

not as widely appreciated as it deserves to be. There are few comparable or equally comprehensive outlines of the subject published in English. This chapter may serve also to illustrate the effectiveness of the revision which has been practised in the new edition. Not only are new facts introduced (American investigations not being overlooked), but discarded and unsubstantiated views have been conservatively eliminated. For example, there are found detailed allusions to the studies in "artificial" nutrition, Michaud's experiments on the protein minimum, Rubner's recent discussions on nutrition, Murlin's study of gelatin feeding, and the disputed problem of the specific dynamic action of foods. The discussion of obsolete obesity "cures," etc., has been omitted.

In the translator's preface Professor Mandel writes: "The work of translating and editing has been a labor of love, inasmuch as I feel that it will be of aid in the advance of this department of chemical science." He is right, and deserves a renewed expression of appreciation from biochemical workers for the faithful and correct execution of an uninviting task.

LAFAYETTE B. MENDEL

SHEFFIELD SCIENTIFIC SCHOOL,
YALE UNIVERSITY

SPECIAL ARTICLES

ON THE NATURE AND SEAT OF THE ELECTROMOTIVE FORCES MANIFESTED BY LIVING ORGANS¹

1. When an organ of an animal or a plant is injured an electromotive force develops between the injured and the non-injured surface, the latter being, as a rule (if not always), positive to the former. Loeb suggested in 1897 that this difference of potential might be due to the fact that the injured spot formed an acid and that on account of the H-ion moving faster than the anion a difference of potential was produced. This assumption accounted for the sense of the E.M.F. in a partially injured organ. It was, however, pointed out that the order of magnitude of such dif-

fusion elements is too small to account for the E.M.F. found in living organs. Wilhelm Ostwald had suggested the possibility that living organs form concentration elements with a solid phase interposed, the solid phase—the membrane—being permeable for certain ions only. Bernstein starting from Helmholtz's conception of free energy conceived the idea that measurements of the effect of temperature on the E.M.F. of a partially injured muscle or nerve might indicate the nature of the elements to which these systems belong. He reached the conclusion that the partially injured muscle belongs to the type of concentration element in which a solid phase—the membrane—separates the two liquids, the solid phase being only or more permeable to kations than to anions, thus corroborating Ostwald's suggestion.

Bernstein found that the E.M.F. of the muscle rises in general with the temperature and that it approaches a value in proportion to the temperature. The agreement was, however, not as good as should be desired to put the theory of concentration cell of the muscle current on an absolutely safe basis. Moreover, experiments on nerve were less satisfactory and in both cases accessory assumptions were required to make the actual results agree with the theory.

2. Muscles and nerves are, perhaps, too variable or rather perishable to investigate quantitatively with any degree of satisfaction the nature and origin of their E.M.F. We selected for this purpose a hardier and more constant object, namely, apples, the surface film of which is strong and which remains sufficiently constant during such an investigation. Instead of testing the effect of temperature on the E.M.F., we selected the effect of the concentration of the solutions in contact with the apple. The limit within which the temperature can be safely changed without injuring or modifying the living organ is very small and this is one of the reasons why Bernstein's figures are not quite satisfactory, as he himself recognized; while we can change the concentration on such living ob-

¹ Preliminary communication.

jects as the apple in very wide limits without injury or complicating alterations.

We found the following method most satisfactory. An apple with perfect skin was put into a glass dish containing a small amount of a liquid *a*. On the opposite side of the apple a piece of the skin and the underlying tissue was removed and into the hole was put a small quantity of a liquid *b*. The latter remained constant throughout the experiment, while *a* was changed according to the nature of the experiment. Both liquids *a* and *b* were connected with calomel electrodes and the E.M.F. was determined by Poggendorf's compensation method (capillary electrometer). The temperature was kept practically constant (about 19° C.).

We, therefore, were studying the E.M.F. of the following system: liquid *a*; apple; liquid *b*, the membrane of the apple being the solid phase between liquids *a* and *b*. According to the theory a fivefold dilution of *a* should always give a constant decrease of E.M.F., namely, .040 volt; and a tenfold dilution should decrease the E.M.F. always by a value of .058 volt. These values may be expected to be smaller if the ideal conditions of semi-permeability are not fulfilled.

In the following experiment liquid *b* (in the apple) was *m*/10 KCl. Liquid *a*, on external surface, varied in concentration. The calomel electrodes contained *m*/10 KCl. The

TABLE I

Concentration of Liquid <i>a</i> .	E.M.F.
<i>m</i> /10 KCl	+ .040 volt
<i>m</i> /50 KCl	+ .068 volt
<i>m</i> /250 KCl	+ .103 volt
<i>m</i> /1,250 KCl	+ .137 volt
<i>m</i> /6,250 KCl	+ .169 volt

sign + means that liquid *a* was positive to liquid *b*. In this experiment each successive liquid was five times as diluted as the previous one and the theory demands that the difference of E.M.F. between two successive solutions should be identical. If we compare the interval from *m*/50 to *m*/6,250 this is true. A dilution from *m*/50 to *m*/250 increases the

E.M.F. by .035 volt; from *m*/250 to *m*/1,250 by .034 volt; from *m*/1,250 to *m*/6,250 by 0.32 volt. By diluting from *m*/10 to *m*/50 we find .028 volt, which is a little too small. We have found it to be generally true that as soon as we work with more concentrated solution than *m*/50 the differences are a little smaller than the theory demands. Whether this is due to the decrease in ionization or to an injurious effect of the solutions of higher concentration upon the skin of the apple we are not yet prepared to state.

The values observed are all a little smaller than we should expect. According to Nernst's formula the difference of potential for a dilution of five should be .040 volt, while we found a difference of .033 volt. The fact that we did not get the maximum potential difference is, perhaps, due to the fact that the skin of the apple is not completely impermeable for anions.

Experiments with other salts gave similar results as far as the effect of concentration was concerned.

3. The sign of the E.M.F. of the system electrolyte; apple; electrolyte was always in that sense as if the membrane of the apple were more permeable for kations than for anions. In order to test this possibility the electromotive effects of NaCl were compared with those of Na₂SO₄. If our assumption were correct the E.M.F. of a NaCl solution should always be equal to the E.M.F. of a Na₂SO₄ of half the concentration of the former. The following example shows that this is actually the case. The internal liquid *b* remained constant throughout the experiment *m*/10 KCl. The external liquid *a* varied according to Table II.

TABLE II

Liquid <i>a</i> .	E.M.F.
<i>m</i> /10 NaCl	+ .038 volt
<i>m</i> /100 NaCl	+ .090 volt
<i>m</i> /1,000 NaCl	+ .139 volt
<i>m</i> /2,000 Na ₂ SO ₄	+ .141 volt
<i>m</i> /200 Na ₂ SO ₄	+ .092 volt
<i>m</i> /20 Na ₂ SO ₄	+ .044 volt

This experiment shows that whether the anion is Cl or SO₄ as long as the concentra-

tion of the kation remains the same the E.M.F. remains unaltered. The E.M.F., if liquid *a* is *m*/1,000 NaCl, is .139 volt, and if it is *m*/2,000 Na_2SO_4 is .141 volt; practically identical values. If the liquid *a* is *m*/100 NaCl it is .090 volt, if it is *m*/200 Na_2SO_4 it is .092 volt; again practically identical values.

4. It was necessary to convince ourselves that we were not dealing with purely osmotic effects (and possibly diaphragm currents). In one experiment the external liquid *a* was *m*/100 NaCl, the liquid *b* on the injured side of the apple was *m*/10 KCl. The E.M.F. was +.092 volt. Then enough cane sugar in crystals was added to the liquid *a* to make its total concentration about *m*/2. After the sugar was dissolved the E.M.F. was .093 and remained so. Changes in concentration by a non-electrolyte like cane sugar, therefore, do not alter the E.M.F.

5. Continuing an investigation by Haber and Beutner on "Phasengrenzkraft," Haber and Klemensiewicz described a concentration cell for H-ions of the type, acid; glass; alkali, the acid representing the solution with a high, the alkali with a low concentration of H-ions. Haber pointed out that this type of concentration element might correspond to the type represented by muscle, the muscle fibrils corresponding to the glass in the acid alkali element. Since the liquids in the cells are practically neutral a slight production of acid in the fibril (or the injured spot) would give rise to a considerable E.M.F. We fully expected at the beginning of these experiments to find that the E.M.F. of living organs was of the type of that found by Haber. We found, however, that for the apple this is not the case, as the following experiment shows. The internal liquid *b* was throughout the whole experiment *m*/10 KCl (neutral). The external liquid *a* was in succession *m*/20 NaCl, neutral, alkaline, acid and alkali again. It was rendered acid through addition of enough HCl to render the *m*/20 NaCl solution *m*/1,000 acid, and it was rendered alkaline through the addition of enough NaHO to render the *m*/20 NaCl solution *m*/1,000 alkaline.

TABLE III

Liquid <i>a</i> .	E.M.F.
<i>m</i> /20 NaCl, neutral	+ .051 volt
<i>m</i> /20 NaCl, <i>m</i> /1,000 alkaline	+ .052 volt
<i>m</i> /20 NaCl, <i>m</i> /1,000 acid . .	+ .048 volt
<i>m</i> /20 NaCl, <i>m</i> /1,000 alkaline	+ .052 volt

The differences found between neutral, acid and alkaline *m*/20 NaCl are slight and within the limits of purely accidental variations. If we were dealing with a reversible cell in regard to H-ions we should expect a difference of almost .5 volt between *m*/1,000 acid and *m*/1,000 alkali.

6. It may be of interest to mention also that acids and alkalis behave in regard to the E.M.F. to which they give rise like the salts. The experiments we made in this respect show also that if the concentrations of these substances are a little too high the regularity of the results suffers, and the irregularities are always of such a nature as should be expected if the injury or the etching effect of acids and alkalis increased the anion permeability of the skin of the apple. Liquid *b* in the apple was *m*/10 NaCl throughout the experiment.

TABLE IV

Liquid <i>a</i> .	E.M.F.
<i>m</i> /10 NaCl	+ .003 volt
<i>m</i> /10 NaHO	+ .009 volt
<i>m</i> /100 NaHO	+ .041 volt
<i>m</i> /1,000 NaHO	+ .085 volt
<i>m</i> /10,000 NaHO	+ .125 volt
<i>m</i> /100 NaHO	+ .042 volt
<i>m</i> /1,000 NaHO	+ .085 volt
<i>m</i> /100 NaHO	+ .044 volt
<i>m</i> /10,000 HCl	+ .126 volt
<i>m</i> /1,000 HCl	+ .064 volt
<i>m</i> /100 HCl	+ .021 volt

The *m*/10 NaHO and the *m*/100 HCl act as if they had a slight etching effect on the skin, otherwise we notice the same influence of dilution as in the case of salts.

7. We believe that these and other experiments, which will be published in the full report, show that the influence of the concentration of electrolytes on the E.M.F. of living organs agrees quantitatively with the values to be expected if the skin is permeable for

kations, impermeable or less permeable for anions.

JACQUES LOEB,
REINHARD BEUTNER

DEPARTMENT OF EXPERIMENTAL BIOLOGY,
ROCKEFELLER INSTITUTE

THE PERMEABILITY OF THE OVARIAN EGG-MEMBRANES OF THE FOWL

I

VERY ordinary eggs have long been the subject of very much noise-making; the cackling hen, the cold-storage man, and the public each playing in this an individual, different and discordant part. One wonders therefore whether the quiet of the earth might be in any measure restored if *ordinary* eggs were made different; particularly if the egg were so much metamorphosed as to be born in a fully preserved and stable state. Would the noisily expressed solicitude of the persevering egg-maker then abate? Would the cold-storage man then "fold his tent like the Arabs, and as silently steal away"? Would the voiceful public then wait with less impatience "for its ships to come from sea"?

With something of this thought—of possibly contributing a modicum to the quiet of our planet—the undersigned, in an odd moment, set to the present task many months ago. Despite the generally rough exterior of the common barn-yard fowl, is it not possible to bring about some very nice adjustments between its blood and its growing ova, such as will effect the formation of eggs thus capable of maintaining themselves against the ravages of time and the decomposing influences of temperature?

To some veteran doubters, however, it may seem that the triumph of the experiment would bring no blessings whatever; and some there may be who would even assert that its success and utilization really but spells new calamity to egg-users. We do not know; we repose in our innocent intentions, in our wonder, and in our questions.

II

Can hexamethylenetetramine leave the blood and penetrate the cells which guard the germ

—the germ-plasm? Supposing that it can do so, will this substance decompose spontaneously within the egg—as it is known to do in some tissues—setting free formaldehyde? And will not the formaldehyde thus liberated exercise a preserving action on the elements of the egg? Again, can sodium benzoate pass through the egg-envelopes and enter the growing egg? If so, will it do duty as a preservative there? What will sodium salicylate do in a similar way?

The answer to these questions in so far as it is supplied by our experiments may be given at once; the details and the evidence being presented later.

When hexamethylenetetramine (urotropin) is fed to laying hens it passes through the follicular and vitelline membranes surrounding the egg and is deposited in the egg. It undergoes decomposition there; formalin being set free. It acts as a preservative; *i. e.*, it lengthens the time which normally intervenes between the fresh and the unpalatable egg.

Numerous chemical tests have failed to demonstrate the presence of either benzoate or salicylate in eggs from birds fed with these substances. Whether the latter actually entered the egg, but in another form or combination, *e. g.*, as hippuric and salicyluric acids respectively, has not been determined; our supply of eggs having been exhausted in making other tests. Quite probably the benzoate would give rise to ornithuric acid, since it is known that this acid is formed when benzoate is excreted through the kidneys of birds. Some other evidence, however, is afforded by the eggs from birds fed with sodium benzoate and sodium salicylate that such eggs, particularly those supposed to contain salicylate, withstand the effects of summer temperatures better than do the untreated control eggs.

III

Something is intimated above as to reasons for the expectation that the feeding of urotropin to birds would result in its penetration and preservation of the growing egg. A