Summary. 1. A method is presented for the preparation of sterile closed intestinal loops. 2. The animal does not show signs of toxemia. 3. Evidence is offered that absorption continues in the sterile loop.

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Utilization of the Labile Factor During Normal and Abnormal Coagulation of Blood.* (17911)

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The study of the extent of utilization of the different factors known to be necessary for the formation of thrombin is a fruitful technic of investigation of the clotting process. Thus, in the hands of Quick and associates, the quantitative determination of the consumption of prothrombin has given conclusive evidence of the existence of a preliminary phase in the coagulation of blood, namely the formation of thromboplastin from an inactive plasmatic precursor through the action of a platelet factor(1). It has also helped to identify the mechanism of the coagulation defect in hemophilia and thrombocytopenic purpura(1,2). The factors taking part in the formation of thrombin are not totally utilized during coagulation. Examination of the serum by one-stage technics, after the thrombin formed has been neutralized by the natural antithrombin, reveals the presence of at least 3 proteins active in promoting the coagulation of blood: (a) unconverted prothrombin; (b) unconverted labile factor (factor V, plasma Ac globulin); and (c) a new agent, capable of accelerating the conversion of prothrombin to thrombin (serum Ac globulin, factor VI, spca, etc.). The concentration of these 3 factors varies according to the normality or abnormality of the coagulation process.

The present paper introduces data on the utilization of the labile factor during coagulation in healthy subjects and in patients with different disorders of the clotting mechanism. The relationship of the utilization of the labile factor to the consumption of prothrombin and to the development of the accelerator in serum has also been investigated, since previous findings have suggested that the accelerator may derive from the labile factor through the action of thrombin(3).

Materials and methods. A 20-gauge needle on a glass syringe was neatly inserted into a vein and a few ml of blood were drawn. The glass syringe was then exchanged for a second one coated with Silicone and chilled in ice prior to use. Four ml of blood, apparently uncontaminated with tissue thromboplastin, were collected and divided into 2 samples of 2 ml each. The first was decalcified by the addition of 1/10 volume of sodium oxalate 0.1 M and the second allowed to clot in a glass test tube in a water bath at 37°C. Plasma was obtained from the first sample by centrifugation at 1,500 r.p.m. for 5 minutes and its labile factor activity determined by the method of Quick and Stefanini(4) as described by Stefanini(5). This consists essentially in determining the ability of the plasma under observation to reduce towards normal values the

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1. Quick, A. J., *Am. J. Med. Sc.*, 1947, v214, 272.

2. Quick, A. J., Shanberge, J. N., and Stefanini, M., *J. Lab. and Clin. Med.*, 1949, v34, 761; and *Am. J. Med. Sc.*, 1949, v217, 198.

3. Stefanini, M., and Crosby, W. H., *Fed. Proc.*, 1950, v9, 233.

TABLE I.
Consumption of Prothrombin During Coagulation and Its Relation to Utilization of Labile Factor and Development of Accelerator in Serum.*

Diagnosis	No. cases	Serum/plasma prothrombin activity ratio, ×100	Labile factor activity of plasma, %	Labile factor activity of serum, %	Serum/plasma labile factor activity ratio, ×100	Accelerator effect of serum, %
Normal	15	2.4	98	14.2	14.3	89
Polycythemia vera	10	4.3	100	19.6	19.6	87
Pseudohemophilia	4	5.1	100	11.3	11.3	91
Hemophilia	5	84.3	94.8	68.5	72.5	12
Thrombocytopenic purpura	7	75.0	97.0	74.2	75.9	15
Symptomatic thrombocytopenia	21	81.4	74.0	63.0	84.6	9
Dicumarol hypoprothrombinemia	10	11.4	92.6	45.4	49.2	24
Cirrhosis of liver	5	42.8	51.4	29.7	58.0	39

* Prothrombin activity, labile factor activity and accelerator were determined in serum one hour after the completion of coagulation.

prolonged prothrombin time of stored human oxalated plasma(4). The clotted sample was centrifuged at 1,500 r.p.m. for 2 minutes, one hour after the completion of coagulation and the serum was separated and treated with the addition of 1/10 volume of sodium oxalate solution 0.1 M and then absorbed with $Ca_3(PO_4)_2$ gel 0.008 M. This treatment was found necessary to neutralize the thrombin still present in the serum and to adsorb all prothrombin and accelerator agent from the serum. The removal of the latter substance was found to be erratic and incomplete if the serum was not oxalated prior to adsorption. The labile factor of the supernatant serum was then determined with the previously described method(5). The concentration of serum accelerator was determined with the one-stage method of deVries *et al.*(6); the plasma prothrombin activity with the method of Quick(7); the prothrombin activity of the serum with the method recently described, in which correction is made for the accelerator effect of serum(8). In this paper we give the ratio between prothrombin activity of serum and prothrombin activity of plasma, which is a more correct indication of the consumption of prothrombin during coagulation than the original values. The ratio was calculated by dividing the values of the serum and plasma prothrombin activities and multiplying the result × 100 (8). Healthy subjects and patients with different disorders of the hemostatic mechanism were investigated. The number of individuals studied for each condition is indicated in Table I. Cases of polycythemia vera and pseudohemophilia were included in this series to show the relation of the utilization of the labile factor to the utilization of prothrombin and development of accelerator in serum in conditions in which the bleeding tendency is not due to demonstrable deficiency of the coagulation mechanism.

Results and discussion. The findings are summarized in Table I. About 85% of the labile factor was utilized during coagulation in healthy subjects and smaller amounts in those presenting various pathological conditions. The values of the serum/plasma prothrombin activity and the serum/plasma labile factor activity ratio ran a fairly parallel course, indicating that the percentage of labile factor utilized during the coagulation is roughly proportional to that of prothrombin consumed. On the other hand, the labile factor activity of serum was in definite inverse proportion to the concentration of the accelerator developed in serum. These relationships were well in evidence in all cases

4. Quick, A. J., Stefanini, M., *J. Lab. and Clin. Med.*, 1948, v33, 819.

5. Stefanini, M., *Am. J. Clin. Path.*, 1950, v20, 233.

6. de Vries, A., Alexander, B., and Goldstein, R., *Blood*, 1949, v4, 247.

7. Quick, A. J., *Am. J. Clin. Path.*, 1945, v15, 560.

8. Stefanini, M., and Crosby, W. H., *Blood*, 1950, in press.

studied. In patients with a normal coagulation mechanism we constantly found a low prothrombin and low labile factor activity but a high accelerator effect in serum. These findings were reversed for patients in which the coagulation mechanism was defective because of deficiency of thromboplastin, prothrombin or labile factor. Thus, high serum prothrombin and serum labile factor activity but low serum accelerator were found in patients with: (a) hemophilia or thrombocytopenia, where very little thromboplastin was available(2); and (b) liver dysfunction, where deficiency of prothrombin and labile factor were associated. Defective formation of thrombin suggests itself as a common denominator (see later). A special condition was represented by the hypoprothrombinemia due to dicumarol therapy. The little amount of prothrombin available was well utilized, as labile factor and thromboplastin were normal, but a comparatively small amount of labile factor appeared consumed and little accelerator effect developed in serum.

The observations that: (a) the amount of labile factor utilized during coagulation is directly proportional to that of prothrombin; and (b) little labile factor is utilized during coagulation when either prothrombin or thromboplastin are deficient, clearly suggest that prothrombin, thromboplastin and labile factor react among themselves to form thrombin in definite quantitative proportions. These findings complement the previous report by Quick and Stefanini(9) that, in the presence of excess thromboplastin and an optimum amount of calcium, the addition of increasing quantities of labile factor (in the form of diluted deprothrombinized rabbit plasma) to stored human oxalated plasma induced a progressively increasing consumption of prothrombin.

Less clear is the significance of the inverse relationship between utilization of prothrombin and labile factor during coagulation and development of serum accelerator. It has been previously reported that the latter agent derives from the labile factor through the action of thrombin(3). It may then be suggested that little accelerator is formed whenever the formation of thrombin is slow and incomplete. This interpretation is confirmed by the findings in dicumarol hypoprothrombinemia. In this case the utilization during clotting of the limited amount of prothrombin available is high, but, as only a little thrombin can be formed, a high percentage of labile factor will be left unconverted and little accelerator found in the serum.

Summary. The percentage of labile factor and prothrombin activities and accelerator effect found in serum after completion of the coagulation of blood are in inverse quantitative proportions. A very small percentage of labile factor and prothrombin activity can be found in sera of healthy subjects; in this case high values of accelerator are the rule. Low values of accelerator and a large percentage of prothrombin and labile factor activity are found whenever the formation of thrombin is deficient because of the depletion of either thromboplastin (hemophilia, thrombocytopenic purpura) or prothrombin and labile factor (liver dysfunction). In dicumarol hypoprothrombinemia the available prothrombin is well utilized during coagulation, but the percentage in serum of unconverted labile factor is high and that of the accelerator low.

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9. Quick, A. J., and Stefanini, M., *Am. J. Physiol.*, 1950, v160, 572.