Nous tirons de ces expériences les conclusions suivantes:

1° Certaines fractions protidiques d'un sérum normal sont susceptibles de donner une réaction au thymol positive (contrairement aux observations faites par MACLAGAN).

2° Les α- et β-globulines riches en lipides peuvent entrer en réaction.

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Summary

The fractions of human plasma obtained by COHN'S method (ethanol-water mixtures-low salt-low temperature) have allowed of establishing the following facts:—

(1) Certain proteinic fractions of a normal blood serum are able to give a positive thymol test (contrary to the observations made by MACLAGAN).

(2) The α- and β-globulins which are rich in lipids are also able to react positively with thymol.

The Speed of Consumption of Prothrombin and of Inactivation of Thrombin in Human Native Plasma

The effectiveness of the coagulation of blood is directly related to the speed and completeness of the formation of thrombin. Detecting the percentage of prothrombin still present in serum at different intervals of time, the prothrombin consumption test, recently introduced by QUICK[1], is a reliable index of the sufficiency of formation of thrombin. Theoretical aspects, technique and clinical and experimental applications of the test have been described in a previous paper[2] to which reference is made for full details. This communication will discuss briefly: (a) the speed and extent of consumption of prothrombin during and after coagulation; (b) the influence of the formation of active thrombin during the clotting process on the results of the test. Moreover, interesting information on the speed of inactivation of thrombin in human serum has been obtained in the course of this work and is also presented.

Unlike in blood, in plasma the end point of coagulation can be detected with accuracy[3]. Since it was essential to the reliability of the results of this study to determine exactly the completion of clotting, plasma was used in all experiments.

Experimental Techniques.—Needles, syringes, and centrifuge tubes were coated with silicone[4]. Syringes were chilled before use. Venous blood was drawn from normal subjects without addition of anticoagulants. The first ml was extracted in an ordinary syringe and discarded; the silicone-coated syringe was then substituted and the required volume of blood obtained. Blood was immediately transferred to centrifuge tubes kept on ice. The various samples of "native blood" were centrifuged at different speeds and different periods of time, to study the effect of these variations on the prothrombin consumption test. The plasma was then transferred to "Pyrex" tubes of the uniform internal diameter of 11 mm, kept in water bath at 37°C, and allowed to coagulate; in these experimental conditions it usually clotted from the air-plasma interface downwards, at a speed directly proportional to the number of platelets present. Coagulation was considered completed when the plasma appeared uniformly opaque. From this moment, the prothrombin left in serum and the residual thrombin activity were determined at regular intervals, as specified in the figures accompanying the article.

The prothrombin consumption of plasma was measured with the modification of the original method of QUICK[1] currently employed in this laboratory[3]. The thrombin activity of serum was determined at regular intervals starting from the moment of completion of clotting, until it reached negligible values. The test was carried out in water bath at 37°C. The thrombin activity was expressed as the clotting time of a mixture of 0·1 ml of the serum under investigation and 0·2 ml of deprothrombinized plasma (as source of fibrinogen). It is obvious that the curve which expresses the various values obtained in function of time indicates the speed of inactivation of thrombin in human serum. "Thrombin neutralization curves" were calculated for plasmas obtained with centrifugation of varying duration and speed.

Results and discussion.—The direct relationship between the number of platelets and the speed of prothrombin consumption in native human plasma is indicated in Fig. 1. In samples of plasma obtained by centrifugation at low speed for a limited period of time the final values of prothrombin consumption are reached very promptly, with a critical ascent between 8 and 14 minutes after the completion of clotting. When, on the other end, the number of platelets is reduced by centrifuging the native blood at higher speed for longer time, maximum values of prothrombin consumption are also reached, but only slowly and considerably later. Thus has been confirmed the observation of QUICK, SHANBERGE, and STEFANINI[3] that the number of platelets influences more the speed than the final percentage of prothrombin consumed during the coagulation of plasma, which, after twenty-four hours, is essentially the same, independent of the number of platelets present. From a practical viewpoint, these

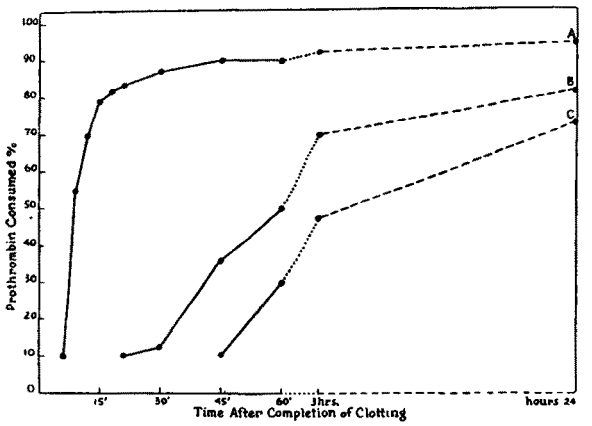

Fig. 1. – The influence of the number of platelets on the speed of the prothrombin consumption in native human plasma (average results of several experiments).

A plasma containing 300,000 platelets per mm³ (blood centrifuged at 800 r. p.m. for 5 minutes)

B plasma containing 150,000 platelets per mm³ (blood centrifuged at 2,000 r. p.m. for 5 minutes)

C plasma containing 100,000 platelets per mm³ (blood centrifuged at 2,000 r. p.m. for 10 minutes)

[1] A. J. QUICK, Amer. J. Med. Sci. *214*, 272 (1947).
[2] M. STEFANINI, Policlinico (sez. prat.) *55*, 1298 (1948).
[3] M. STEFANINI, Minerva Medica *2*, 528 (1948).
[4] Methyl-chloro-silone (General Electric Dri Film 9987).

[1] A. J. QUICK, Amer. J. Med. Sci. *214*, 272 (1947).
[2] M. STEFANINI, Policlinico (sez. prat.) *55*, 1298 (1948). – A. J. QUICK, J. N. SHANBERGE, and M. STEFANINI, Amer. J. Med. Sci. *217*, 198 (1949).
[3] A.J. QUICK, J. N. SHANBERGE, and M. STEFANINI, Amer. J. Med. Sci. *217*, 198 (1949).

results suggest that, in order to obtain correct information on the completeness of the conversion of prothrombin to thrombin as an indication of the effectiveness of the clotting mechanism, the prothrombin consumption should be determined thirty and sixty minutes after the completion of coagulation.

Fig. 2 shows the speed of neutralization in serum of thrombin formed during the coagulation of plasma. About 15 minutes after the completion of clotting, the thrombin activity of serum appears negligible. Speed

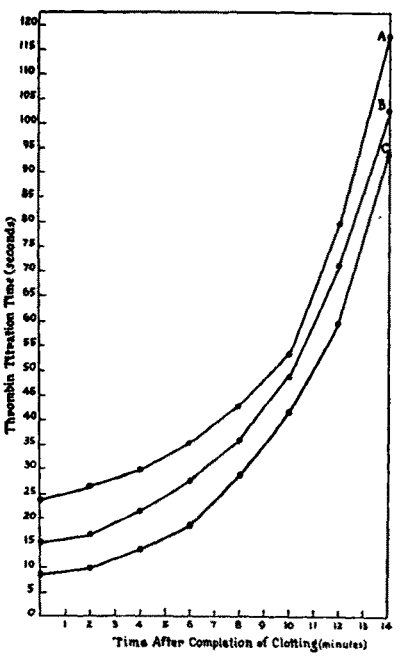

Fig. 2. – The rate of inactivation in serum of thrombin formed during the clotting of fresh human native plasma ("Thrombin inactivation curve"). (Average results of several experiments.)
A plasma from blood centrifuged at 2,000 r. p.m. for 10 minutes
B plasma from blood centrifuged at 2,000 r. p.m. for 5 minutes
C plasma from blood centrifuged at 800 r. p.m. for 5 minutes

and duration of centrifugation of plasma do not influence remarkably the velocity of neutralization of thrombin (*"thrombin neutralization curve"*). It can be seen, however, that the concentration of thrombin which can be determined at the completion of clotting is lower when the plasma has been subjected to progressively higher and more prolonged centrifugation. An explanation of this result is probably that, with a lesser number of platelets, the activation of thromboplastinogen is incomplete and so subsequently will be the conversion of prothrombin to thrombin. Furthermore, it should not be forgotten that in relatively platelet-free plasma the coagulation process proceeds slowly and is completed in the upper part of the test tube much sooner than in the lower part. It is then clear that, when the clotting is considered complete (uniform opacity of the plasma), part of the thrombin formed has already been neutralized.

Findings not presented here for considerations of space indicate that, even during the period immediately following coagulation, the thrombin present influences very limitedly the results of the prothrombin consumption test. The results presented in Fig. 2 show that fifteen minutes after the completion of clotting the thrombin formed has been completely neutralized and, from that time forward, will not influence the results of the test.

Preliminary experiments have indicated that the "thrombin neutralization curve" shows definite delay in patients with thrombotic tendency. This observation is particularly promising as no reliable diagnostic tests are available today for the diagnosis of states of hypercoagulability, but more work is required to determine wheter significant and constant variations from the normal of the "thrombin neutralization curve" may be found in these pathological conditions.

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Zusammenfassung

Mit neuartigen Methoden wurde die Geschwindigkeit untersucht, mit der das Prothrombin verbraucht und das Thrombin nach Beendigung der Gerinnung frischen menschlichen Plasmas inaktiviert wird. Die Geschwindigkeit des «Prothrombinverbrauchs» hängt von der Zahl der vorhandenen Blutplättchen ab. Sie wächst kritisch bei optimaler Blutplättchenzahl zwischen der 8. und 14. Minute nach Beendigung der Gerinnung.

Die Inaktivierung des während der Gerinnung gebildeten Thrombins wird nur sehr wenig von den im Plasma verbliebenen Plättchen beeinflußt. Sie geht rasch in den ersten 15 Minuten nach Beendigung der Gerinnung vor sich. Bei krankhaften Zuständen zeigt die Thrombininaktivierungskurve Veränderungen, die vielleicht diagnostisch bei der thrombotischen Diathese nützlich sein könnten.

Die Resultate des Prothrombinverbrauchtests werden durch das restliche Thrombin des Serums nicht beeinflußt, wenn die Bestimmung 30 bis 60 Minuten nach Beendigung der Gerinnung vorgenommen wird.

Influence de la folliculine sur le métabolisme calcique du pigeon étudiée à l'aide du radiocalcium

Une série de recherches exécutées à l'aide du radiocalcium[1] chez le pigeon en repos sexuel, en activité ovarienne spontanée ou en activité provoquée nous a permis de confirmer ou d'établir les faits suivants:

1° L'excrétion du calcium injecté au pigeon en repos sexuel suit une courbe absolument régulière contrastant avec les écarts journaliers de l'élimination entéro-rénale du calcium total. La première ne dépend en effet que du métabolisme calcique tissulaire, tandis que la deuxième est soumise à des variations d'ordre alimentaire ou digestif.

2° L'excrétion du calcium total chez le pigeon soumis à la folliculine atteint un minimum au 17e jour d'administration de l'hormone; par contre, celle-ci provoque la rétention maximum du radiocalcium injecté vers le 7e jour. La folliculine exercerait donc sur la résorption intestinale du calcium une action indépendante de son influence sur le métabolisme tissulaire de ce dernier.

3° La cessation des injections de folliculine provoque une décharge calcique dans les excrétats: celle-ci dépend d'un arrêt de la résorption intestinale autant que d'une

[1] Le radiocalcium nous a été fourni par la Commission américaine de l'énergie atomique.