(*5*) without the use of deoxycholate. The pellet was resuspended and then centrifuged at 0°C in a linear sucrose gradient (15 to 30 percent) at 25,000 rev/min in the SW-25.1 rotor of a Spinco L-2 preparative centrifuge. Optical densities were read at 260 $m\mu$ in a Gilford recording spectrophotometer.

Polysome profiles of cytoplasmic skin and feather extracts of 15-day-old hypophysectomized embryos which had been treated with pituitary extract are shown in Fig. 1*A*. The patterns of untreated hypophysectomized birds are very similar to those of either control or operated embryos at 12 days of incubation. In contrast, skin and feathers of the treated 15-day embryos yield intermediate patterns, approaching those of the 15-day control birds in which a marked shift from the monosome region to the heavier polysome region of the gradient profile occurs.

When one embryo of a double-yolked egg is hypophysectomized, vascular anastomoses between the two embryos are extensive and apparently allow hormonal replacement to occur (Fig. 2*A*). The pattern of feathering of the control is similar to that of the operated birds (Fig. 2*B*). This similarity in feather development is also reflected in the polysomes; there is complete differentiation of the polysome pattern in the hypophysectomized embryo (Fig. 1*B*).

In the normal course of events, maturation of down feathers is completed by a rapid deposition of the structural protein keratin between day 13 and day 17. The underlying molecular events are reflected by changes in the polysome distribution and morphology (*1*). It has been proposed that these molecular events are under endocrine control (*2*), since extirpation of the pituitary gland prevents the normal gross anatomical and intracellular changes. That there is an increased quantity of heavier polysomes and, by inference, of messenger-RNA after hormonal treatment of hypophysectomized embryos indicates that differentiation of skin and feathers is hormonally controlled. Whether new species of messenger-RNA are synthesized when pituitary hormones are replaced is not known.

Thus, keratinization of feathers requires the presence of pituitary hormones. Hypophyseal control of feather differentiation might be exerted by one or a combination of the following: increased rate of synthesis, rate of translation, or enhanced stability of messenger-RNA already present in skin and feathers. A final possibility is that pituitary hormones operate via cistron derepression which allows the synthesis of entirely new species of messenger-RNA.

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3 May 1966

Lipid Films as Transducers for Detection of Antigen-Antibody and Enzyme-Substrate Reactions

Abstract. *The transverse electrical impedance of thin lipid films separating two aqueous saline solutions containing a small concentration of antibodies or enzymes decreases markedly and reversibly after immunological and enzymatic reactions involving such protein molecules, which presumably are adsorbed to the lipid-water interface.*

Development of techniques to form stable phospholipid films between two aqueous phases (*1*) offers an enticing opportunity to experiment with artificial systems which resemble, after a fashion, the structure of cell membranes—that is, thin lipid leaflets coated by protein molecules adsorbed to the lipid-water interface.

The work we now summarize was undertaken to find out whether reactions involving such affixed protein molecules would influence the electrical impedance of the lipid phase. This possibility was suggested by a number of published observations showing that immunological and enzymatic reactions, in which the layers of protein coating participate, often result in large changes in membrane permeability.

It is known, for instance, that specific antiserums, in the presence of com-

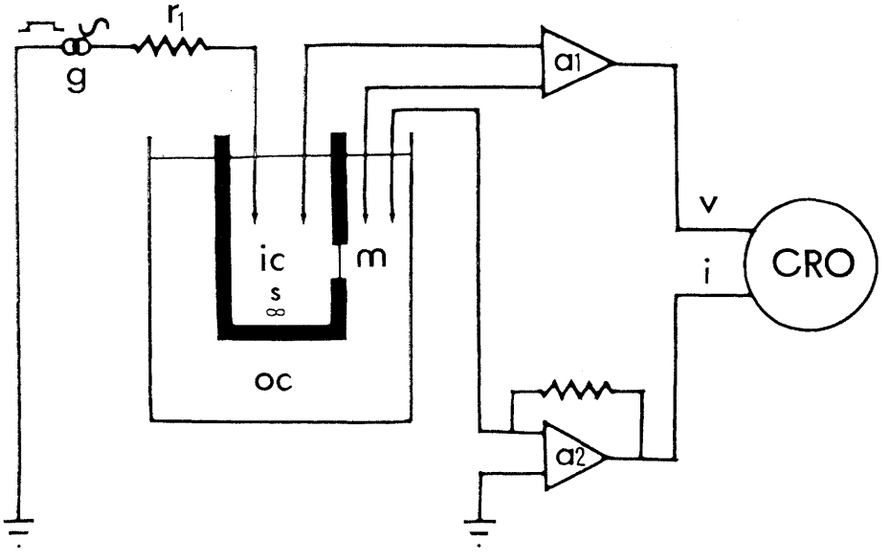

Fig. 1. Experimental arrangement. The current (*i*) is made to flow from the generator through a resistor (r_1), up to 250 Mohm, across the lipid film (*m*) separating the inner (*ic*) from the outer (*oc*) compartments (*s*, stirring device). The current electrode in the outer compartment is connected to the summing point of an operational amplifier (*a2*). The potential changes (*v*) across film *m* are differentially recorded with help of a high-input-impedance amplifier (*a1*). Both potential *v* and current *i* are displayed on a dual-beam cathode-ray oscilloscope (*CRO*).

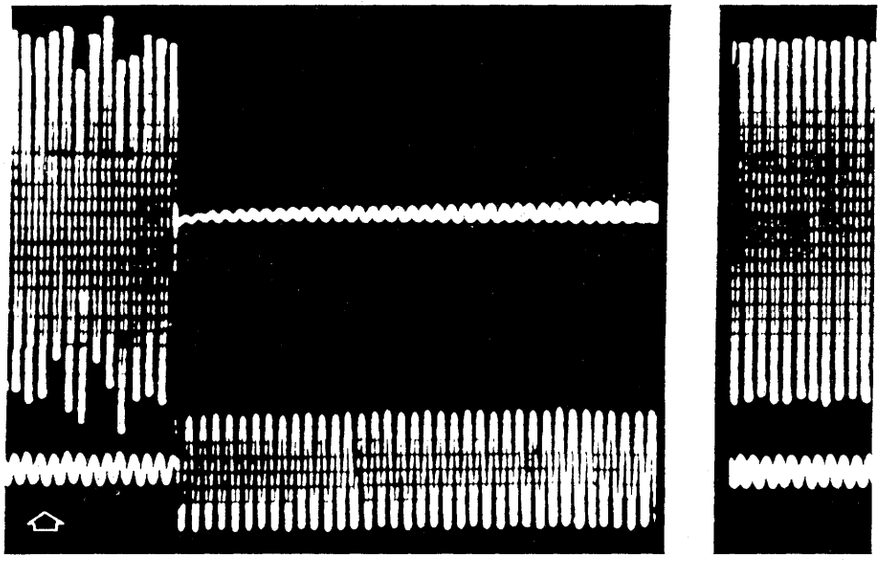

Fig. 2. Effect of bovine serum albumin on the transverse impedance of an antibody-treated lipid film to a 300 cy/sec a-c current. Before the beginning of this record antiserum to bovine serum albumin had been added to the inner compartment (see Fig. 1) in a concentration of 1/40 (vol./vol.) with no appreciable change in the properties of the film. The arrow indicates, only approximately (through a manually operated signal), the moment when antigen (in a concentration of 10 μg/ml) was injected into the inner compartment. The lower record is the current between the two compartments; this current increased as the impedance of the film decreased, since the value of the series resistance (r_1, Fig. 1) was only 15 Mohm. The record on the right shows full recovery of the initial impedance of the film.

plement, have a profound effect on the surface membrane of ascites tumor cells and erythrocytes; this membrane not only becomes more permeable to ions but actually develops "holes" which permit passage of molecules as large as those of hemoglobin and ribonucleotides (*2*). Another example of such interaction between protein and lipid layers is the evidence presented by Lehninger (*3*) that conformational changes in the respiratory assemblies of the mitochondrial membrane, determined by their oxidoreduction state, can bring about marked changes in the permeability of the membrane.

Lipid films are formed, following the technique of Mueller *et al.* (*1*), across a small window punched in the wall of a polyethylene cup clamped within a larger container (see Fig. 1). Both compartments are filled to the same level with a solution of 100 m*M* NaCl and 5 m*M* histidine maintained at 36°C. A small amount of a chloroform-methanol extract of ox brain (for preparation, see *1*) to which α-tocopherol and cholesterol have been added is

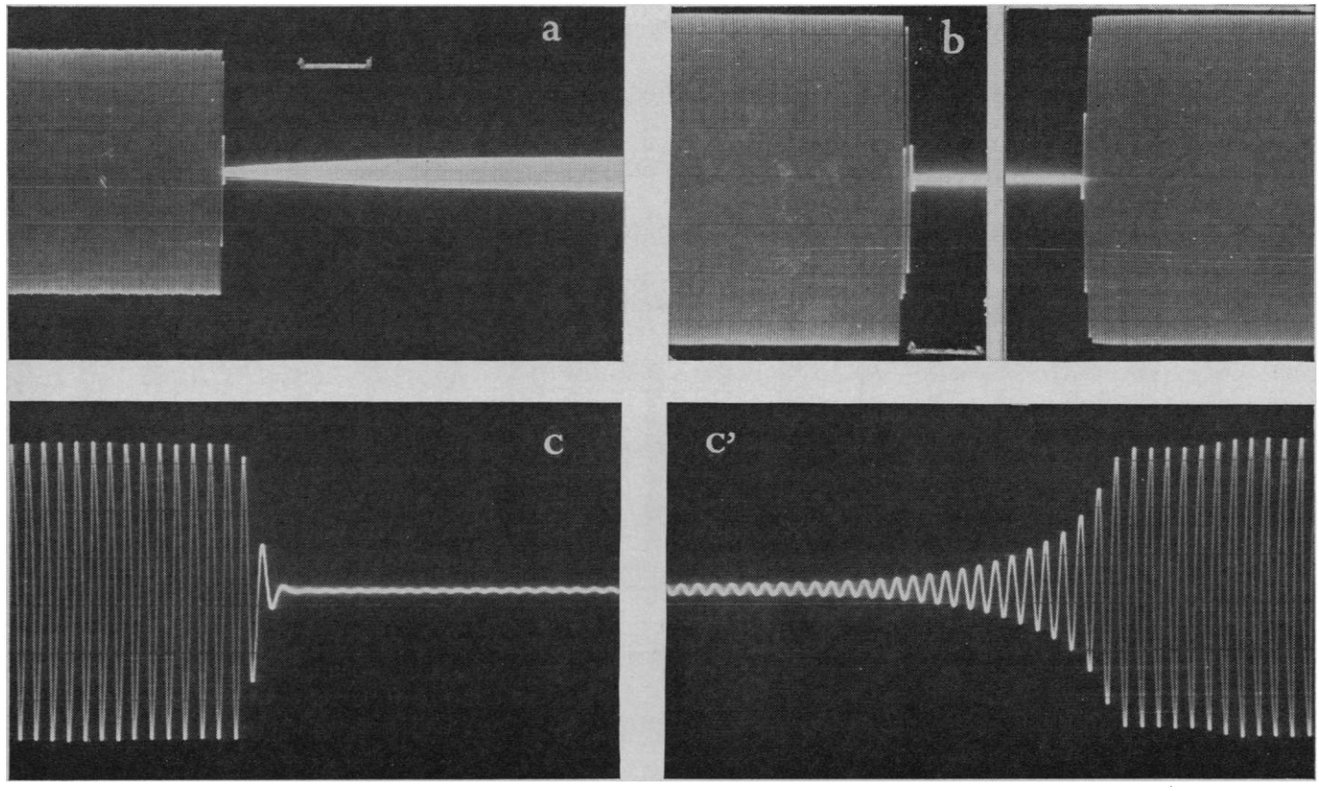

Fig. 3. Three examples of sudden reductions in the transverse impedance of enzyme-treated lipid membranes following the addition of substrate. (*a*) Effect of 10 μg of benzoylarginine ethyl ester HCl on a membrane exposed to trypsin (30 μg/ml). Time calibration, 0.2 second. (*b*) Sudden reduction and recovery of the impedance of a membrane treated with lactic acid dehydrogenase (30 μg/ml) upon addition of 0.5 mg of pyruvate and 10 μg of β-diphosphopyridine nucleotide. Total duration of the effect, 0.2 second; time calibration, 20 msec; frequency, 1000 cy/sec. (*c*) This record and its continuation (*c'*) show the effect of adding 10 μg of denatured ovalbumin on a membrane exposed to chymotrypsin (30 μg/ml). Intervals between cycles, 5 msec; total duration of effect, about 0.6 second; volume of the enzyme solution in the cup, 3.5 ml.

spread with a thin brush across the window. As the solvent diffuses into the aqueous phase, a thin film is formed which first shows interference colors and then black areas.

The electrical impedance of such films was monitored throughout by recording the voltage drop produced by a sinusoidal current (from 200 to 1000 cy/sec) between the two compartments. Its d-c resistance was measured at certain times by superimposing long rectangular current pulses of known intensity on the a-c signal.

The potential across the lipid film was recorded with silver–silver chloride electrodes joined to the saline solution by agar bridges. These electrodes were connected, through preamplifiers with high input impedance, to one of the channels of a cathode-ray oscilloscope. In some experiments the current through the membrane was displayed on the other beam as the output of an operational amplifier whose summing point was connected to the current electrode in the outer compartment.

In the experiments involving immunological reactions, crystallized albumins (human and bovine serum albumins, ovalbumin, and human serum albumin diazotized with sulfanilic acid) were used as antigens. In some instances purified antibodies were made to react with those proteins, but more often immune serums were employed. To study the effect of enzymatic reactions, trypsin, chymotrypsin, pinguinain, lactic acid and glutamic acid dehydrogenases, urease, uricase, and cholinesterase, together with appropriate substrates, were used.

Separate addition of albumin (to a final concentration of up to 1 mg/ml) or immune serum (up to 1/20 vol./vol.) to the cup had no appreciable influence on the lipid films. However, addition of small amounts of a protein, after the corresponding specific antigen or antibody was in contact with the membrane, caused a sudden reduction in the electrical impedance of the lipid barrier. This occurred regardless of the order in which the two proteins were applied, although particularly consistent results were obtained if the antibody was first injected into the cup. Marked changes in impedance could be observed when the concentration of immune serum in the inner compartment was between 1/40 and 1/100 (vol./vol.) and the antigen albumin was added to give final concentrations of about 10 μg/ml. An example of such

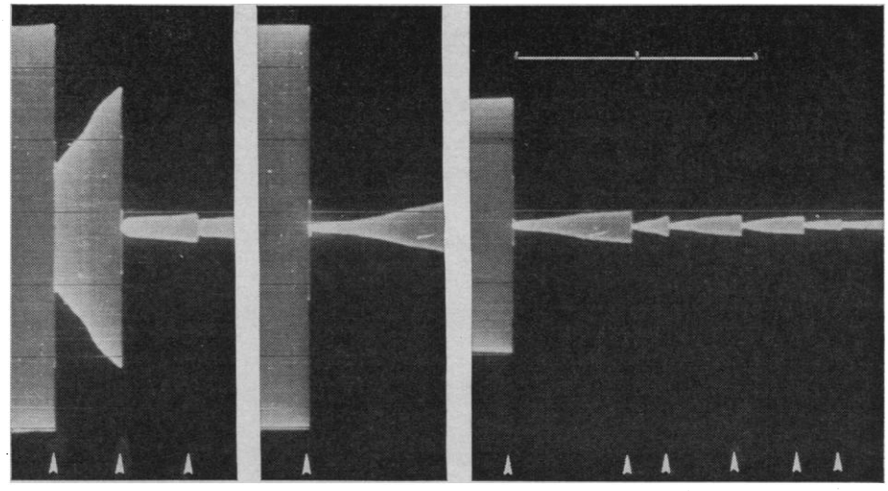

Fig. 4. The arrows mark the effects of the addition of droplets containing 10 μg of denatured ovalbumin on the impedance (to 200 cy/sec a-c) of a lipid membrane exposed to chymotrypsin (30 μg/ml). The experiment was performed on a single membrane; two sections of the record have been omitted. Volume of the solution in the cup, 3.5 ml; time calibration, 2 seconds.

effect is illustrated in Fig. 2. As shown in this record, the impedance of the membrane returns to its initial level after its sudden reduction. A second addition of the same protein after an impedance drop has taken place has a smaller effect or no effect at all.

If the antigen and antibody are mixed before they are added to the system, no impedance changes are produced. This observation suggests that the factor responsible for such effects is the formation of antigen-antibody complexes—the process itself, rather than the result of such combination.

Equally marked changes in impedance have been observed when small amounts of appropriate substrates were brought into contact with films exposed to the aforementioned enzymes. Examples of such changes are illustrated in Fig. 3. One record (*a*) shows the effect of the addition of benzoylarginine ethyl ester HCl on a membrane exposed to trypsin (30 μg/ml) Another (*b*) was produced when a mixture of pyruvate and β-diphosphopyridin nucleotide was added to a membrane exposed to lactic acid dehydrogenase (30 μg/ml). Finally, two records (*c* and *c'*) illustrate the action of denatured ovalbumin on a film exposed to chymotrypsin (30 μg/ml).

Whereas the addition of specific antigens to antibody-treated membranes is followed by a rapid desensitization, the effects of substrates on membranes exposed to enzymes can be demonstrated over and over again (Fig. 4).

The time course of the transient changes in impedance which follow the initial application of small amounts of substrate to the inner compartment appear to reflect the waxing and waning of the substrate concentration at the lipid-water interface; both waxing and waning are determined by mixing and diffusion as well as by enzymatic action. However, the addition of comparatively higher amounts, or the accumulation of substrate in the cup, leads to more persistent effects.

Preliminary measurements with a logarithmic amplifier have shown that the impedance of the lipid film may drop by three orders of magnitude during such responses.

A number of experiments were performed to find out the immediate cause of the impedance changes elicited by enzymatic reactions; these results can be summarized as follows. (i) Such changes could only be observed if an appropriate substrate for the enzyme in contact with the membrane was added to the system. (ii) The presence of urea at concentrations of 0.2 mg/ml and higher completely blocked the effects. (iii) The responses of chymotrypsin-treated membranes were inhibited by Cu^{++} and Hg^{++} ions at concentrations of $10^{-5}M$. (iv) To discard the possibility that the observed impedance changes might be due to compounds arising from the enzymatic reactions, such products (for example, appropriate amino acids when working with proteolytic enzymes) were added to the system, but no impedance changes could be detected.

These observations suggest that we are dealing with the effects of an interaction of genuine catalytic nature between the enzyme present in the system, presumably adsorbed to the lipid-water interface (see *4* for evidence on absorption), and the added substrate molecules. It also appears that the enzymatic reaction is effective as a process and not as a source of active ions or molecules.

It should be emphasized that not all the phospholipid extracts produce films which show the impedance changes described. In fact, many of them yield films which are "inert" but otherwise indistinguishable from the "responsive" ones.

As yet we cannot explain such differences, although we have found that they depend on the particular brain material employed rather than on small variations in technique. It is, therefore, advisable to work with a number of brains at the same time, preparing and testing small amounts of chloroform-methanol extract from each. Larger volumes of solution can then be obtained from those brains which produce responsive membranes.

Both the quantity and quality of the cholesterol added to the phospholipid extracts are critical. Best results were obtained working with a mixture of 1 ml of extract, 150 mg of *dl*-α-tocopherol, and 30 mg of cholesterol; "99%+" cholesterol standard for chromatography (Sigma Chemical Company) was employed, since the products of oxidation of this compound, even in small amounts, block the responses of the lipid films (*5*).

It has been found possible, as well as convenient, to preserve the mixture of phospholipid extract, cholesterol, and tocopherol in sealed ampoules under a nitrogen atmosphere; these ampoules have been kept without appreciable deterioration for periods of up to 2 months at 5°C (*6*).

The appearance of the film and the absolute value of its transverse resistance are not critical for the occurrence of the impedance changes. Though most membranes show distinct black areas and their d-c resistances are of the order of 10^6 ohm cm^2, large responses have been observed both in relatively thick membranes where those areas could not be discerned and in films with resistances lower than 10^5 ohm cm^2. Therefore we are inclined to believe that such changes do not occur in the regions of the film where the phospholipid molecules form highly organized smectic structures, but rather at the level of more permeable areas with, perhaps, a predominantly micellar structure. However, no impedance changes were observed when specific antigens and substrates were added to the opposite side of the lipid film from that exposed to the corresponding antibody or enzyme.

Apart from the problems posed by the mechanisms responsible for such impedance changes, the effects are interesting for two main reasons. First, they can be used for extremely fast detection of antigen-antibody reactions and are already being used routinely in this laboratory for such purposes. Second, the fact that marked impedance changes are produced in enzyme-complexed membranes upon exposure to substrates of low molecular weight is interesting, since these systems may be regarded as analogs of chemical synaptic mechanisms in which the presynaptically liberated transmitter substance changes the impedance of the postsynaptic membrane upon its combination with receptor sites on its surface.

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5. *Note added in proof.* Analysis by thin-layer and vapor-phase chromatography has shown that oxidation derivatives of tocopherol also block the film responses. Separate chromatographic analysis of the chloroform-methanol extract and the above-mentioned additives is strongly recommended.
6. We shall be glad to supply small amounts of this material to interested investigators.
7. Supported in part by PHS grant NB-05235.
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Diagenesis of Carbonate Sediments: Interaction of Magnesium in Sea Water with Mineral Grains

Abstract. *Samples of natural fine-grained carbonate sediment from Florida Bay, Florida, undergo mole-for-mole cation exchange with aqueous solutions of $MgCl_2$ and $CaCl_2$ in the laboratory. The exchange reaction, which involves the surface of the grains of sediment, can be essentially described by a simple mass action–law equation. Enrichment of Mg^{++} beyond the amounts found within particle interiors should take place on the surface of $CaCo_3$ sediments immersed in sea water; it may be on both exchangeable and unexchangeable sites.*

There is considerable evidence (*1, 2*) for the generalization that recrystallization of the common carbonate minerals of Recent marine sediments, aragonite and highly magnesian calcite, to the carbonate minerals characteristic of ancient rocks, calcite and dolomite, is not rapid in sea water of average salinity; in other words, carbonate diagenesis, with consequent formation of limestones and dolostones, is inhibited by sea water. This phenomenon cannot be explained simply by the bulk thermodynamic properties of the individual minerals. From the data of Lerman (*3*) and the thermodynamic data summarized by Langmuir (*4*) it can be shown that, in sea water of average Mg:Ca ratio, stoichiometrically ordered dolomite is more stable than low-magnesium calcite (calcite containing less than 4 mole percent $MgCO_3$ in solid substitution) and that low-magnesium calcite is more stable than aragonite or high-magnesium calcite (calcite containing more than 10 mole percent $MgCO_3$). Absence of recrystallization must result from surface effects that retard rates of solution of metastable phases or prevent nucleation and growth of low-magnesium calcite and dolomite.

The most common explanation of the absence of diagenetic formation of low-magnesium calcite in sea water is that Mg dissolved in the water acts as a specific inhibitor of crystallization. The role of Mg ion in retarding the rate of recrystallization of aragonite to calcite, even in the presence of calcite seeds, has been directly demonstrated in the laboratory (*5*). Other laboratory studies have shown that dissolved Mg: (i) severely inhibits precipitation of low-magnesium calcite from artificially