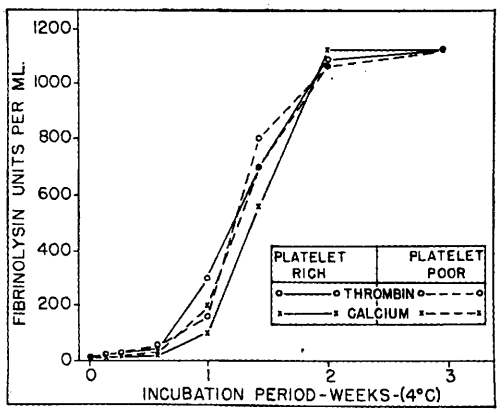

FIG. 1. Appearance of fibrinolysin in chloroformed sera prepared from platelet-rich and platelet-poor plasmas.

contents determined at intervals. In Fig. 1 it is shown that fibrinolytic activity develops at approximately the same rate in each serum regardless of whether the serum has been obtained from platelet-rich or platelet-poor plasma and also regardless of whether the serum is obtained by recalcification or by thrombinization. Control sera, not treated with chloroform, develop no lytic activity.

II. *Does chloroform act as a fibrinolysokinase?* To test whether chloroform itself exerts a kinase-like action on profibrinolysin we used the development of fibrinolytic activity in our standard Prolysin. This serum fraction contains no antifibrinolysin, and does not usually activate spontaneously. The profibrinolysin content of different lots varies between 4000-4600 units per ml.

A solution of Prolysin was divided into 2 lots. One lot was treated with chloroform by shaking with 1/5 volume for 2 minutes. Both lots were incubated at 4°-6°C for 4 weeks and fibrinolysin contents titered twice a week. Neither the plain nor the chloroformed Prolysin developed detectable fibrinolysin titers, although, even after 4 weeks, both still could be shown to contain high profibrinolysin concentrations. Four similar experiments confirmed this result.

III. *"Spontaneous" activation of serum fractions.* Fresh oxalated plasma was recalcified and the resulting serum divided into 2 lots which were fractionated in the following manner. All fractionation procedures were carried out at 5°C. 1. *Euglobulin fraction.* 100 ml serum was diluted to 2000 ml with distilled water and the pH adjusted to 5.35 with 1% acetic acid. After standing cold for one hour, the precipitate was collected by centrifugation and dissolved in 20 ml borate buffer, maintaining pH at 7.6. 2. *Prolysin fraction.* To 100 ml serum was added 49 ml ammonium sulfate (saturated at 5°C). The mixture was allowed to stand for 30 minutes and the precipitate collected by centrifugation. This precipitate was dissolved in distilled water, and dialyzed against running tap water overnight. The resulting precipitate was collected by centrifugation and dissolved in 20 ml borate buffer (pH 7.6). Both fractions were tested for antilysin and profibrinolysin. They were then incubated for 6 weeks at 4°-6°C and fibrinolysin contents titered at intervals. No antifibrinolysin was present in either fraction. Profibrinolysin titers, as determined by staph activation at 5°C were respectively, Euglobulin: 4628 units per ml and Prolysin: 4580 units per ml. As shown in Fig. 2, fibrinolysin appeared spontaneously in the Euglobulin fraction, reaching a titer of 3000 units. The Prolysin fraction did not activate. After the Prolysin fraction had incubated for 6 weeks a sample treated with staphylokinase for 20 minutes at 37°C yielded 1800 units per ml. Similar staphylokinase treatment of the incubated Euglobulin fraction did not yield any increase in fibrinolysin concentration.

From this experiment we concluded that the two fractions, Euglobulin and Prolysin, pre-

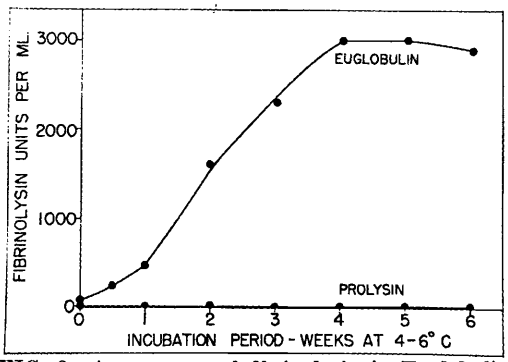

FIG. 2. Appearance of fibrinolysin in Euglobulin and Prolysin fractions of the same serum.

pared from the same serum, were similar in that both were free from antilysin and contained like amounts of profibrinolysin capable of activation by staphylokinase, but they differed in that the Euglobulin activated spontaneously whereas the Prolysin did not. Two explanations seem possible: (1) that the profibrinolysin was so changed in the Prolysin fraction that it could not activate autocatalytically or (2) that an activator, a fibrinolysokinase, was present in the Euglobulin fraction, but absent from the Prolysin fraction.

IV. *Isolation of a fibrinolysokinase fraction from serum.* The last possibility was explored and many attempts were made to isolate from serum a fraction, free from profibrinolysin and antifibrinolysin, but capable of activating Prolysin. Several such fractions, prepared by different methods, were found. The experiment illustrated below describes such a fraction. Pooled whole dog serum was chilled to 5°C and an equal volume of cold absolute ethyl alcohol added. The precipitate so formed was discarded and an additional volume of alcohol equivalent to 24% of the original serum volume was added. The resulting precipitate was collected by centrifugation and dissolved in 1/3 the serum volume of saline. Traces of alcohol were removed by passing a stream of air over the solution. The pH was adjusted to 7.3. Tests of this fraction (K) showed that it contained 82 units of antifibrinolysin (normal whole serum, by this method = 3625 antilysin units per ml) and less than 20 units of profibrinolysin. Mixtures of this fraction (K on Fig. 3) were made with equal volumes of Prolysin and of saline, and a control mixture of Prolysin plus saline was prepared. All pH's were found to be 7.35 ± 0.05. Each mixture was incubated at 5°C and fibrinolysin content determined at intervals.

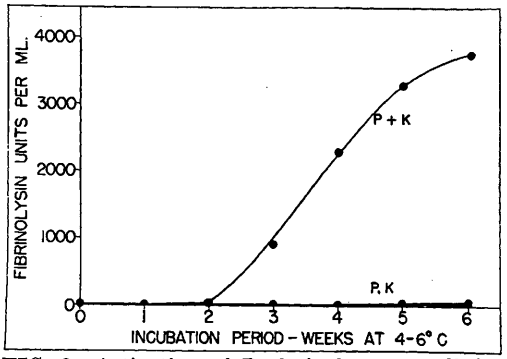

FIG. 3. Activation of Prolysin by serum fibrinolysokinase.

Fig. 3 illustrates the development of fibrinolysin activity in the various mixtures. After an initial lag the Prolysin plus fraction K mixture (P + K) developed a high titer of fibrinolysin (3730 units) comparable to that developed with this Prolysin plus staphylokinase (4200 units). The Prolysin plus saline (P) developed 49 units of fibrinolysin and the fraction K plus saline (K) developed less than 20 units of fibrinolysin during the 6 weeks incubation period.

Discussion. The "spontaneous" appearance of fibrinolysin in whole serum which has been treated with chloroform might be explained in any of the following ways: A. The splitting of a fibrinolysin-antifibrinolysin complex with liberation of an active fibrinolysin(6,22). B. A direct action of chloroform on the profibrinolysin molecule. C. The removal of antifibrinolysin followed by the autocatalytic activation of profibrinolysin, as suggested by Christensen and MacLeod(23), or D. The removal of antifibrinolysin (? also antifibrinolysokinase) followed by the activation of profibrinolysin by serum fibrinolysokinase. The first of these possibilities seems unlikely as, immediately after chloroform treatment, antifibrinolysin is greatly reduced or absent, but active fibrinolysin develops only gradually (Exp. I), reaching its maximum in about 2 weeks at 5°C. The second possibility also seems unlikely as chloroform will not activate an isolated prolysin fraction (Exp. II). The third possibility has been explored by attempting to activate our Prolysin by addition of potent fibrinolysin preparations. Activation has been rarely demonstrable and in those cases in which some activation was observed we were unable to eliminate the possibility that the fibrinolysin preparation itself con-

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tained a kinase.

From our first experiment and from previous experiments(3) in which no kinase activity could be found in isolated platelets, leukocytes or erythrocytes, we concluded that if a fibrinolysokinase was responsible for the "spontaneous" activation of profibrinolysin it must be a serum component independent of cellular elements.

Experiment III strongly suggests that the Euglobulin fraction of serum contains a fibrinolysokinase which is absent in the Prolysin fraction. The isolation and action of such a substance is shown in Experiment IV. Fraction K was able to activate the Prolysin almost completely in the 6 week experimental period. The slow initiation of this activation may be due to the presence of traces of antifibrinolysin in Fraction K. Other serum fractions, prepared from whole serum and from the serum residual after Prolysin has been removed, have also proved active as fibrinolysokinases. Apparently very exact technic is necessary to separate the kinase from profibrinolysin as duplicate experiments on different serum lots will not always give a good separation. Studies are underway to elucidate further this fractionation problem.

Conclusion. Serum, independent of cellular elements, contains a fibrinolysokinase which is capable of converting profibrinolysin to active fibrinolysin.

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Relation of Vitamin E to Acute Physiological Stress.* (19015)

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According to Selye(1), the normal physiological response of the body to varied forms of stress, such as cold, heat, emotional excitement, etc., proceeds via a common pathway. The pathway seems to involve stimulation of the pituitary gland by epinephrine or other agents. The pituitary then releases adrenocorticotropic hormone (ACTH), which causes the adrenal glands to secrete various steroid hormones (*e.g.* cortisone) which mediate the physiological response of the body to stress. The entire system is designated the "general adaptation syndrome."

The nutritional status of the body has been shown to affect the capacity to respond to stress in the case of several nutrients such as pantothenic acid(2) and vit. C(3). The possibility of a causal relationship between vit. E and the stress response via the anterior pituitary-adrenal cortical pathway seemed likely in view of the known protective effect of α-tocopherol in various different types of stress, such as carbon tetrachloride poisoning (4), exposure to high altitude(5), or dietary restriction of either protein or total calories (6). Also, vit. E has been implicated as a factor in maintaining the anatomical integrity of the adrenal cortex by Tonutti(7) who found that vit. E-deficient rats showed degenerative changes in the exterior and interior zones of the adrenal cortex similar to those following hypophysectomy.

Two experiments were performed to test the possibility that vit. E is related to the initial phase (alarm reaction) of the general adaptation syndrome.

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