

## A Prothrombin Conversion Accelerator in Serum<sup>1</sup>

Benjamin Alexander, Andre de Vries,<sup>2</sup> Robert Goldstein, and Greta Landwehr

*Medical Research Laboratory, Beth Israel Hospital and the Department of Medicine, Harvard Medical School*

Recently substances have been described in serum which activate or accelerate the conversion of prothrombin to thrombin by thromboplastin plus ionized calcium (8, 5). These observations are of fundamental importance, since they help explain the autocatalytic process underlying the formation of thrombin.

We have found that human serum contains a substance which arises during blood coagulation and which is capable of accelerating the conversion of prothrombin to thrombin. This agent, distinct from thromboplastin or thrombin, can be separated from prothrombin-free serum and has been purified to the extent that all of the accelerating activity is contained in 20 mg of protein from 100 cc of serum. The substance is measured by its ability to accelerate the velocity of prothrombin conversion to thrombin when added to fresh normal plasma, the mixture then being suitably diluted with prothrombin-free plasma (6). The prothrombin time of the mixture is determined by the one-stage procedure and its prothrombic activity computed from a standardization curve obtained on normal plasma.

Some of the biochemical and physiological properties of the serum prothrombin conversion accelerator (spca) have been delineated: 1) It can be quantitatively adsorbed from serum by BaSO<sub>4</sub> or BaCO<sub>3</sub>. 2) It can be eluted from the BaSO<sub>4</sub> by solutions of sodium citrate. 3) It is non-dialyzable. 4) It is removed from serum by Seitz filtration. 5) It is not precipitated from diluted serum at pH 5.8. 6) It is destroyed in serum at 56° C in 2 min. 7) It is stable in serum at 4–5° C for at least 3 days, in purified fractions for at least 9 days, but is less stable in media which are free of electrolytes. 8) In serum it is destroyed below pH 5 and above pH 9. 9) It can accelerate the coagulation of normal or hemophilic blood. 10) Its effect on prothrombin conversion is not obviated by moderate amounts of heparin capable of retarding coagulation. 11) The amount of spca evolved during blood clotting is related to the amount of prothrombin consumed in the process. 12) It is increased by mechanical agitation of, or thromboplastin supplements to, clotting blood. 13) It is decreased by removal of platelets or by allowing blood to clot in siliconized tubes. 14) Preparations of spca obtained by BaSO<sub>4</sub> adsorption and elution with citrate have an ultraviolet absorption spectrum which is indistinguishable from prothrombin fractions obtained in the same manner from fresh human plasma.

<sup>1</sup> Supported by a grant from the Commonwealth Fund.

<sup>2</sup> Aided by a fellowship from the Rothschild Hadassah University Hospital, Jerusalem.

In serum from patients with idiopathic hypoprothrombinemia, or with hypoprothrombinemia induced by dicoumarol, the amount of the serum accelerator is abnormally small. Similar observations were found also in thrombocytopenia, either idiopathic or secondary to other pathological conditions; in these instances residual serum prothrombic activity was much greater than normal.

Hemophilic serum frequently shows a prothrombic activity, by the modified one-stage procedure (6) that is considerably higher than that of the parent plasma. This is probably due, at least in part, to some spca which has evolved during coagulation.

It appears that the serum prothrombin conversion accelerator, described above, is different from the serum A<sub>2</sub> globulin of Ware and Seegers, which is reported to be remarkably unstable in human serum (7). Nothing can be said regarding its identity or non-identity with factor VI of Owren (5). Detailed reports on some of these observations are in press elsewhere (1–4).

### References

1. ALEXANDER, B., and DE VRIES, A. *Blood*. In press.
2. ———. *Blood*. In press.
3. ALEXANDER, B., DE VRIES, A., and GOLDSTEIN, R. *Blood*. In press.
4. DE VRIES, A., ALEXANDER, B., and GOLDSTEIN, R. *Blood*. 1949, 4, 247.
5. OWREN, P. A. *Acta Med. Scan.*, 1947, 1, Suppl. 194.
6. ROSENFELD, R. E., and TUFT, H. S. *Amer. J. clin. Path.*, 1947, 17, 405.
7. SEEGER, W. H., and MURPHY, R. C. *Amer. J. Physiol.*, 1948, 154, 134.
8. WARE, A. G., and SEEGER, W. H. *Fed. Proc.*, 1948, 7, 131.

## A Linear Diffusion Method Suitable for Large Scale Microbiological Antibiotic Assay

W. W. Davis, T. V. Parke, and W. A. Daily

*The Lilly Research Laboratories, Indianapolis*

A diffusion method of microbiological antibiotic assay has been developed which appears to offer advantages of economy, accuracy, and concentration range of validity over Petri dish methods. This method involves linear diffusion of antibiotics into inoculated, agar-filled, glass capillaries from solutions of antibiotic in nutrient broth.<sup>1</sup>

Pyrex glass capillaries having an internal diameter of approximately 1 mm and length of 3½ in. are ground flat on one end. These are partially filled with melted agar seeded with test organisms. These capillaries are filled in an automatic device which molds the end of the agar column flat and flush in the end of the capillary. This flat end of the agar column serves as the initial boundary

<sup>1</sup> Our attention has recently been directed to a paper—Torii, Toshio, Yasuo Kawakami, and Hiro Kozima. *Journal of Penicillin (Japan)* 1947, 1, No. 5, 281—describing the use of a system of linear diffusion for assay of antibiotics using test tubes partially filled with agar overlaid with a broth solution of the antibiotic.