Table I compares the effect of sulfanilamide (SA), sulfapyridine (SP) and sulfathiazole (ST) on the warming time following feedings in the indicated amounts over the indicated number of days. The rabbits used were New Zealand White males averaging 3 kg in weight. They had not been losing weight before the experiment was begun and had normal rectal temperatures. The average warming time before the drug feedings was 35. The drugs were given in tablet form with water.* The blood level estimations[3] were made on samples drawn from the marginal vein of the ear 2 to 3 hours after the final drug feeding and immediately before the test for degree of warming time lengthening induced.

The lengthenings observed did not persist following discontinuance of the drug feedings.

Summary. Sulfanilamide, sulfapyridine and sulfathiazole produced a lengthening of the warming time, in the rabbit, roughly proportional to the amount fed and the blood level reached. Serious (>60%) lengthening occurred to an extent paralleling the incidence of rapid weight loss and death.

11897

Stability of Prothrombin in the Presence of Thrombin.

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In earlier numbers of these PROCEEDINGS, Mertz, Seegers and Smith[1, 2] allege that thrombin can inactivate its precursor prothrombin. We have published evidence[3] that a trypsin-like enzyme is not only a thromboplastic agent[4] but also a *progressive* antithrom-

* The tablets were pushed through the side of the mouth, and as far back as possible, with the right forefinger. The mouth was kept moist and the finger held against the disintegrating material until swallowing was complete.

3 Bratton, C. A., and Marshall, E. K., *J. Biol. Chem.*, 1939, **128**, 537.

1 Mertz, E. T., Seegers, W. H., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 657.

2 Mertz, E. T., Seegers, W. H., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 604.

3 Ferguson, J. H., and Erickson, B. Nims, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 625.

4 Ferguson, J. H., and Erickson, B. Nims, *Am. J. Physiol.*, 1939, **126**, 661.

bin[5] of major importance in natural thrombin destruction. It is present, to a varying extent, in serum and in most prothrombin and thrombin preparations. There is doubt whether such a factor was ruled out in the Iowa experiments. It should be pointed out that Smith, *et al.,* use extremely potent thrombic agents and assay them by a dilution technic which yields values up to several thousands. In our experience, strong thrombins (and prothrombins) *appear* unusually stable but turn out to be much less so when diluted to, say, about 5-25 Iowa units. In Table I of another Iowa communication,[6] there is a 9% loss of activity of thrombin in the presence of prothrombin as compared with a much more startling 95% fall in the prothrombin titer during the same 4-hour period.

Reagents and Methods. Our routine prothrombins,[7] when maximally activated with $CaCl_2$ and brain thromboplastin, usually clot a test fibrinogen in some 5-20+ seconds (temp. = 37.5°C, pH = 7.5). A typical prothrombin solution, assayed through the courtesy of Dr. H. P. Smith (Iowa) gave 47 units per cc (38 units per mg N). Persisting in our unwillingness to use any system of thrombin "units" for comparing one preparation with another, without convincing proof of control of *instability factors,* we restrict the assay to a single prothrombin preparation and the thrombins freshly prepared from it. Under these narrowed conditions, thrombin concentrations are measured, with a 5-10% accuracy, in a relative manner from the clotting-times obtained with a series of dilutions tested under strictly comparable conditions. In favorable cases, there is close approximation to the "inverse law,"[5] *viz.,* thrombin concentration varies inversely as the clotting-time. Since there is often difficulty in establishing the simple linear relation, it is more practicable to read the thrombin strengths directly off a *dilution curve* plotted graphically, as in Fig. 1 (Curve 0).

In the current experiments, typical of several such series, the prothrombin solution gave a thrombin which may be regarded as relatively stable, since it suffered only a 15-20% deterioration in 4 hours (*cf.* supra[6]) and some 50-60% loss in 24 hours (at 10°C).

We have observed increased thrombin formation on adding prothrombin to thrombin solutions, and it seemed reasonable to attribute this to the action of excess Ca and thromboplastin in the thrombin preparation. Hence the prothrombin solution was heavily citrated with one-tenth volume of M/1 trisodium citrate. This necessitated

[5] Glazko, A. J., and Ferguson, J. H., *J. Gen. Physiol.,* 1940, **24**, 169.

[6] Smith, H. P., *Am. Assn. Adv. Sci. Publ.,* 1940, **13**, 10.

[7] Ferguson, J. H., *J. Lab. Clin. Med.,* 1938, **24**, 273.

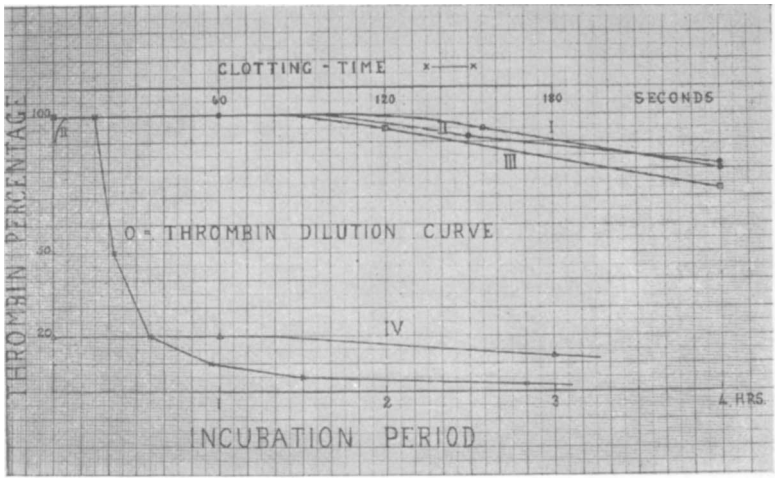

FIG. 1.

Thrombin content (relative) of aging thrombin and prothrombin mixtures. 10°C; pH 7.5.

a stronger $CaCl_2$ (N/2 instead of the usual N/10) for subsequent reactivation. There was no thrombin increase (curve IV) in the citrated mixture. Our confirmation of this Iowa finding lends further support to the view that prothrombin is *not* activated by thrombin and denies the so-called "autocatalytic" process, variously reported in the coagulation literature.

Taking the maximum thrombic potency (C.T. = 15 sec.) of full strength thrombin as 100% on our dilution scale (curve 0), 80% (four-fifths volume) of prothrombin solution, citrated as noted above, was mixed with 20% (one-fifth volume) of stable (75 min.) thrombin.

Data. Curve I is the prothrombin control made, be it noted, with citrated prothrombin containing saline, $CaCl_2$, and thromboplastin in the same amounts and proportions as the ingredients of the thrombic mixture used in curve III. Samples, removed at intervals, were maximally activated with additional Ca plus thromboplastin and the clotting-times converted into thrombin percentage by reference to the dilution curve. Curve II is the thrombin control. Both controls show a minor deterioration amounting to the loss of some 15-20% of activity in the 4-hour test period. Curve IV represents the thrombin content of the untreated mixture. It maintains the theoretical (20%) level for an hour or more and gradually falls off to some 13% in 3 hours. Curve III is the total thrombin yield (prothrombin plus thrombin) on reactivating samples of the mixture

with additional $CaCl_2$ and thromboplastin. The theoretical level (100%) is present at the start, and the fall to 95% in 2½ hours and 75% in 4 hours is again of a minor order, quite unlike the dramatic prothrombin disappearance reported in the Iowa experiments.[6]

Discussion. It is concluded that thrombin and (citrated) prothrombin can co-exist with no more destruction of either than can readily be attributed to the natural instability of both preparations. Thrombin itself is neither an activator nor a destroyer of prothrombin. The lytic factor is an independent entity which, from previously reported data,[5] we believe to be an enzyme factor, probably serum tryptase.

11898 P

Influence of Sulfathiazole Therapy on Plasma Lipids in Pneumonia.*

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Previous investigations[1, 2] by the author have shown that during the febrile periods of acute infections such as pneumonia, there is a definite fall in the values for total and ester cholesterol, total fatty acids and phospholipids. The iodine numbers of the total fatty acids and of the phospholipid fatty acids also are lowered.

Further studies[3, 4] have revealed the interesting fact that the early administration of specific serum for pneumonia in children leads to a critical drop in the fever with rapid recovery which prevents the development of any significant changes in the levels of the plasma lipids. On the other hand, chemotherapy with sulfapyridine also causes a fall in the fever with improvement in the patient's condition but with little or no influence on the lipids of the serum which continue to undergo changes similar to those found in untreated cases of pneumonia. This latter observation raises the question as to whether

* This work was supported by a grant from the Medical Research Fund of the University of Minnesota.

1 Stoesser, A. V., and McQuarrie, Irvine, *Am. J. Dis. Child.*, 1935, **49**, 658.

2 Stoesser, A. V., *Am. J. Dis. Child.*, 1938, **56**, 1215.

3 Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 168.

4 Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 201.