stem reticular formation and the cord site of the patellar reflex arc had been severed. Post-mortem examination of the cord showed complete severance. This inhibitory effect has been obtained in 3 preparations. It is characterized by inconsistency of elicitation; and it is at no time so strong, so complete, or so rapid in appearance as when the cord is intact. Occlusion of the femoral artery may depress bulbar inhibition of the patellar reflex, decrease the degree of inhibitory after-discharge, and increase the extent of "escape" from prolonged bulbar inhibitory stimulation.

It appears obvious from these results that bulbar facilitation of somatic reflex activity involves the production of adrenalin and the activation of the sympathetic supply to the limb. It is not yet clear that such facilitation precludes any additional or substitutive facilitatory mechanisms in the orthodox sense at the site of the patellar reflex arc within the cord. It is apparent that the sympathetic system and adrenalin liberation may be activated by stimulation of the bulbar reticular facilitatory system in such a fashion as to resemble a *piqure d'Orbeli* in the Bernardian sense (*5*). The presence of some circulatory factor in the bulbar inhibitory phenomenon also seems apparent, but the nature of this effect is as yet obscure.

Ever since Orbeli's first report on the effects of adrenalin on neuromuscular fatigue (*6*), various investigators have reaffirmed and extended his original findings. Thus Corkill and Tiegs (*7*) showed that both adrenalin and sympathetic stimulation would enhance the height of muscular contraction, and Gruber (*8–10*) has shown that adrenalin enhances muscular contraction, delays the onset of fatigue, and introduces significant variations in the time relations of various phases of muscular contractions. Tuttle (*11*) claimed that adrenalin increased the tonus of the quadriceps and of the patellar reflex. Recently Bülbring and Burn and collaborators have carried out intensive and significant investigations concerning the mode of action of adrenalin on neuromuscular and central nervous system activity (*12–16*). Their results indicate that the phrenic nerve-diaphragm preparations show an increased tension duration to maximal stimulus volleys due to the direct action of adrenalin on the muscle fiber. They also find that flexor and extensor movements induced by descending motor tract stimulations can be depressed or enhanced under different circumstances by adrenalin, and that nerve action potentials may be enhanced by a non-circulatory effect of intra-arterially injected adrenalin. From additional evidence that adrenalin may enhance transmission at the neuromuscular junction in the phrenic nerve-diaphragm preparation, they conclude that adrenalin may increase the nerve-muscle response in three ways: (1) to a minor extent by augmenting the contraction of the muscle fiber itself; (2) by improving neuromuscular transmission; and (3) by improving excitability and conduction in the motor nerve fiber. It is tempting to draw the conclusion that the effects described here constitute a central nervous system activation of the effects described by Bülbring and Burn, but much work remains before this point can be confirmed.

References

1. Bach, L. M. N. *Federation Proc.*, **7** (1948).
2. Thompson, W. C., and Bach, L. M. N. *J. Neurophysiol.*, **13**, 455 (1950).
3. Bach, L. M. N. *Federation Proc.*, **11** (1952).
4. ———. *Am. J. Physiol.* (in press).
5. Bernard, C. *Leçons de physiologie expérimentale*, 296 (1855).
6. Orbeli, L. A. *Pavolov Jubilee Volume*, 1924.
7. Corkill, A. B., and Tiegs, O. W. *J. Physiol. (London)*, **78**, 161 (1933).
8. Gruber, C. M. *Am. J. Physiol.*, **62**, 438 (1922).
9. *Ibid.*, 475.
10. *Ibid.*, **69**, 157 (1924).
11. Tuttle, W. W. *Ibid.*, 446.
12. Brown, G. L., Bülbring, E., and Burns, D. *J. Physiol. (London)*, **107**, 115 (1948).
13. Bülbring, E., Burn, J. H., and Skoglund, C. R. *Ibid.*, 289.
14. Bülbring, E., and Whitteridge, D. *Ibid.*, **99**, 201 (1941).
15. Bülbring, E. *Brit. J. Pharmacol.*, **1**, 38 (1946).
16. Bülbring, E., and Burn, J. H. *J. Pharmacol. Exptl. Therap.*, **68**, 150 (1940).

Manuscript received October 30, 1952.

On the Use of Calomel Half Cells to Measure Donnan Potentials

K. L. Babcock and Roy Overstreet

Division of Soils, University of California, Berkeley

Since their use by Loeb (*1*), calomel half cells have been widely employed in measuring interface potentials that arise between suspensions of charged colloids and solutions of electrolytes with which they may be in equilibrium (i.e., Donnan potentials). In making these measurements it has been commonly assumed that no junction potential exists at the KCl saturated bridge which is inserted in the suspension. Recently, this assumption has been questioned by Jenny *et al.* (*2*) who interpret their data to mean that the junction potential may be appreciable. This has led the present writers to re-examine the assumption that two calomel half cells can measure a Donnan potential even if one explicitly assumes that the transference numbers of K^+ and Cl^- are equal.

In order to predict the emf which will result from the insertion of electrodes into any system, the proper procedure is to write down each individual process that occurs when n faradays pass through the circuit in question, to add these processes and to obtain the net cell reaction. The emf for the cell is then given by,

$$\Delta F = -nF_y E_{\text{cell}}$$

where F_y is the faraday. This procedure is applied below to the classical model of a Donnan system.

Assume that a suspension of potassium colloid, KR, (phase I) is in equilibrium with a solution of KCl (phase II) across a membrane permeable to KCl but not to colloid. The equilibrium condition is:

$$a_{(KCl)}{}^{(I)} = a_{(KCl)}{}^{(II)}$$

We now inquire whether calomel half cells with salt bridges can measure an interface potential which may exist across the membrane (Donnan potential).

Let us insert calomel half cells with saturated KCl bridges. The cell is

			A		M	B			
Hg	Hg_2Cl_2	KCl (sat)	I KCl + KR		II KCl	KCl (sat)	Hg_2Cl_2	Hg

We shall assume that the transference numbers of K^+ and Cl^- are equal across boundaries A, B, and M.

In order to obtain the net cell process, we must write the electrode reactions and the transference processes at the interfaces *A, M,* and *B* and obtain their sum. When 2 faradays pass, the following processes occur:

(*a*)	$Hg_2Cl_2 + 2e^-$	$\rightarrow 2Cl^-(I_s) + 2Hg$
(*b*)	$K^+(I) + Cl^-(I_s)$	$\rightarrow Cl^-(I) + K^+(I_s)$
(*c*)	$K^+(II) + Cl^-(I)$	$\rightarrow Cl^-(II) + K^+(I)$
(*d*)	$K^+(II_s) + Cl^-(II)$	$\rightarrow K^+(II) + Cl^-(II_s)$
(*e*)	$2Hg + 2Cl^-(II_s)$	$\rightarrow 2e^- + Hg_2Cl_2$

The subscript *s* denotes that the ion in question is in the salt bridge so that (*b*) corresponds to the transfer of 2 faradays from bridge I to solution I, (*c*) corresponds to the transfer of 2 faradays across the membrane, and (*d*) corresponds to the transfer of 2 faradays from solution II to bridge II. Adding the processes we obtain the net cell reaction,

$$KCl(II_s) \rightarrow KCl(I_s)$$

That is, the net process is simply the transfer of KCl between the two bridges. The ΔF for this process must be zero since KCl is transferred between solutions of the same composition, and hence $E_{cell} = 0$. This same result is obtained even when the transference numbers across the membrane are not the same. If the transference numbers of K^+ and Cl^- across the membrane are t_+ and t_-, then the net cell process becomes,

$$(2t_+ - 1)\,KCl(II) + KCl(II_s) \rightarrow (2t_+ - 1)\,KCl(I) + KCl(I_s)$$

Since the activity of KCl is the same in I as in II at equilibrium, we again find $\Delta F = 0$ and $E_{cell} = 0$.

We are led to the conclusion that if an emf is measured for the cell in the laboratory, the assumption of equal transference numbers at either *A* or *B* or both must be in error. When the transference numbers are not equal at the bridge junction, this means that there is a net transfer of salt from the bridge to the suspension. This transfer is the driving force of the cell, and it cannot have a ΔF which is the measure of the membrane potential, since, as may be demonstrated experimentally, its magnitude depends upon the salt concentration in the bridge.

The arguments above show that even where transference numbers at the KCl bridges are assumed to be equal, the calomel half cell assembly does not measure the membrane potential in a Donnan system. Therefore, if an emf is obtained experimentally for such a cell, it must mean that the transference numbers are not equal at at least one of the bridge junctions. In other words, the nonequilibrium bridge junctions are the only possible seat of an emf.

This finding is in accord with the statements of Gibbs and later of Guggenheim who emphasized that it is impossible to measure interface potentials. However, this is not to deny the probability that such interface potentials may exist. In certain kinds of membrane systems interface potentials can be estimated from theory, provided that the solutions are sufficiently dilute.

The foregoing approach has been extended and applied to membrane systems of other types (permselective membranes). A more complete discussion will be published soon.

References

1. LOEB, J. *Proteins and the Theory of Colloidal Behavior.* New York: McGraw-Hill (1922).
2. JENNY, H., *et al. Science,* **112**, 164 (1950).

Manuscript received November 5, 1952.

The Mucolytic and Digestive Action of Trypsin in the Preparation of Sputum for Cytologic Study[1]

Seymour M. Farber, Samuel L. Pharr, David A. Wood, and R. Daniel Gorman

Cancer Research Institute of the University of California School of Medicine; Department of Medicine, University of California School of Medicine, San Francisco Hospital and *San Francisco Department of Public Health*

Cytology is, at the present time, the most promising approach to the problem of early morphologic diagnosis of lung tumors. The most serious limitation to its widespread application has been the lack, heretofore, of a satisfactory means of concentrating the cellular material found in sputum. As a result, it has been necessary for the pathologist or laboratory technician to select, by macroscopic inspection, a very small proportion of the total specimen for microscopic examination.

This method of selection is unavoidably influenced by chance. Nasal and oral materials are always present in sputum; if the amount of true bronchial sputum is minimal, it may be entirely overlooked in the selection of material for screening.

It is now believed that a negative cytological report is not justified unless at least 15 slides have been studied. When 15 slides, from 5 different sputum samples are examined, an accuracy of approximately 92% can be achieved (*1, 2*). If for any reason this series is not complete, accuracy falls off sharply. A satisfactory method of concentration, it is hoped, would not only increase over-all accuracy, but reduce the number of sputum samples required as well, and thus considerably expedite diagnosis.

[1] The trypsin used in this study was Tryptar, kindly furnished by the Armour Laboratories, Chicago, Illinois.