TABLE I.
Effect of Purified Anterior Pituitary Growth Hormone on Tumor Growth.
Wt (in g) at autopsy.

Sarcoma 37						Mammary adenocarcinoma					
Controls			Hormone-treated			Controls			Hormone-treated		
3.45	0.56	1.68	2.14	2.29	0.91	2.37	2.55	2.95	1.60	2.30	1.90
3.02	0.22	1.88	2.25	1.69	1.29	2.03	1.55	1.75	1.10	1.15	1.70
2.27	0.55	1.76	2.98	2.90	1.32	1.30	1.30	1.53	2.40	1.10	0.70
1.89	1.07	2.42	2.42	2.13	2.01	1.60	1.35	2.42	1.00	1.28	0.95
1.71	2.58	1.58	1.41	0.29	1.37	1.20	0.45	1.35	1.75	0.47	1.25
1.64	3.57	1.36	1.37	0.41	2.01	1.55	1.40	1.58	1.95	1.43	0.65
2.14	2.67	1.23	2.00	2.83	2.17	0.80	1.10	1.57	1.20	0.63	1.20
1.06	2.14	1.15	1.74	0.76	1.74	0.77	0.70	0.90	1.25	0.72	1.10
0.73	1.92	1.08	2.01	1.48	0.94	0.13	0.60	1.28	1.25	0.49	0.55
0.88	2.02	1.69	1.39	1.12	1.70	0.12	1.40	0.87	0.75	1.03	0.70
1.73 ± 0.15*			1.70 ± 0.13			1.35 ± 0.12			1.19 ± 0.095		

* Mean values ± standard error.

(Armour). The mice receiving growth hormone showed a marked increase in weight during the first week of therapy. After this time, when the tumors were becoming necrotic, all groups suffered weight losses.

The animals were killed by decapitation; the tumor was peeled away from the subcutaneous connective tissue of the host, the interior exposed, and all visible necrotic material removed. The weights of the tumors are recorded in Table I.

It can be seen that the purified growth hormone did not show any appreciable effect, stimulatory or inhibitory, on the transplantable tumors used. Histological studies, which were kindly performed by Dr. E. Lowenhaupt of the Department of Pathology, revealed that tumors of all groups of animals, hormone-treated and controls, were of essentially similar appearance as regards relative proportions of necrotic tissue, numbers of mitotic figures and cell appearance.

Conclusion. Purified growth hormone does not influence the growth of sarcoma 37 and the mammary adenocarcinoma in "A" strain mice.

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Activation of Purified Prothrombin.* (17540)

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The activation of prothrombin has probably been discussed more extensively than any other problem in blood coagulation. It is an important process and exceedingly complex in nature. Calcium, thromboplastin, Ac-globulin and platelet derivatives participate in this vital function; and although it is not exactly clear how the activators work, it is apparent that they may be divided into two main classes; namely, those with thromboplastin activity in the traditional sense and those with accelerator or thromboplastin co-factor activity. Either class alone is a poor activator of purified prothrombin, but together they cause rapid conversion. To understand more exactly what forces these substances bring to bear on prothrombin it is essential that they be obtained in purified form and in sufficient quantity for study. This still needs to be accomplished, but in the meantime it may be possible to obtain indirect information concerning the mechanism of their action. For example, this

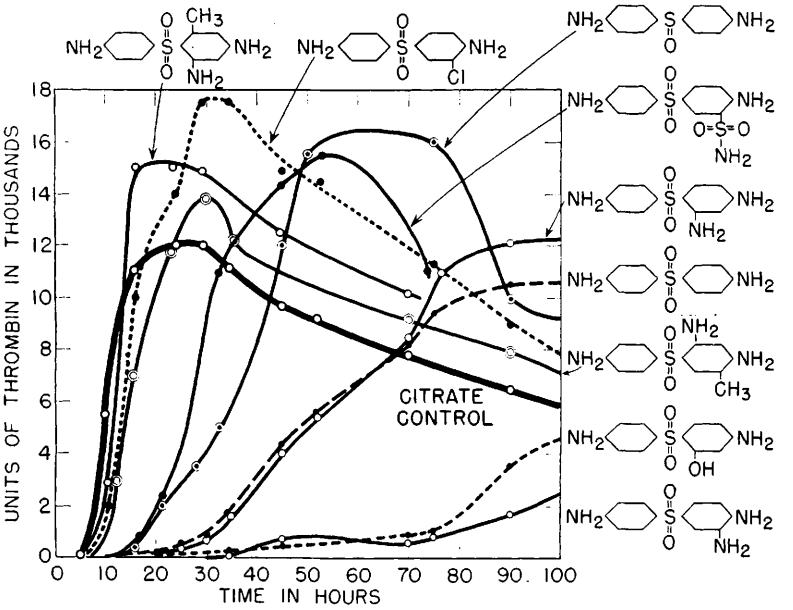

FIG. 1.

Activation of purified prothrombin in 25% sodium citrate solution, and of the same prothrombin in 25% sodium citrate solution together with a saturated solution of the organic compound indicated for each curve. The concentration of prothrombin was 18,000 units per cc.

paper describes prothrombin activation experiments in which sodium citrate, an organic compound of known structure, is the activating agent. This activation can be either blocked or synergized by certain diphenyl sulfones whose structure is also known. It should be possible to elucidate the nature of the forces required for prothrombin conversion by studying the action of these relatively simple compounds as activators and inhibitors, and thus by analogy formulate viewpoints concerning the more complex physiological activators. The work to be described is sufficiently promising to justify a hopeful outlook for the new attack on the problem of thrombin formation.

Experimental. Thrombin Analysis. Quantitative measurement of thrombin activity was done by the method of Seegers and Smith.(1)

Prothrombin. Purified prothrombin was obtained from bovine plasma by methods previously described.(2,3) Only high quality material was used. Generally the specific activity was 1200 units per milligram dry weight or more. It was free of Ac-globulin activity and contained no thrombin. On electrophoresis such products have a main boundary pattern comprising about 90% of the total. Quantitative determination of activity was by the 2-stage method.(4,5)

Diphenyl Sulfones. These were all synthesized in the laboratory and it is expected that the method of synthesis will be described in detail elsewhere. I wish to thank Leonard Doub for placing these compounds at my disposal. The crystalline compounds were added to the prothrombin dissolved in 25% sodium citrate solution. All are sparingly soluble and it required only a few milligrams to give a saturated solution with respect to the sulfone.

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

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

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

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

No attempt was made to have the concentration of the sulfones equal on a molecular weight basis.

Prothrombin Activation. Powdered sodium citrate was added to an aqueous solution of prothrombin so that the concentration of sodium citrate was 25% and the concentration of prothrombin was about 1.5%. Then the crystalline sulfone was added in sufficient quantity so that crystals could be seen suspended in the prothrombin solution. The mixture was allowed to stand at room temperature, and samples were taken for thrombin analyses. The results presented in Fig. 1 were obtained with the same prothrombin preparation and, were obtained simultaneously so as to obtain the best possible comparable results with the various sulfones.

Results. With the 25% sodium citrate solution prothrombin activation was slow during the first 5 hours. After this induction period activation was rapid, and within a day the maximum yield of thrombin was obtained. This was, however, only 67% of the potential thrombin which could be obtained from the prothrombin with the use of calcium, thromboplastin and Ac-globulin. The thrombin titer was followed for 3 more days and showed a progressive decline similar to that commonly encountered with thrombin solutions which are not stabilized with glycerol or carbohydrates.(6) By adding 3, 4, 4′-triaminodiphenyl sulfone, or 2-hydroxy-4, 4′-diaminodiphenyl sulfone to the prothrombin in 25% sodium citrate solution the induction period was prolonged to more than a day and even thereafter only small amounts of thrombin formed so that in 5 days the thrombin yield was less than 25%. These compounds can thus be considered powerful inhibitors of prothrombin activation under the conditions of the experiment.

It is also possible to obtain the opposite effect; namely, an acceleration of prothrombin activation. The most interesting compound studied was 3-chloro-4, 4′-diaminodiphenyl sulfone. It prolonged the induction period about 2 hours. Then thrombin formation was far more rapid than with the citrate control and the final yield was almost 100% as compared with 67% for the control. The deterioration of the thrombin solution was at a rate typical of other thrombin solutions. Several other compounds were also found to be accelerators. Two of these, 4, 4′-diaminodiphenyl sulfoxide and 3-sulfonamide-4, 4′-diaminodiphenyl sulfone doubled the induction period but once thrombin began to form it continued until a high yield was obtained on the third day. Two others, 2, 4, 4′-triaminodiphenyl sulfone, and 4, 4′-diaminodiphenyl sulfone prolonged the induction period to one day. The thrombin titer then rose very slowly so that a 67% yield was obtained on about the fourth day.

Discussion. There are two practical benefits which can be derived from these experiments. (1) The preparation of thrombin from prothrombin is simplified. By adding 3-chloro-4, 4′-diaminodiphenyl sulfone to purified prothrombin in 25% sodium citrate the yield is increased to a maximum. There is thus no loss of an expensive prothrombin product, or remnants of proenzyme with the desired thrombin. (2) The preparation of prothrombin is simplified. In the purification procedures it has been difficult to eliminate thrombin from the final product. Failure to do so causes the prothrombin to be altered by the small amounts of thrombin.(7) This hazard can now be eliminated, for the ammonium sulfate used for fractionation work, like sodium citrate, causes slow activation of prothrombin, and this effect of ammonium sulfate can be blocked by adding small amounts of 3, 4, 4′-triaminodiphenyl sulfone to the ammonium sulfate solutions used in the fractionation procedures.

It has been discussed repeatedly that thromboplastin and Ac-globulin together activate prothrombin more rapidly and give a higher yield of thrombin than when the former is not supplemented with Ac-globulin. This general observation also applies to these artificial activators. Sodium citrate alone, like thromboplastin, acts slowly and the yield of thrombin is incomplete, but sodium citrate with 3-

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chloro-4, 4′-diaminodiphenyl sulfone, like thromboplastin with Ac-globulin, activates prothrombin more rapidly and the yield of thrombin is increased. The similarity between the action of the artificial activators and the physiological ones would seem to be of more than coincidental significance, even though the time intervals involve hours in one instance and minutes in the other.

Perhaps the most remarkable result of these experiments is the difference in action of 3, 4, 4′-triaminodiphenyl sulfone and 3-chloro-4, 4′-diaminodiphenyl sulfone. One has an amino group in the three position and the other a chlorine atom. One is an inhibitor and the other an accelerator. Thus, for one set of conditions, the difference between a "coagulant" and an "anticoagulant" has been reduced to a locus on the benzene ring where placement of an amino group produces one effect and a chlorine atom the opposite. This subtle detail is easily brought into focus, and perhaps it is here where we can hope to see, for the first time, what forces physiological anticoagulants and coagulants bring to bear on prothrombin.

Summary. Purified prothrombin can be activated by dissolving it in a 25 per cent solution of sodium citrate, but the yield of thrombin is usually low. By adding a small amount of 3-chloro-4, 4′-diaminodiphenyl sulfone to the activation mixture the yield of thrombin is increased to the maximum. Several other diphenyl sulfones also increase the yield. In contrast, 3, 4, 4′-triaminodiphenyl sulfone and 2-hydroxy-4, 4′-diaminodiphenyl sulfone act as inhibitors of the activation process.

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Cultivation of Influenza Virus in the Chorio-Allantoic Membrane of Deembryonated Eggs.* (17541)

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Fertilized chicken eggs have proved to be an excellent medium for the growth of many viruses and other pathogenic agents. A number of viruses grow in the various tissues of the embryo and in the membranes enveloping it in an indiscriminate manner while others show specific affinities for certain structures in the egg. For a number of viruses, among them those of the influenza group, the chorio-allantoic membrane(1-3) is the main site of virus multiplication. Most other structures offer less favorable conditions. It seemed an obvious step, therefore, to use only the chorio-allantoic membrane *in situ* for virus growth and to eliminate the other constituents of the egg. For this purpose the following technic was found practicable.

Methods. Eggs which have been incubated for 14-15 days are candled and the outline of the air-sac is marked. One-half centimeter above this line the egg shell is cut with an electric drill and the piece of shell is removed. The free-lying shell membrane and the marginal zone of the shell both on the inside and outside along this opening are covered with hot paraffin. The shell membrane together with the piece of underlying chorio-allantoic membrane is then cut away with a pair of curved scissors. By gently tilting and rotating the egg the embryo and the yolk sac are poured out and their connections with the chorio-allantoic membrane are severed with

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