

Positive reactions of varying intensity were obtained with a number of these sera, although they were in no case so pronounced as with the antisera for *B. enteritidis* or its culture filtrates.

Although both of these fractions apparently possess elements in common with other members of the colon-typhoid group, they seem to be antigenically distinct from each other; *i. e.*, antisera obtained with the water-soluble fraction was precipitated by the water-soluble fractions in relatively high dilutions, but by the water-insoluble fraction was very slightly precipitated in dilutions of 1 to 10. Since these crude fractions have not been purified, it would be surprising if some cross reactions did not occur.

The relation of the antigenic activity of culture filtrates to the proteins contained in these crude fractions, and the relation of these proteins to the colon-typhoid group as a whole, cannot be determined until the materials are purified.

This is a preliminary report.

¹ Branham, S. E., and Humphreys, E. M., *J. Infect. Dis.*, 1927, xl, 516.

² Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1925, lxvi, 495.

³ Branham, S. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1927, xxiv, 349.

⁴ Koeh, F. C., *J. Lab. and Clin. Med.*, 1926, xi, 774.

⁵ Branham, S. E., *J. Infect. Dis.*, 1925, xxxvii, 291.

3679

Comparison of Electromotive Effect of Concentration on Tissues, Proteins and Other Substances.

R. BEUTNER, P. O. HUTH AND C. K. BUSH.

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville.

Previous work in this laboratory¹ led us to conclude that proteins cannot be the essential cause of animal electricity. We then investigated the relation of salt concentration to electromotive forces which is characteristic for tissues. This seemed particularly important in relation to the recent work of Mond.²

Uninjured green plants show the greatest electromotive effect of the concentration, and exhibit also a striking regularity.³ With animal organs, such as excised muscle, etc., this effect is much smaller and variable owing to unknown conditions, *but it is almost always in the same direction, viz., the dilute solution is on the positive side.* Mond,² working in Höber's laboratory, has tried to match

this effect of concentration on tissue with gelatin; he observed $+1/1 \text{ KCl} // \text{gelatin (acid or neutral)} // 1/512 \text{ KCl} = 0.06 \text{ volt}$.

It is clearly seen that the direction of e. m. f.* is *opposite* to that of almost any tissue. With alkali added to gelatin (amount not given) Mond observed, $-1/1 \text{ KCl} // \text{gelatin (alkalin)} // 1/512 \text{ KCl} + 0.02 \text{ volt}$. This is in the same direction as tissue, but too small, since the uninjured plants (used in place of gelatin) produce an e. m. f. *four to five times* as large in this cell arrangement. It is true that most tissues exhibit a small electromotive effect of concentration, and some of them none at all. It is possible, therefore, to find some tissue which will resemble Mond's alkaline gelatin cell, however small its e. m. f. is. But it should be remembered that the number of substances which exhibit some effect of concentration is almost unlimited. This is evident from many former investigations. According to R. Beutner² water immiscible organic liquids will produce electromotive forces of 0.03 to 0.03 volt when used as central conductors between 1/10 and 1/1000 mol. KCl solutions. All these forces are in the same direction as Mond's alkaline gelatin cell (or tissues). Such immiscible liquids are cresol, guaiacol or other OH-compounds, acetophenon, benzophenon or other ketones; also esters like ethyl acetate, butyl acetate, aceto acetic ester, and many other similar compounds. Considering, however, that the chemical nature of all these substances is different from that of tissue constituents, we tested a true lipoid, lecithin, with no addition, for the same interval (1/10 to 1/1000 mol.). This produced 0.03 volt. These measurements, which were repeated several times with the same result, were performed by immersing a lecithin plug, first into 1/1000, and then into 1/10 mol. KCl solution, using the technique described previously.¹ The difference between these measurements then gives the figures quoted.

Such small electromotive effects of concentration are not limited, however, to water immiscible substances as middle conductors. It is quite well known that water and dilute aqueous solutions produce an effect in the same direction as tissues. The attention of physiologists has been called long ago to the fact that the insertion of a slightly acid solution between two KCl solutions of different concentration produces an electromotive force.⁴ More recently Rohonyi⁵ again demonstrated a small and inconstant effect of the concentration with dilute solutions of lactic acid, K_4FeCN_6 , HCl, or KOH[†]

* e. m. f. = electromotive force.

† By comparing these experiments of Rohonyi with Mond's alkaline gelatin cell, we may conclude that the latter acts due to its alkali content alone, that the gelatin plays no part.

as central conductors between 1/1000 and 1/10 mol. KCl solutions, and that these were in the same direction as Mond's alkaline gelatin cell, amounting to 0.04 to 0.06 volt (extrapolated). We have corroborated these findings by new experiments with our own technique,¹ using now as central conductor between 1/1000 and 1/10 KCl a *plug of agar*, either with or without traces of acid and alkali in it. In this way we have observed the same effect and the same magnitude or even higher than Rohonyi or Mond did with alkaline gelatin or tissue, as the following figures show. One per cent agar gel with no addition as a central conductor between 1/10 and 1/1000 molecular KCl = solutions: 0.02 volt. With a slight addition of an acid (viz. 0.1 cc. 1/1 normal HCl to 50 cc.) the e. m. f. is 0.006 volt. With a slight addition of an alkali, the e. m. f. is 0.015 volt. Mond's alkaline gelatin cell would exhibit 0.02 volt for the interval 1/10 to 1/1000. (This also shows that acid or neutral agar do not produce opposite effects like gelatin; the same finding had been reported by Fujita⁷). Similar results were obtained with other non-protein colloids such as starch paste or kaolin in the place of agar.[‡]

Summary: It is more difficult to find a material which fails to exhibit any electromotive effect of the concentration than one which does. (Concentr. KCl = solution or similar salt solutions would be about the only kind of central conductors available.) It would be useless, therefore, if we tried to identify the small effect of the concentration on the e. m. f. of animal tissue, which ranges from 0.01 to 0.06 volt, with that of any definite substance or material. Almost anything will work as a model for animal electricity if the low electromotive effect on animal tissue is chosen as criterion. There is nothing typical about Mond's alkaline gelatin cell to justify its use as a model. Moreover, it is doubtful whether its action is not due to the alkali alone.

[‡] Experiments more recently described by Michaelis and his co-workers⁷ further illustrate the fact that almost any material may serve as a bioelectric model as far as a small effect of concentration is concerned. Fujita,⁶ Michaelis and Kokan⁷ have measured the e. m. f. produced by such materials as parchment, caoutchouc, wax, etc., used as central conductors between 1/10 and 1/100 molecular KCl solutions. They found for parchment and paraffin about 0.01 volt, for wax and mastix about 0.02 volt, for caoutchouc 0.03 volt (As the range of concentration is ten times smaller here, the voltage should be doubled for comparison with the measurements cited.) Loeb (in his well known book on colloidal behavior) finds an e. m. f. of 0.02 volt for highly acid gelatin between 1/1000 and 1/10 KCl (extrapolated). This is also not larger than the effects on the majority of materials quoted above. Loeb's figures are not comparable with those of Mond, owing to his entirely different method, and mainly because equilibrium is established at the surface of the gelatin.

The maximal electromotive effect of the concentration as seen in plants is the only suitable object for artificial reproduction, as its magnitude is much higher, ranging from 0.09 to 0.1 volt for the interval 1/1000-1/10 mol. KCl, and particularly because in this case only a somewhat limited number of selected substances is exclusively capable of reproducing it. Such substances are: salicylic aldehyde, nitrobenzene solutions of salicylic acid, picric acid, fatty acid, lecithin, or solutions of these acids in some other solvents like guaiacol,³ also nitrocellulose (collodion) in an air dried state (according to recent investigations of Michaelis and Fujita.⁸ As an example, the effect may be quoted which is observed with a guaiacol + oleic acid mixture as central conductor between 1/1000 and 1/10 KCl; this is 0.1 volt, a value about five times as large as that given by Mond for his alkaline gelatin cell.³)

¹ Beutner, R., and Menitoff, A., *Proc. Soc. Exp. BIOL. AND MED.*, 1927, xxiv, 462.

² Mond, R., *Pflüger's Archiv.*, 1924, cciii, 247.

³ Beutner, R., *Entstehung electr. Stromen in Geweben Stuttgart*, (F. Enke) 1920. *J. Am. Chem. Soc.*, 1913, xxxv, 344, and 1914, xxxvi, 2046. *Am. J. Physiol.*, 1913, xxxi, 343.

⁴ Blom, Oker, *Pflüger's Archiv.*, 1901, lxxxiv, 191.

⁵ Rohonyi, *Biochem. Zeitschr.*, 1914, lxvi, 231 and 248.

⁶ Fujita, A., *Biochem. Zeitschr.*, 1925, clix, 370.

⁷ Fujita, A.; also Michaelis, L., and Dokam, S., *Biochem. Zeitschr.*, 1925, clxii, 255 and 258.

⁸ Michaelis, L., and Fujita, A., *Biochem. Zeitschr.*, 1925, clvii, 47.

3680

The Effect of Hyper- and Hypotonic Solutions on Oxidations by the Red Blood Cell.

G. B. RAY.

From the Department of Physiology, Western Reserve University.

It has previously been reported¹ that under the proper conditions of nutrient supply, the adult red blood cell has a well defined metabolism. At the same time it was shown that dialyzed solutions of hemoglobin have the power to oxidize the same substrate, sodium lactate to CO₂. While the oxidation by the red cell is of long duration and considerable magnitude as compared with the same activity in the case of hemoglobin, one may suppose the blood pigment is associated with this oxidative activity of the cell. That this is the