though the results were not conclusive, owing to considerable error in the method. When potassium (in amounts similar to those normally present in serum) was added to the outside fluid described in the last experiment and the system was allowed to come to equilibrium, then it was found that—

Concentration K in serum > concentration K in fluid.
Concentration Cl in serum < concentration Cl in fluid.
Concentration Na in serum = concentration Na in fluid.

In other words, when working with pure serum proteins and electrolyte solutions, we are able to reproduce in vitro the conditions existing in the body and the relationships determined are in the nature of the Donnan equilibrium. Furthermore, as might be predicted from the Donnan equilibrium, the quantitative differences of ion concentrations in the serum and salt solutions are proportionate to the protein concentration of the serum.

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Electrometric acid-base titrations by means of the quinhydrone electrode and its application under physiological conditions.[1]

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It has recently been shown that an acid solution containing equimolecular proportions of benzoquinone and hydroquinone, a condition which is most easily obtained by dissolving the crystalline addition product, benzoquinhydrone, in the medium under examination, promptly produces a stable and reproducible oxidation-reduction potential on a properly prepared inert electrode, preferably of gold. In acid solutions the observed po-

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[1] Read before the Physiological Society, London, January 20, 1923.

[2] William Bayard Cutting, traveling fellow, Columbia University.

[3] Working under a grant from the Medical Research Council of Great Britain.

tential has been shown to be a linear function of the P_H and may, therefore, serve as a very simple means of determining hydrogen ion concentrations. For measurements on this system and references to the literature see[4]. Using this method, Biilmann[5] has shown that the potential is stable in a phosphate buffer at P_H 6.81, but unstable in an alkaline borate solution at P_H 9.24, and further that the method possesses the distinct advantage that it may be used where the platinum black hydrogen electrode is inapplicable, *e.g.*, in the presence of certain mild oxidizing agents as 0.1 M nitric acid or unsaturated compounds of the acryhic acid type.

We have made a study of the quinhydrone electrode in order to determine more definitely the range of its applicability under physiological conditions. The validity of the method depends upon maintaining the ratio of the concentrations or more correctly of the activities of the quinone and hydroquinone strictly equal to unity or to some other constant and known value.

The factors which may upset this relationship are: (A) Deviations from the simple oxidation-reduction equation of Peters due to hydroquinone acting as a weak dibasic acid. (B) Changes in the activities of the dissolved quinone or hydroquinone molecules due to the presence of salts. (C) The presence of other oxidizing or reducing substances which react with the quinone substances with measurable velocity.

In order to show the relative importance of these factors we have derived the following general form of the expression for the oxidation-reduction potential (electron pressure) at an inert electrode which will take into account the factors (A) and (B) at varying hydrogen ion and salt concentrations:

$$\text{Observed E.M.F.} = \pi_0 + 0.059 \log A_{H^+} + \frac{0.059}{2} \log \left(1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^{+2}]}\right) + \frac{0.059}{2} \log \frac{A_{\text{quinone}}}{A_{\text{hydroquinone}}}$$

where π_0 is the normal potential of the quinhydrone system and has the value +.6990 volts on the hydrogen electrode

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[4] LaMer and Baker, *Jour. Amer. Chem. Soc.*, 1922, xliv, 1954.

[5] *Ann. Chim.*, 9th Ser., 1921, xv. 119.

scale or +.4250 volts when measured against the saturated calomel cell at 25° C. The temperature coefficient is —.77 millivolts per degree, K_1 and K_2 are the primary and secondary acidic (phenolic) ionization constants of hydroquinone. The symbol "a" refers to the activity or thermodynamic concentration of the respective substances.

Subject to the restrictions given below this general equation reduces to the simplest form

$$P_H = -\log A_{H^+} = \frac{\pi_0 - \text{observed E.M.F.}}{0.059}$$

which differs from the usual hydrogen electrode formula only in the magnitude of π_0; namely, by .6990 volts.

From the values for K_1 and K_2, determined by Sheppard[6]; namely, 1.8×10^{-10} and 4×10^{-12}, it can readily be seen that the term involving these constants, which is the correction for factor (A), becomes negligible (less than 0.2 mv.) when the reaction is more acid than P_H 8.0, but that it rapidly increases in magnitude as the solution is made more alkaline.

With regard to factor (B) our experiments have shown that the total salt concentrations present in physiological solutions, except in certain special cases, are seldom sufficient to affect the activities of the quinone substances appreciably, but in any given case the magnitude of this effect may be evaluated and taken into account by determining the activity coefficients from the solubilities of the quinone and of the hydroquinone in pure water and in the salt solution under examination, according to the equations:

$$\text{activity } (a) = \text{activity coefficient } (f) \times \text{molar concentration } (f)$$

$$\text{in salt solution} = \frac{\text{solubility in salt solution}}{\text{solubility in pure water}}$$

In contrast to (A) and (B), factor (C) is important for solutions that are exposed to oxygen owing to the conversion of hydroquinone to quinone which alters the equimolecular ratio between these two substances. The as yet unpublished results of kinetic experiments by LaMer and Rideal on the mechanism of the autoxidation of hydroquinone have shown

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[6] *Trans. Amer. Electrochem. Soc.*, 1921, xxxix, 428.

that the velocity of this reaction varies inversely as (hydrogen ion concentration)$^{3/2}$ and further that it becomes of appreciable magnitude on shaking vigorously in air when the reaction is more alkaline than about P_H 7.8. Apparently hydroquinone will not absorb oxygen until the alkalinity is sufficient to cause phenolic ionization through salt formation.

In order to get more definite information on the stability of the quinhydrone electrode potentials to air over a wide range of P_H, we have carried out careful parallel electrometric titrations at 25° C. of 0.2 molar hydrochloric acid, acetic acid, potassium acid phosphate and borate buffer mixtures with 0.2 molar caustic soda, using in one case the hydrogen electrode and in the other the quinhydrone electrode in the presence of air. We have directed particular attention to the point in the alkaline range at which the quinhydrone electrode first begins to give values that are different from those given by the hydrogen electrode.

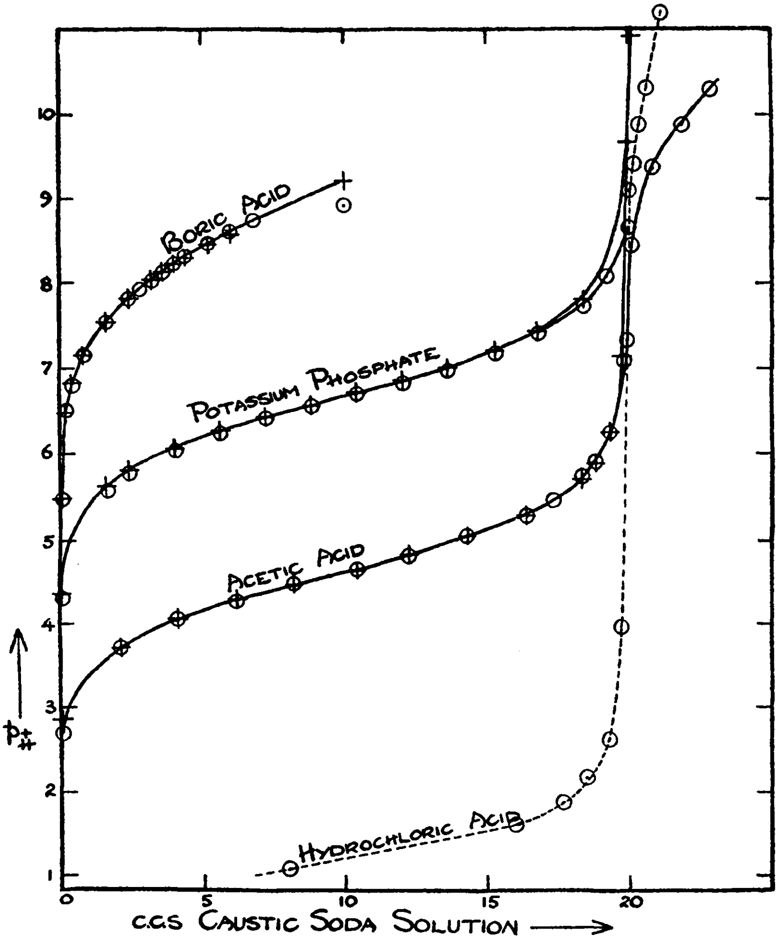
The result of these experiments, some of which are represented graphically in the figure, may be summarized as follows:

1. The quinhydrone electrode furnishes an admirably simple and rapid way of determining the end points and titration curves of acids that are stronger than monopotassium phosphate; *i.e.*, having values of K_2 greater than 10^{-7}. The method should be valuable in determining the ionization constants of unsaturated acids.

2. The divergence between the hydrogen electrode and the quinhydrone electrode values is negligible up to P_H 8.0.

3. Since the quinhydrone electrode is a one phase system and therefore attains electrode equilibrium promptly, it can be used for the determination of electrometric end points by continuing the titration curve beyond P_H 8, provided that one works rapidly and avoids undue shaking in air. See boric acid curve for concordance beyond P_H 8.0.

We are extending these investigations to the substituted hydroquinone-quinone systems with the object of choosing appropriate electrode materials for more alkaline ranges and particularly for the determination of the reaction of oxygenated blood.



Titration curves obtained with the hydrogen and with the quinhydrone electrodes in the presence of air.

Ordinates—Values of P_H.

Abscissæ—c.c.'s. of 0.2 MNaOH added to 20 c.c.'s of the acid component.

+ hydrogen electrode values.

⊙ Quinhydrone electrode values.