dency toward a decreasing daily excretion from Sunday to Saturday. 3. These results are interpreted as supporting the claim of a fatigue effect upon protein consumption. 4. It is shown that in spite of this tendency specimens collected on any day of the week may be used to give an accurate idea of the protein consumption of the group if the group is large enough.

4594

Source of Bioelectricity, Investigated by the Relation Between Stainability and Electric Charges in Tissues and Artificial Models.

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Little is known about the electrical action inside of living tissue. Its nature and cause can be elucidated to a certain degree by comparing stainability and electromotive forces.[1]

Numerous previous experiments have demonstrated the following relation between the stainability of tissues and bioelectric currents. Structures bearing a relatively negative charge are preferably stained by eosin and certain other acid dyes, while electrically positive struc-

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[1] It may be added that electrical potential differences must be present in tissue everywhere, *viz.*, at every phase boundary and also at any place where diffusion occurs. The electromotive property of the skin of plants is analyzed by its extraordinarily large and regular variations following changes in the concentration of the solutions in contact with it. This effect can *not* be reproduced by means of protein as has been maintained by J. Loeb. (Loeb, J., *Proteins and Colloidal Behavior*, 1922; Höber, *Zeitschr. physik chemic.*, 1924, cx, 142.) None of the values given by Loeb, Höber, and their collaborators amounts to more than one-fifth of the maximal effect of concentration obtainable in plant and animal tissue.

The so-called 'protein' effects demonstrated by Höber with various salts are, moreover, just water effects. His assertion that this of itself exclusively should explain bioelectricity is contradicted by numerous facts. With a *few selected* substances only the maximal effect of concentration can be reproduced. Among these substances is dried collodion. The electromotive action of this substance need not necessarily be explained as L. Michaelis (*Biochem. Zeitsch.*, 1925-26, nine papers; also *J. Gen. Physiol.*, 1927-29, eight papers) suggest, as due to pores of molecular dimensions which cause selective ionic permeability. This case does not necessarily require an entirely different theory from other similar cases.

tures seem to attract methylene blue and other staining bases, as expressed by the following scheme:

+ Saline solution	*basophilic* structure (stained by *methylene blue*)	*acidophilic* structure (stained by *eosin*)	saline solution —

G. W. Crile was the first to indicate a similar relation in his so-called Bipolar Theory, which is the expression of his finding that the stainability of tissue runs parallel to the electric potential of its current of injury.[2] Crile found that both stainability and potential are decreased in exhaustion, chronic poisoning, hemorrhage, shock, infection, etc. J. Gicklhorn, R. Kellar and others have offered definite experimental proofs of the above relation by studying the stainability and electromotive forces of plant tissues.[3]

Artificial systems have now been found which exhibit a relation between stainability and electromotive forces similar to that observed in living tissues. One such system is the following:

+ Saline solution pH = 7.4	layer of olive oil + oleic acid (with or without amine basophilic) I	layer of olive oil + amylamine (acidophilic) II	saline solution — pH = 7.4

When shaken with a mixture of methylene blue and eosin (Wright's stain or Pianese's mixture), I stains like basophilic tissue; for example, the nucleus; II stains like acidophilic tissue; for example, the cytoplasm.

Various other oils may be used, cocoanut oil, tributyrin, or triacetin; and also various esters, ethyloleate, ethylcaproate, ethylbutyrate, ethylacetate or amylacetate, cetylacetate, etc. The use of lecithin or cholesterol as a neutral fatty solvent offers considerable experimental difficulties, but it has been observed, at least qualitatively, that similar electric effects can be produced by the addition of oleic acid or amine. Amylalcohol, or other higher alcohols with or without the addition of hydrocarbons, can also be used. It appears to be the rule in a system of the kind described that any neutral water-immiscible solvent becomes electrically positive by the addition of an oil-soluble acid, and electrically negative by the addition

[2] Crile's original publication on the "Bipolar Theory" fails to contain direct measurements of potential differences; these were made a short time later by him and his collaborators and the results were found to agree with the theory as stated in his book. (Crile, *Arch. Surgery*, 1921-25, six articles.)

[3] R. Keller has suggested that this relation should exist and has induced J. Gicklhorn to perform the measurements. (Keller, *Elektr. in der Zelle*, 1925; Gicklhorn and Umrath, *Protoplasma*, 1928, iv, 228.)

of an oil-soluble base—positivity being associated with basophilic staining and negativity with acidophilic staining.

From the standpoint of physical chemistry this finding must be expected, since *phase boundary potentials* are located at the junction between any of these water-immiscible layers and the aqueous solutions in contact with them. These potential differences must be differentiated in the direction actually observed on account of a soap content of the oleic acid layer; which leads to an excess of Na ions in that layer. On the other hand the presence of amine has no such action. The amine, on the contrary, combines with any oil-soluble acid constituents which may be present, *e. g.*, oleic acid from saponification of olive oil—and thus tends to diminish the concentration of the Na+ ions in the olive oil. Consequently the cell arrangement, mentioned above, is really a concentration cell in regard to Na+ ions:

	Na+ in water	Na+ in oil	Na+ in oil	Na+ in water
+	about 0.1 molecular	high (Na oleat)	low (amine)	about 0.1 molecular —

According to well known laws, this system must have an e.m.f. in the direction observed.†

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Composition of Bone IX. Equilibration of Serum with $CaHPO_4$.

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Because of the complications introduced by the presence of proteins in serum, we studied first the solubility equilibria in protein-free solutions. We[1] found that, at the pH of serum, solutions with the inorganic composition of ricketic serum are markedly undersaturated with respect to $CaHPO_4$. Solutions with the calcium and phosphorus content of normal blood serum are slightly undersaturated; it is only solutions which have Ca x P products greater than about 50 which are supersaturated with respect to $CaHPO_4$.

Since part of the calcium in serum is bound to protein, it was

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[1] Shear, M. J., Washburn, M., and Kramer, B., *J. Biol. Chem.*, 1929, lxxxiii, 697.