DETECTION OF ANTI-INSULIN ANTIBODIES WITH A NEW ELECTRICAL TECHNIQUE: LIPID MEMBRANE CONDUCTOMETRY[1]

E. Toro-Goyco, A. Rodriguez and J. del Castillo
Departments of Biochemistry and Nutrition and Pharmacology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico 00905

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A new technique (lipid membrane conductometry) based on the impedance changes occurring in thin phospholipid films when antigen--antibody or enzyme-substrate complexes are formed at an aqueous solution in contact with the lipid, has been used for the detection of anti-insulin antibodies in sera from insulin-treated diabetics. The presence of insulinase in sera from cirrhotic patients was also suggested by these experiments.

It has been recently shown in this laboratory (del Castillo et al., 1966) that the transverse electrical impedance of lipid films separating two aqueous phases decreases markedly when immunological reactions take place in one of the latter. Whereas the separate addition of antibodies and antigenic proteins has no appreciable effect, the successive injection of immunologically complementary proteins is followed by sudden reduction in the impedance of the lipid phase. An identical effect is observed when the addition of an activated enzyme is followed by the injection of its corresponding substrate or substrates into the same aqueous phase.

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Leaving aside the problems posed by the molecular mechanisms responsible for such conductance changes and the biological analogies that they may suggest, one should think that these effects might have an empirical application for the instantaneous recording of interactions between antigens and antibodies. Its usefulness could be very significant to study antigen-antibody reactions leading to the formation of soluble complexes which elude detection by routine immunochemical techniques.

An attempt to test the potential value of conductivity measurements in lipid membranes for immunological work has been done by investigating with this technique the reaction between insulin and the sera of insulin-treated patients.

Treatment with insulin elicits, usually, the formation of specific antibodies (Tuft, 1928) which form soluble complexes with this hormone. For this reason, their detection involves sophisticated techniques such as paper electrophoresis of labelled antigen-antibody complex (Berson et al., 1956) or restricted diffusion chromatography in Sephadex G-200 gel columns (Toro-Goyco et al., 1965, 1966). Although quantitative, both techniques are laborious and time consuming.

Materials and methods

To test the reliability of our electrical technique, a total of 45 serum samples were used. Of these, 30 were from diabetic patients who had been treated with insulin. The presence of insulin antibodies in their sera had been determined by the column chromatography technique (Toro-Goyco et al., 1965, 1966). Three were from diabetics who had never received insulin and 12 from individuals with no indication of diabetes and no previous exposure to that hormone were used as controls. The procedure used for the preparation of lipid extracts, the formation of membranes and the experimental set up have already been described (del Castillo et al., 1966). Commercial beef insulin

(Squibb, 40 units/cc) which had been prepared from crystallized zinc insulin was used as antigen.

Results and discussion

The results of these experiments are illustrated on Table I. They show a very good discrimination between the sera from insulin-treated diabetics and those treated with oral hypoglycemic agents.

Table I

	Number studied	Chromatographic technique (+)	L.m.c.t* (+)
Controls	12	0	0
Insulin-treated diabetics	30	30	30
Diabetics never treated with insulin	3	0	0

*L.m.c.t. = lipid membrane conductivity test.

The changes in the impedance of the lipid membrane seem to follow the changes in the concentration of insulin at the lipid-water interface, showing a sudden rise as the droplet is injected into the solution and a slower decay. This effect is illustrated in Figure 1 which shows the effect of insulin on the impedance of a membrane exposed to the 7S fraction of the serum of an insulin treated patient (see details in legend). Since the time course of such concentration changes are determined mainly by mixing and diffusion factors, this technique, at least in its present form, can only be used as a purely qualitative test.

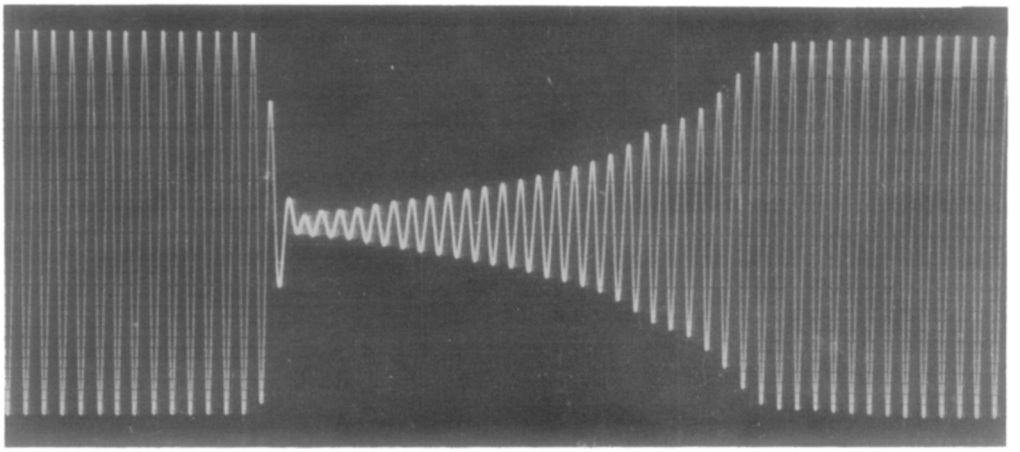

Figure 1.- Transient reduction of the impedance (to a 200 c/s sinusoidal current) of a lipid membrane exposed to the 7S fraction of the serum of an insulin-treated patient (0.14 mg/ml) following the injection of 10 µl of regular insulin solution (Squibb, 40 units/ml) at the point marked by a manually operated signal (arrow). This response was followed by desensitization of the membrane to further application of insulin.

It must be emphasized that a reduction in the impedance of a lipid film, such as that illustrated in Figure 1, can be caused by the formation of either of two types of complexes at the lipid-water interface: antigen-antibody and enzyme-substrate. Due to the irreversibility of the antigen-antibody combination, impedance changes take place only once or twice following the repeated addition of antigen to a system containing antibody. Yet, when small amounts of a substrate are added to enzyme-treated membranes, many successive responses can be shown.

In the course of the tests described above, we found that a membrane treated with a control serum gave multiple successive reactions to the repeated addition of insulin droplets (see Fig. 2).

A search of the records showed that the serum came from a patient suffering from liver cirrhosis. Hence, there was the possibility that insulinase, instead of anti-insulin antibodies, was responsible for such effect; i.e. that we were detecting the formation of an enzyme--substrate (insulin-insulinase) complex rather than an immunological

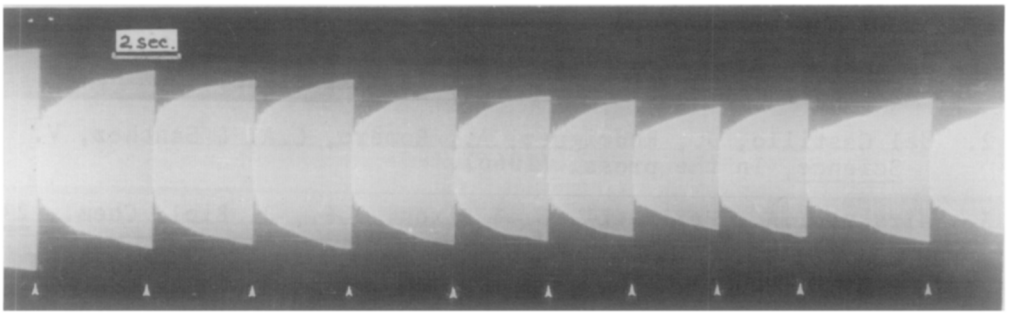

Figure 2.- Multiple successive reductions in the impedance (to a 200 c/s a.c.) of a lipid membrane treated with the serum of a cirrhotic patient at a concentration of 1/70 (v/v) upon repeated addition of insulin, 10 µl droplets (Squibb, 40 units/ml). Time calibration, 2 sec.

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reaction. Indeed, the presence of insulinase in the human liver has been previously reported (Mirsky, Perisutti and Dixon, 1955; Mirsky, 1959), and damage to hepatic tissue may lead to its leakage to the blood, as it is the case with other enzymes contained in the liver cells.

Subsequent tests have shown that the sera from each one of 14 cirrhotic patients gave such multiple positive reactions. The marked difference in the patterns between the antigen-antibody and enzyme--substrate reaction leave no ground for confusion between diabetic and cirrhotic sera.

The rapidity and simplicity of this screening method for the detection of insulin antibodies promises to be very useful for routine clinical laboratory work.

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