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A Polarographic Study of Insulin.*

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Polarographic features of insulin were first studied by Brdicka.[1] Later, Mulli and Werner,[2] and Tropp[3] also made polarographic studies of insulin. Mulli and Werner claimed that they could determine the activity of any sample of insulin from the polarographic curves. Tropp, who made a more substantial study of the problem, maintains that it is possible to compare activities and height of polarographic steps of a given brand of insulin.

The experiments to be reported here show that the height of polarographic steps is solely a function of the SS or SH concentration of the insulin; it may be independent of its activity. This investigation of insulin is based on 4 assumptions: (1) that it is possible to determine polarographically the concentration of SS and SH groups of a protein, (2) that this concentration is approximately proportional to the protein concentration if similar proteins are concerned, (3) that the insulin solutions contained no elements that interfered with the development of the characteristic steps, and (4) that the activity of the tested solutions of insulin corresponded to the data given by the manufacturer.

The first assumption is justified if a constant fraction of the SS groups present in insulin is polarographically evident. The second assumption presupposes that all insulins contain nearly equal proportions of polarographically active SS groups.

In any given case it is possible to verify assumption three because experience has shown that substances tending to suppress the characteristic waves may be practically eliminated by appropriate dilution.

The polarograph is an instrument for automatic registration of current-voltage curves obtained by electrolysis of the investigated substance with a dropping mercury cathode and an unpolarizable mercury anode. Cations, reducible molecules, or catalytic sub-

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1 Brdicka, R., *Collection of Czechoslovak Chemical Communications*, 1933, **5**, 112.

2 Mulli and Werner, *Deutsche Med. Wochenschr.*, 1937, **63**, 1941.

3 Tropp, C., *Klin. Wochenschr.*, 1938, **17**, 465.

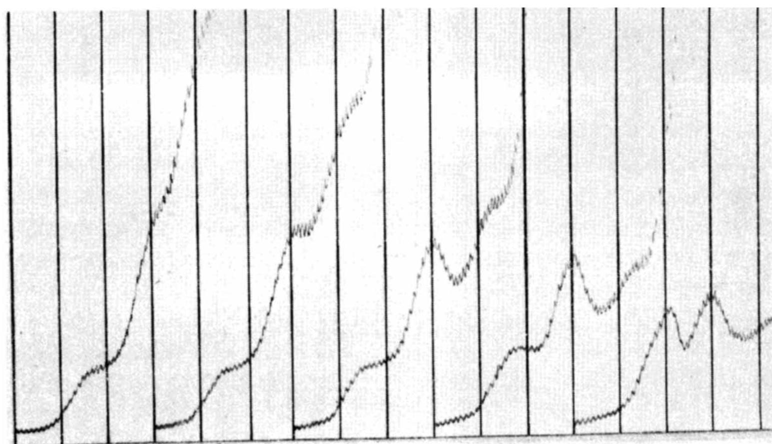

FIG. 1.
Solutions of crystalline insulin in concentrations 20 mg%, 10 mg%, 5 mg%, 2.5 mg%, 1.2 mg%.
The first step of each curve indicates cobalt, added to induce the catalytic protein steps. The second double step indicates protein. Galv. sens. 1/200.

stances are characterized by the voltage at which steps occur on the current-voltage curve. Their concentration is indicated by the height of these steps.† Confirming Brdicka, it was observed that insulin yields curves which are not distinguishable from those of other polarographically active proteins. With available methods, polarographic determination at best gives only an approximation of the protein contents of any given sample of insulin.

The insulin step, like other protein steps, is a double step characterized by 2 waves which follow each other in close succession (Fig. 1). When the height of these steps is plotted against concentration, a curve is obtained which has the shape of an adsorption isotherm. This fact agrees with polarographic theories. With decreasing concentration, the second part of the double step tends to decrease first, while the first part varies but little. On further dilution, the second part of the double step disappears completely and the first part develops a maximum, the relative height of which depends upon the dilution. Finally, in the greatest dilutions a cobalt maximum appears. This latter dilution is the minimum dilution at which, if the liquid is shaken, no foam appears.

This report deals primarily with the comparison of various samples of insulin. For the sake of comparison the measurements are based upon the height of the insulin double step of a 4.5 mg % solution of

† For theory and application of polarography, see Heyrovsky,[4] and Brdicka.[1]

[4] Heyrovsky, J., Polarography in *Physikalische Methoden der analytischen Chemie*, Vol. II, Leipzig, 1936.

TABLE I.

Brand	A	B	C	D	E	F	G	H
Height of protein wave in mm	26	27	33	23	28	27	26	26

insulin prepared by the authors from crystals supplied by various manufacturers. Eight different brands were examined. Table I indicates the results.

Four samples of crystalline insulin in solution (commercial product) were obtained from manufacturers. All of the ampules were labeled 40 U per cc. These insulin solutions were then diluted 50 times. The average values of 10 measurements were the following:

Brand A	Lot 1: 25.5 mm	Lot 2: 25.5 mm
Brand B	Lot 1: 23.5 mm	Lot 2: 23.5 mm

There is no reason to doubt that the higher step corresponds to a higher concentration of available SS groups, as all single measurements were within ±1 mm of the average. These findings tend to show that different brands of crystalline insulin may have a different chemical composition or structure.

A comparison between solutions prepared from solid insulin and the commercial solutions of the same insulin brought out the following facts: The insulin concentration of the market product was determined by a polarographic comparison with the authors' solutions. From this concentration and the known activity of the sample (40 U per cc, according to the label), the activity of one mg of the solid insulin could be calculated. Table II gives the results of this experiment.

TABLE II.

	Brand A Crystalline	Brand B Crystalline	Brand A Amorphous
(I) Sample dissolved by the authors			
Concentration of solutions prepared by the authors	4.5 mg %	4.5 mg %	4.5 mg %
Height of polarographic step (Average of 5 readings)	25.5 mm	27 mm	18 mm
(II) Insulin solution, commercial, (40 U per cc), diluted 50 times			
Height of polarographic step (Average of 5 readings)	25.5 mm	23.5 mm	27.5 mm
Concentration of solid insulin calculated from height of polarographic step	4.5 mg %	3.9 mg %	6.9 mg %
Units per 100 cc (after diluting 50 times)	80	80	80
Units per mg of solid insulin	17.8	20.5	11.6

It is a well known fact that the activity of an insulin sample can be decreased or entirely destroyed by various physical and chemical agents. One of these is heat. A comparative analysis was made of 2 different lots of insulin before and after prolonged application of heat, which kept the temperature at 52°C for 10 days. In both instances, the activity of the sample dropped to about one-half of its original value; yet the polarographic behavior did not change at all. Fig. 2 illustrates this. One of the curves was obtained with the unheated sample labeled 40 U per cc. The other curve was obtained under the same conditions and represents the heated sample. According to the manufacturer the activity of this sample had dropped from 40 U per cc to 18.1 U per cc because of the application of heat. The polarographic curves obtained with these samples, so different in physiological activity, are identical. This tends to show that, so far, polarographic methods are inadequate as a basis for the determination of the physiological activity of an insulin sample.

From the work of Abel and Geiling[5] and du Vigneaud, *et al.*,[6] it appears that the reduction of SS to SH groups affects the activity of insulin, the SS groups thus being related to the active principle

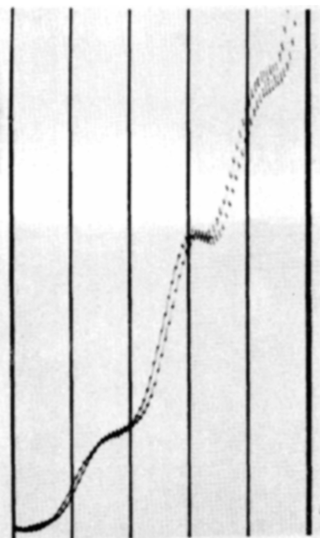

FIG. 2.
Curves of heated and unheated insulin sample. Written closely together for better comparison. First step indicates cobalt. Following double step indicates insulin protein. Galv. sens. 1/200.

[5] Abel, J. J., and Geiling, E. M. K., *J. Pharm. and Exp. Ther.*, 1925, **25**, 423.
[6] du Vigneaud, V., *J. Biol. Chem.*, 1927, **75**, 393; 1931-32, **94**, 233.

of insulin. With the polarograph, SS as well as SH groups are registered. Based upon the findings of du Vigneaud, it could be assumed that insulin contains SS groups, but no free SH groups.

It is very unlikely that the heat treatment of the insulin samples changed about half of the SS groups to SH groups, and yet the findings do not contradict the assumption that the activity of insulin is related to its SS groups. Many substances contain SS groups without any physiological activity comparable to that of insulin. Inactivated insulin may be one of them. Therefore, it appears that if the SS groups are related to the activity, their presence constitutes but one of a series of conditions which must be fulfilled in order to obtain insulin activity.

Conclusions. 1. The height of polarographic steps of an insulin solution is a function of the concentration of SS (or SH) groups; it is independent of its intrinsic physiologic activity. 2. Polarographic analysis permits the determination of the concentration of insulin solutions, for a given brand, and after calibration.

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Lack of Carcinogenic Potency of Sulfanilamide and Prontosil Soluble in Mice.

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Because of the structural resemblance of sulfanilamide and Prontosil Soluble to o-amido-azotoluol,[1] B-naphthylamine,[2] "light green FS",[3] and other benzene derivatives which have been found to be carcinogenic,[4] the question of possible carcinogenic properties of these compounds naturally arises. Lewis reported on the lack ot carcinogenic potency of sulfanilamide in mice.[5] The present experiments were in progress at that time, and may be considered confirmatory evidence for Lewis's findings.

1 Shear, M. J., *Am. J. Cancer*, 1937, **29**, 269.

2 Hueper, W. C., and Wolfe, H. D., Am. Assn. Pathol. Sci. Proc., *Am. J. Path.*, 1937, **13**, 656.

3 Schiller, W., *Am. J. Cancer*, 1937, **31**, 486.

4 Cook, J. W., and Kennaway, E. L., *Am. J. Cancer*, 1938, **33**, 50.

5 Lewis, M. R., *Am. J. Cancer*, 1938, **34**, 431.