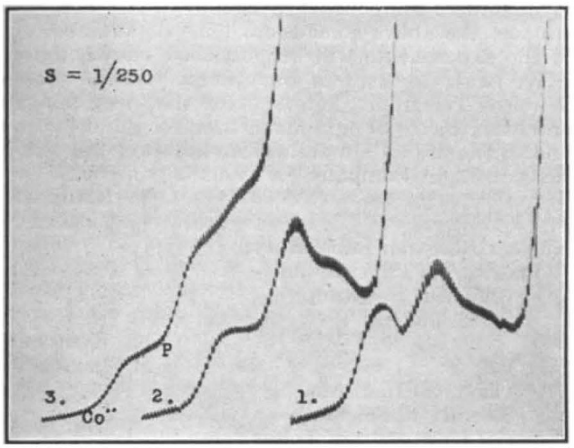
carefully sterilized, through membrane filters "impermeable to proteins" and we used the filtrate for the modified Fuchs reaction. It has been shown by the above-named authors that the proteolytic activity of the serum is increased when the serum is freed from proteins. A further advantage of using the serum filtrates consists in the fact that the liquid, after the proteolysis, does not need to be deproteinated. Such a deproteination in the case of serum leads to the precipitation of higher proteolytic products with proteins, whereby the effect of the proteolysis is lessened.

We put 0·5 c.c. of the ultra-filtrate into each of three sterilized test-tubes. The first sample served as a control; to the second sample 5 mgm. of fibrin prepared from the blood of a normal man was added, and to the third one the same amount of a carcinomatic fibrin. After an incubation period at 37° C., the samples were filtered through a hard filter paper and a certain amount of the filtrates were mixed with an equal amount of the Brdička buffered cobalt



Polarographic evidence of proteolysis in the Fuchs modified reaction.

Curve 1. Polarographic effect of the serum ultrafiltrate. Curve 2. Proteolysis of carcinomatic substrate with carcinomatic serum (slightly positive). Curve 3. Proteolysis of normal substrate with a carcinomatic serum (strongly positive). The serum was taken from a patient suffering from Ca mammæ.

solution. These solutions were then submitted to polarographic analysis and the usual current-voltage curves were recorded. It should be mentioned that the fibrin filtrate alone does not show any protein reaction.

The result of such an experiment is shown in the accompanying curves.

The first current-voltage curve, due to the ultra-filtrate in the Brdička buffered cobalt solution, proves that the membrane filter does let some small amount of protein substances, giving the Brdička protein test, pass through. Curves 2 and 3 are due to solutions of ultra-filtrates incubated with carcinomatic and normal fibrin respectively; in the test solution of ultra-filtrate where the proteolysis of fibrin took place, an appreciable 'double wave' (*P*)—the Brdička protein reaction—appears on the current voltage curve (see Curve 3), whereas in the ultra-filtrate which reacted with the corresponding substrate to a small extent only, this effect is slight (Curve 2). Thus it is evident that the proteolysis proceeds in both ultra-filtrates, but the effects differ in the height of the 'double wave' caused by the products of proteolysis. Hence the height of this 'wave' indicates objectively the degree of proteolysis in a way similar to the determination of the increase of non-protein nitrogen, the latter method being beyond comparison more difficult from the technical point of view.

From similar experiments—altogether fourteen have been carried out—it follows that the Brdička polarographic test for proteins and their decomposition products enables one to prove in a simple and exact way that proteolysis occurs in reactions of serum filtrates with substrates. A similar mode of procedure was used by Brdička and Klumpar[4] in their study of proteolytic cleavage of proteins with pepsin. We believe that these findings will lead to far-reaching applications of the polarographic effect to various reactions based on the proteolysis of specific substrates. It will be useful also for the study of different organic dysfunctions and physiological disorders.

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Colorimetric Determination of *dl*-α-Tocopherol (Vitamin E)

Investigations of Evans[1] and Karrer[2] have demonstrated that α-tocopherol possesses reducing properties, so that it can be determined by potentiometric titration with gold chloride.

Our colorimetric determination is based upon the reducing power of α-tocopherol against ferric chloride. The ferrous salt which is formed has been determined by us with α-α′ dipyridyl. The reaction is carried out in ethanol and the red colour of the ferrous-dipyridyl complex has been determined with a Zeiss-Pulfrich photometer (screen 50 and 1 cm. cell). The quantities of α-tocopherol which have been determined varied from 0·01 to 0·4 mgm.

We also have determined α-tocopherol (Hoffman – La Roche) by Karrer's potentiometric titration with gold chloride, and the two methods give results in good agreement. In both methods carotene may cause difficulties.

Details and applications will be published elsewhere.

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