

I showed that with a Wheatstone bridge with equal ratio arms the only impedance that would balance the erythrocytes with a change of frequency of the measuring electric current from 500 to a million cycles

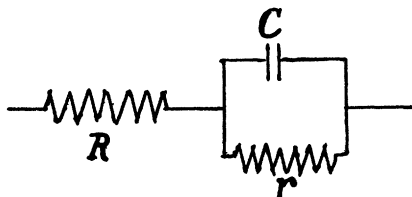


FIG. 1

per second (Fig. 1) was a resistance R in series with a "leaky condenser" consisting of a capacity C and a parallel resistance r , which must be discovered by trial and error. But time was saved by first setting R equal to the total impedance at the highest frequency. For instance, when the total impedance at a million cycles was 124 ohms, R was found to be 105 ohms. The closeness of these values is striking when it is noted that the total impedance at 500 cycles was 734 ohms.¹⁰ At infinite frequency the total impedance would equal R , but technical difficulties with the Wheatstone bridge method and theoretical difficulties such as change of specific inductive capacity of water at very high frequencies led me to limit the high frequency at 1,500,000 cycles¹¹ and assume the total impedance at this frequency to equal R .

In a study of frog's skeletal muscle I showed¹² that the electric impedance decreased on stimulation, and in a later paper,¹³ that the electric impedance of turtle's curarized skeletal muscle decreased when it was stimulated. This decrease in impedance was localized in the plasma membrane by showing that, on stimulation, R remained unaltered but r decreased whereas C appeared to increase.¹⁴ This has been denied by Bozler¹⁵ but confirmed by Dubuisson,¹⁶ who made a great improvement in the technique by the use of the cathode ray oscillograph, with which he could determine the changes in impedance during a thousandth of a second (σ).

Dubuisson used the high frequency impedance for R , and then at low frequency determined C and r . On stimulating the muscle there was no change in R , but there were two time-changes in the other values.

¹⁰ McClendon, *Jour. Biol. Chem.*, 69: 745, 1926, written before the paper by Philipsson was read.

¹¹ McClendon, *Protoplasma*, 7: 561, 1926.

¹² *Am. Jour. Physiol.*, 29: 302, 1912.

¹³ *Am. Jour. Physiol.*, 82: 525, 1927.

¹⁴ McClendon, *Protoplasma*, 7: 561, 1926.

¹⁵ The variation of electrical resistance of muscle during contraction. *Proc. Am. Physiol. Soc., Am. Jour. Physiol.*, 109: 14, 1934.

¹⁶ Ionogrammes de la contraction musculaire: Actualités scientifiques et industrielles 246, Paris (1935) contains references to other workers.

Three σ after stimulation r dropped 0.2 per cent. of its former value and rose again at 13 σ but fell again at 16 σ 8 per cent., and then rose again at 1000 σ . The changes in C were equal and opposite to those in r . The first wave is apparently due to increased permeability of plasma membranes of muscle *fibers* (corresponding to R wave of electromyogram), and the second wave to increased permeability of the *fibrillae* (corresponding to T wave of electromyogram).

It thus appears that the decrease in reactance (increased capacity) of the plasma membrane, as shown by Cole and others, represents an increased conductivity of the plasma membrane. The same conclusion was drawn by Hemingway and Collins.¹⁷ They state that the tissue may be considered as a series of leaky condensers (namely, the cell membranes) and the low resistance cell interiors. In such a case one condenser, if considered as a thin plate of specific conductivity s and dielectric constant k , would have a resistance r and a capacity C .

$$C = \frac{ks}{4\pi} \frac{1}{r}$$

In other words, the less the resistance r the greater the apparent capacity C .

By spectral analysis I showed that the permeability of the unfertilized frog's egg to ions is low¹⁸ but when fertilized, Na, K, Mg, Ca, Cl came out of them at an increased rate. Fahr¹⁹ showed that resting skeletal muscle is impermeable to Na and K, but Fenn and Cobb²⁰ showed that stimulated muscle lost about 15 per cent. of its K, which was replaced by Na from the medium. In other words, the electric impedance studies are harmonious with chemical studies of permeability in the same way as I have shown for the blood corpuscles. Although the plasma membrane of the blood corpuscle is probably as highly resistant as serpentine stone, which may be used in electrical insulation, still it is sufficiently conducting to account for the diffusion of chloride ions (chloride shift) which takes place in the blood during its passage through the lungs.

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EXTRACTION OF VITAMIN B₁ FROM ADSORBATES

IN studies of concentration of vitamin B₁, the adsorption method of Seidell has been extensively used. There have been several methods of extraction of the vitamin from the adsorbate, including the original Seidell method of extraction with alkaline solutions, the aqueous alcoholic hydrochloric acid method of

¹⁷ *Am. Jour. Physiol.*, 99: 338, 1932.

¹⁸ *Am. Jour. Physiol.*, 38: 163, 1935.

¹⁹ *Z. Biol.*, 52: 72, 1909.

²⁰ *Am. Jour. Physiol.*, 115: 345, 1936.

Stuart¹ and the quinine sulfate method of Williams.² Recently, Cook and Carroll³ have reported the successful use of pyridine solutions.

We have been studying the concentration of vitamin B₁ for the last two years and have made an extensive study of the extraction from the adsorbate. In general, the published methods have extracted the vitamin, but in most cases the yields were not high. The quinine sulfate method of Williams probably gave the best yields. Early in our work we observed that pyridine solutions extracted some vitamin from the adsorbate, but the yields were far from satisfactory. In marked contrast, we found that the acid salts, such as the hydrochloride of pyridine, quinoline, aniline and certain other nitrogen bases gave very high yields of vitamin; yields of 90 per cent. or better were ordinarily obtained. Even solutions of ammonium chloride gave better yields of vitamin than solutions of either ammonia or hydrochloric acid.

Aqueous, aqueous alcoholic or alcoholic solutions of some of the acid salts of these bases have been extensively used in our studies. A more detailed description of the procedure of extraction will be contained in a later publication.

Our studies appear to show that the acid salts of certain nitrogen bases have the property of freeing the vitamin B₁ from the fuller's earth or Lloyd's reagent. These salt solutions have a much more marked effect than either the base or the acid alone. In fact, our evidence indicates that a part at least of the extractive power of the nitrogen base or hydrochloric acid is due to the formation of acid salts with either the acid or the nitrogen bases adsorbed with the vitamin on the fuller's earth.

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VITAMIN C IN AN ESTRIN PRODUCING OVARIAN TUMOR

It has been already indicated by the authors¹ that vitamin C seems to be related to the formation of progesterone in the corpus luteum, but not to the production of estrin in the ovary. Weight is added to this latter idea by an examination of a rare estrin-producing ovarian tumor which we were fortunate enough to obtain from an operation upon a 19-year-old girl.

The pathological and clinical aspects of this case are

¹ E. H. Stuart, R. J. Block and G. R. Cowgill, *Jour. Biol. Chem.*, 105: 463, 1934.

² R. R. Williams, R. E. Waterman and J. C. Keresztesy, *Jour. Am. Chem. Soc.*, 56: 1187, 1934.

³ C. A. Cook and R. H. Carroll, *Ind. and Eng. Chem.*, 28: 741, 1936.

¹ G. R. Biskind and D. Glick, *Jour. Biol. Chem.*, 113: 27, 1936.

to be reported in detail by Drs. G. Y. Rusk, R. Rypins and A. Palmer. Hormone studies carried out by Dr. Allan Palmer² showed that the tumor contained the estrogenic equivalent of 11.7 γ theelin per gm desiccated tissue, and 50.0 γ per liter cyst fluid. The daily excretion of estrogenic substance in the urine was about 66.0 γ per 24 hours before operation, and fell to normal limits after removal of the tumor. All estimations for gonadotropic hormones were negative.

The vitamin C concentration was determined by titration of a 2 per cent. metaphosphoric acid extract of the tissue with 2, 6-dichlorophenol-indophenol, and was found to be 0.20 mg per gm fresh tissue. Using a technique previously employed,³ it was estimated that fibrous tissue composed 18 per cent. of the weight of the tumor; hence if the reasonable assumption is made that practically all the vitamin C is contained in the parenchymal cells, it may be calculated that there is 0.24 mg of vitamin C per gm of parenchymatous tissue.

From the cell-counting procedure⁴ the number of parenchymal cells per mg was found to be 712×10^3 .

It follows, then, that there is $\frac{0.24}{712 \times 10^3}$ or $0.34 \times 10^{-6} \gamma$ vitamin C per cell.

Up to the time of operation the patient was on a high vitamin C diet.

The comparatively low concentration of vitamin C in this tissue, which has been generating estrin in large amounts, would emphasize the previous suggestion that vitamin C is unrelated to estrin formation in the ovary.

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AN ARTHROPOD VECTOR FOR EQUINE ENCEPHALOMYELITIS, WESTERN STRAIN¹

IN a forthcoming report² we have described experiments which suggest that the "gopher," *Citellus richardsonii* (Sabine), may serve in nature as a reservoir host for the virus of equine encephalomyelitis, Western strain. We record this supplementary note at the present time to permit workers in the field to use

² Hormone studies were made possible by a grant from the Christine Breon Research Fund, the Department of Obstetrics and Gynecology of the University of California Medical School.

³ D. Glick and G. R. Biskind, *Jour. Biol. Chem.*, 114: 1, 1936.

⁴ D. Glick and G. R. Biskind, *Jour. Biol. Chem.*, 110: 1, 1935.

¹ From the Department of Bacteriology, University of Rochester, School of Medicine and Dentistry, Rochester, New York.

² Jerome T. Syverton and George Packer Berry. *Proc. Soc. Exp. Biol. and Med.* In press.