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AN IMMUNOSENSOR FOR SYPHILIS

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An immuno responsive membrane was prepared by immobilizing Wasserman antigen to acetylcellulose. The antigen-binding membrane was used in developing an immunosensor for determining specifically corresponding antibody in serum. The senor is characterized by electrochemical determination of the antigen–antibody complex formed at the membrane–solution interface. Experimental data on the applicability of the sensor to serology tests for syphilis are given as well as an outline of a possible mechanism for evaluating the electrical potential difference across the antigen-binding membrane.

INTRODUCTION

It has been shown that immobilized enzymes may be used as effective tools for measuring the concentration of specific substances. There have been great advances in enzyme electrodes, in which immobilized enzymes are coupled as functional parts to electrochemical devices (1–7). Enzyme electrodes have already been evaluated in some clinical laboratories for use in measuring important body fluid constituents, such as blood glucose, to aid medical diagnosis.

We have developed an immunoresponsive membrane, to which an antigen is attached (8). The antigen-bound membrane generates a transmembrane potential in association with the immunochemical reaction with free antibody in a solution. In the present work, the antigen-bound membrane was utilized in the development of a new sensoring system for a specific protein.

If an antigen is immobilized to a membrane, and corresponding free antibody is present in a solution, the immunochemical reaction (the antigen–antibody complex formation) will take place at the membrane–solution

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interface with a resulting change of the surface charge. Since the potential difference across the membrane is dependent on the surface charge, the antigen–antibody complex formation can be directly followed by measuring the membrane potential.

In order to test this idea, we have developed an immunosensor for syphilis. Most nontreponemal serology tests for syphilis, such as the Venereal Disease Research Laboratory (VDRL), Unheated Serum Reagin (USR), and Rapid Plasma Reagin (RPR) tests (9), have been based on microagglutination of cardiolipin particles, which react with the Wasserman antibody, forming a strong antigen–antibody complex. In the present investigation cardiolipin was immobilized to an acetylcellulose membrane, and the membrane-bound cardiolipin was used in developing the immunosensor for syphilis.

MATERIALS AND METHODS

Materials

Wasserman antigen for the nontreponemal tests for syphilis was obtained from Sumitomo Chemicals Co. (Osaka). The antigen contained 0.01% cardiolipin, 0.04% phosphatidyl choline, and 0.20% cholesterol in ethanol. Moni-Trol IX Serum and Positive Control Serum for quality control of nontreponemal tests for syphilis, supplied by the DADE Division, American Hospital Supply Co. (Miami, Fla.), were used as negative and positive sera, respectively.

Preparation of Membrane-Bound Antigen

Acetylcellulose (250 mg) was dissolved in 6 ml of acetone. One milliliter of the antigen was homogeneously mixed with the solution by stirring. The resulting solution was cast on a glass plate (18×10 cm^2). The cat membrane was left at 25°C under reduced pressure to dry. The membrane, whose thickness was about 7 μm, was cut into small pieces (2×2 cm^2 each) for use in the potential measurement.

Assembly of the Immunosensor

The experimental setup for measuring membrane potential is presented in Fig. 1. A plastic cell composed of three compartments was used throughout the investigation. These compartments, I, II, and III, are connected through 1 cm ϕ holes. The antigen-binding membrane was fixed

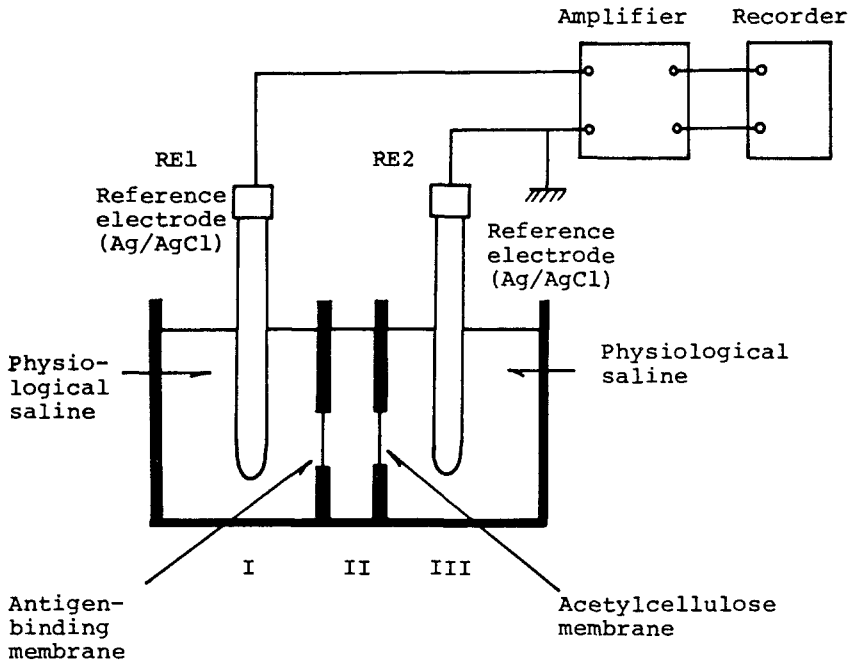

FIG. 1. Experimental setup for measuring the membrane potential.

between I and II, and an acetylcellulose membrane between II and III. I and III contained 3 ml of physiological saline. One milliliter of test serum solution was injected into II. A pair of Ag/AgCl electrodes used as reference electrodes were led to an electrometer (Model HE-101A, made by Hokuto Denko Co., Tokyo).

RESULTS AND DISCUSSION

In order to follow the immunochemical reaction at the membrane–solution interface, the potential difference between a pair of electrodes was measured. The effect of nonspecific adsorption of serum proteins to the antigen-binding membrane cannot be neglected. An acetylcellulose membrane was mounted between compartments II and III for eliminating the effect of the adsorption on the membrane potential. The acetylcellulose membrane also inhibits the direct contact of serum with the electrode.

There was no potential difference when the central compartment was filled with physiological saline. The saline in the central compartment was

then replaced with positive serum, which was diluted with physiological saline in the range of 1 : 10 to 1 : 1000. The concentration of electrolytes in the three compartments can be assumed to be equivalent. The positive serum developed significant potential change, which increased with time, reaching a constant value. The constant potential was attained in approximately 5 min. In sharp contrast, little potential change was produced by negative serum containing no Wasserman antibody.

The extent of the membrane potential change was found to depend on the concentration of Wasserman antobody and temperature. The membrane potential decreased with the dilution of the positive serum. The antibody concentration dependence of the membrane potential is presented in Fig. 2. The serum solution was prepared by diluting the positive serum with physiological saline to the proper dilution. The membrane potential generated by the negative serum solution at the proper dilution is also shown for comparison in Fig. 2. The negative serum gave little membrane potential at any dilution. Therefore, positive serum can be easily distinguished from

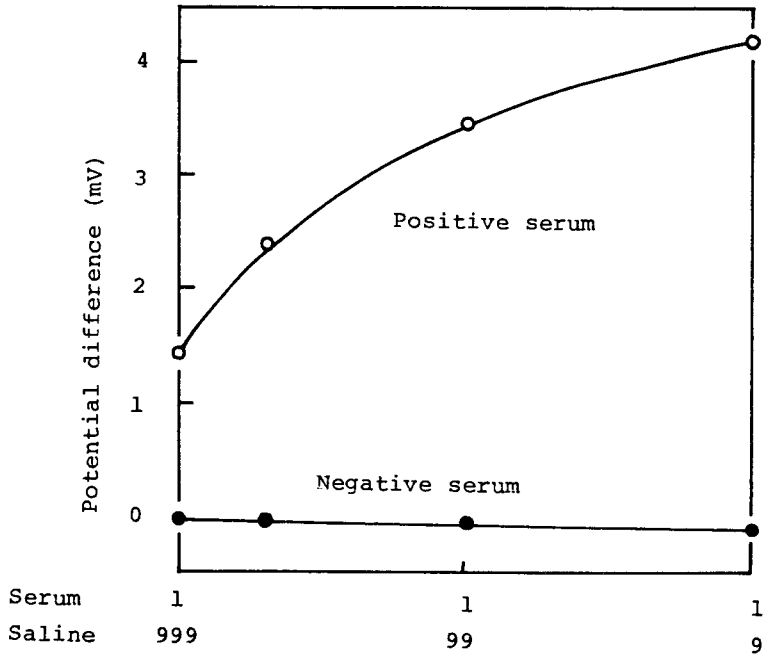

FIG. 2. Serum concentration dependence of the membrane potential developed by negative (—●—) and positive (—○—) sera. The membrane potential was measured at 25°C using a pair of Ag/AgCl electrodes as reference electrodes. The electrode in compartment III was grounded.

negative serum by the membrane potential of the antigen-binding membrane.

These results indicate that a membrane potential change resulted from the antigen–antibody complex formation at the interface of the antigen-binding membrane and serum solution. The surface charge of the antigen-binding membrane in contact with physiological saline remains unchanged, because neither specific nor nonspecific adsorption to this side of the membrane can occur. The antigen-binding membrane may become an asymmetric membrane in charge density when the one side of the membrane

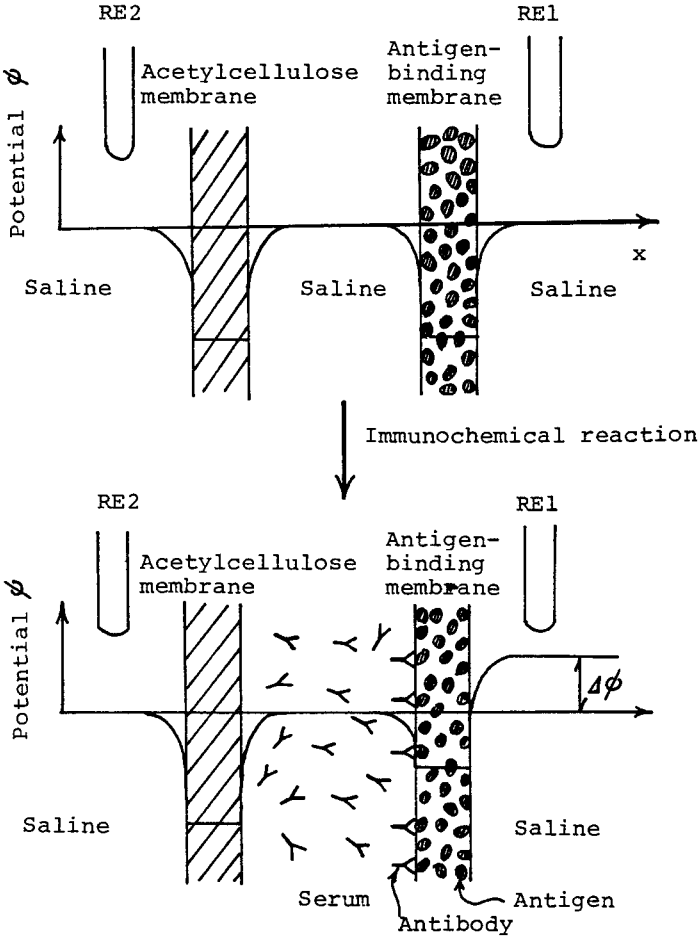

FIG. 3. The postulated potential diagrams of the antigen-binding membrane before and after the immunochemical reaction with the antibody present in serum.

is in contact with serum and the other side with saline containing no serum. Such asymmetric distribution of charge should cause the potential difference observed for positive serum.

The postulated potential diagrams are presented for the antigen-binding membrane before and after the immunochemical reaction with free antibody in Fig. 3. When compartment II contains physiological saline, both acetylcellulose and antigen-binding membranes exhibit no asymmetric distribution in membrane charge. Since there is no concentration gradient across the membrane, no potential difference is generated. If the antigen-binding membrane contacts with corresponding antibody in compartment II, the antibody specifically adsorbs on the membrane surface. Other serum proteins might adsorb on both acetylcellulose and antigen-binding membranes to a comparable extent. Only antibody specifically adsorbed causes an asymmetric distribution of charge across the antigen-binding membrane, thereby generating the membrane potential.

In serology tests for syphilis, the antigen–antibody complex formation is conducted by diluting serum stepwise until no agglutination occurs. It usually takes some time to obtain results from the test. On the other hand, the immunosensor responds to the antibody in several minutes, displaying the result as an electronic signal. This signal could directly correspond to the level of Wasserman antibody in serum. The new immunosensor described here appears applicable to serology tests for syphilis because of simplicity.

CONCLUSIONS

The Wasserman antigen-binding membrane responded in trans-membrane potential to corresponding antibody present in serum. The immunochemically induced membrane potential is thought to have resulted from the antigen–antibody complex formation at the membrane–solution interface with a resulting change in charge density. The immunosensor, which is composed of the antigen-binding membrane, may be applied to serology tests for syphilis.

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