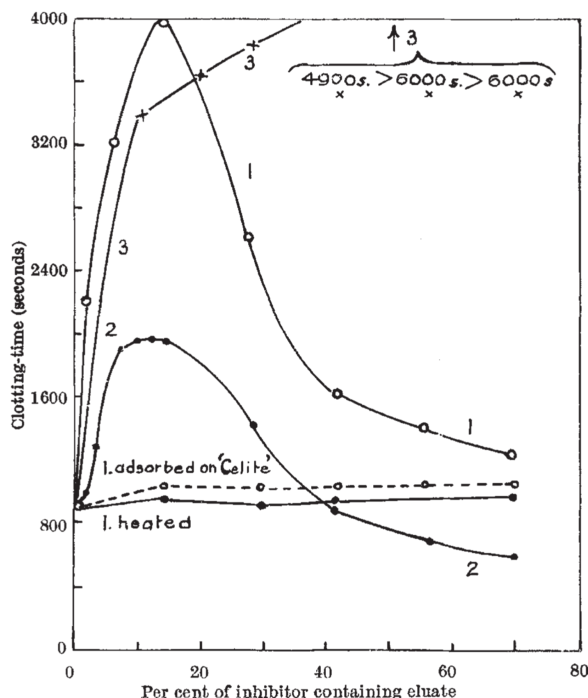
A Thermolabile Inhibitor of Plasma Coagulation

BARIUM carbonate, used as an adsorbent for stable (platelet-free), oxalated, horse plasma (shaking with 10 mgm./ml. for 30 min. at 0° C.) removes prothrombin with an undialysable, thermolabile substance, destroyed by heating to 60° C. for 30 min. This substance, apparently an albumin, when added to non-activated plasma (without addition of brain thromboplastin), acts as an inhibitor of plasma coagulation.

This natural inhibitor of plasma coagulation can easily be shown to exist in the following way: the adsorbent is washed with saline, dissolved with slight excess of acetic acid (10 per cent) and dialysed in cellulose tubing against normal saline at 5° C. for 48 hr. An excess of acid over barium carbonate is necessary, and the *p*H should be below 3·0 after completely dissolving the carbonate. Prothrombin activity is then destroyed by acidity during dialysis, although some accelerator may remain. The dialysed, dissolved, eluate is isotonic, has a final *p*H of 5·5–5·7 and does not contain barium. The resultant solution (for example, 5·0 ml. from the original 50 ml. of plasma) is tested without thromboplastin in regard to its clot-inhibiting activity against a given amount of horse plasma. Calcium added to the system is dissolved in imidazole buffer[1] and corrects the *p*H to 7·1. All glass used in work with plasma and inhibitor must be coated with organic silica[2]. The accompanying curves illustrate the action of two different crude products each with a diphasic curve of activity.



Action of crude inhibitor preparation from dissolved and dialysed barium carbonate on the coagulation of citrated horse plasma.
1. Inhibitor added in increasing amounts (0·01–0·5) to 0·1 ml. citrated horse plasma; 0·1 of 0·02 *M* calcium chloride, *p*H 7·1. Total volume 0·7 ml. 'Dilution effect' of inhibitor.
When heated to 60° C. for 30 min., the inhibiting effect completely disappears. Effect disappears also when inhibitor is adsorbed on 'Celite' (100 mgm./ml., 30 min., 0° C.).

2. Another inhibitor preparation tested on the same plasma. Presence of accelerator evident in higher concentration of inhibitor preparation.

3. Inhibitor from curve 1, after partial purification (without euglobulins). Accelerator removed from inhibitor preparation. System without thromboplastin. All tests were carried out in siliconized vessels at 38° C.

After a partial purification, the inhibitor loses its 'diphasic' character (see graph). Clotting is progressively prolonged as greater amounts of the inhibitor are added. An assay of the partially purified inhibitor shows that 1 ml. of plasma contains less than 2 γ of active protein. The inhibitor can be adsorbed on negatively charged adsorbents, for example, 'Celite' ('Hyflo Super Cel', Johns Manville), kaolin, powdered glass, etc. The inhibitory effect disappears after such adsorption. The same adsorbents shorten the clotting-time of stable plasma (shaking with 10–100 mgm./ml., 30 min., 0° C.) and prothrombin in large amounts can be eluted from them. The acceleration of the clotting time despite loss of adsorbed prothrombin is explained by the excess of free prothrombin in solution as a result of inhibitor being stripped off and held by the adsorbent while free prothrombin comes into solution.

The effects of surface contact, known to accelerate the coagulation of plasma, cannot be detected after adsorption on barium sulphate. The 'dilution effect' of plasma (shortening of the clotting time upon dilution with water or saline) is also absent in a barium-sulphate adsorbed plasma. This can be demonstrated when such a plasma is added in varying amounts to a constant amount of normal plasma, the volume being kept constant.

These experiments show that this inhibitor, while in a complex with prothrombin or a substance related to prothrombin, helps to maintain the fluidity of blood *in vivo*. The agent is completely different from the thermostable 'lipid antithromboplastin' of Tocantins[3] prepared from brain, and it is also different from the pathological globulin of Munro[4] which occurs in certain cases of hæmophilia.

Experiments in an attempt to purify further the inhibitor are in progress. A discussion of the mechanism of prothrombin liberation in the first stage of blood coagulation will be published shortly[5].

SILVIO FIALA

Cancer Research Laboratory,
Pennsylvania Hospital,
Philadelphia 7, Pa.
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[3] Tocantins, L., and Carroll, R. T., Macy Found. II Conf. on Blood Clotting, **2**, 11 (1949).

[4] Munro, F. L., *J. Clin. Invest.*, **25**, 422 (1946).

[5] Fiala, S., *Arch. Internat. Physiol.* (in the press).

New Inhibitors of the First Stage of the Blood-clotting Process

IN the course of a study of the non-protein activator(s)[1] of blood coagulation, which always occur(s) in the cephalin fraction of the phospholipids, without being identical with the classical cephalins, two inhibitors of the first stage of the clotting process, namely, glutamic acid and sphingosine, were discovered by means of paper chromatography. Both compounds are completely ineffective in the second stage of the clotting process.

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