GIANTS IN NEPHROLOGY

Jacques Loeb (1859-1924) and His Forgotten Contributions to Electrolyte and Acid-Base Physiology in The Organism as a Whole

Francesco Sgambato[1], Ester Sgambato[2], Natale Gaspare De Santo[3]

(1) Department of Medicine, Hospital Sacro Cuore di Gesù-Fatebenefratelli, Benevento
(2) Policlinico Casilino, Rome
(3) Department of Medicine, Second University of Naples, Naples, Italy

Address correspondence to:
Francesco Sgambato, MD; Via Appia Antica 344, 81028 S. Maria a Vico, Caserta, Italy; Cell:+39 328 4491155 e-mail: sgambatof@gmail.com
Natale Gaspare De Santo; Emeritus, Second University of Naples, Department of Medicine, Pad. no.17, Nephrology, Policlinico, 5 Via Pansini - 80131 Naples, Italy; Cell:+39 348 4117376 e-mail: nataleg.desanto@unina2.it

Abstract

Jacques Loeb (1859-1924) was the founder of the Journal of General Physiology which he co-directed in association with W.J.V. Osterhout in the years 1918-1924. Having worked (1889-1891) at the Marine Zoological Station of Naples, newly founded by Anton Dohrn, he was imprinted for life. A strong investigator used to perform the experiments personally. Loeb was engaged lifelong in the explanation of life on physico-chemical basis. He touched various fields (being a creative scientist full of ideas), and centered on the exchanges of electrolytes, acids and bases between the body and sea water in fish. He identified two equations:

$$\{[K^+]+[Na^+]\}:\{[Ca^{++}]+[Mg^{++}]\}\& \quad \text{(Loeb's 1}^{st}\text{ equation)}$$

$$\{[K^+]+[Na^+]\}:\{[H^+]+[Ca^{++}]+[Mg^{++}]\} \quad \text{(Loeb's final equation)}$$

Even nowadays these equations may have applications in a wide list of electrolyte and acid-base disturbances. Unfortunately his heredity has been dissipated.

Key words: Zoological Station Anton Dohrn, Ca^{++}, effects of Na^+, H^+, Jacques Loeb, K^+, Mg^{++}, Mg^{++} and H^+, mineral equilibrium, preservation of life, salts

Introduction

Many basic scientists have paved the way leading to our present understanding of electrolyte and acid-base balance, and in finding a rationale for normalizing its disequilibrium. Among them Jacques Loeb (1859-1924) had a preeminent role.

Jacques Loeb (Figure 1), from Maiden in Prussia, was born to a family of Jewish immigrants from Portugal at the time of the Inquisition. He attended the Askanische Gymnasium in Berlin, and studied medicine in Strasbourg where in 1884 he got his MD.

Subsequently he worked with Nathan Zunz (a precursor physiologist of altitude and aviation) in Berlin, and with Adolf Fick (Figure 2) in Würzburg. There he also learned a lot on plant physiology from the charismatic botanist Julius von Sachs (Figure 3). Finally he was ready to take over the assistant position made available by Friedrich Goltz in Strasbourg.

In the years 1889-1891, Loeb worked at the Marine Zoological Station in Naples. At the age of 32 years, he moved to the United States where he later became professor and chief at the Universities of Chicago, California at Berkeley, and the Rockfeller Institute in New York. There he founded and co-directed till death, the *Journal of General Physiology*.

When he moved to his final position in New York, he had clear in mind the concept of translational biology. In fact he had written to Simon Flexner, the first director of the Institute, "in my opinion experimental biology - the experimental biology of the cell

Figure 1.
Jacques Loeb in 1921 (Archives Stazione Zoologica of Naples, Anton Dohrn).

Figure 2.
Adolph Fick (Courtesy of Professor August Heidland)

- will have to form the basis not only of physiology but also of General Pathology and Therapeutics".

One can read this from a letter published in the landmark biography of Loeb by H.J.V.Osterhout [1].

Loeb aimed to apply physico-chemistry methods to the understanding of biological processes and explored the physiological effects of ions. In fact *The Journal of General Physiology* was created for papers "devoted to the investigation of life transport processes from a physico-chemical view-point. [...] The Editors invite contributions relating to the physic-chemical explanation of life phenomenon, no matter in what field of science they originate." [2] (full text).

It should be noticed that he did personally the major part of the experiments that appeared in literature. In the bibliography compiled by Nina Kobelt [3] it emerges that he published a total of 404 papers. For 363 of them he was the only author. In 40 papers there was 1 collaborator (for the majority of them Hardnolph Wasteneys). Only in one paper his name is associated with two coauthors.

Figure 3.
Julius von Sachs (Courtesy of August Heidland).

Figure 4.
Zoological Station of Naples after foundation (Archives Stazione Zoologica of Naples Anton Dohrn).

The importance of Loeb's early studies in Naples for subsequent achievements in life

Loeb went to the Zoological Station of Naples (Figure 4) on October 10, 1889 and left it on April 24, 1811. A working table was granted by the University of Strasbourg. Loeb arrived in Naples to work in a young prestigious institution that was newly established by Anton Dohrn on the sea shore of Naples.

The institution under the prestigious director (Figure 5), a fervent Darwinist, attracted scientists all over the world, because of its system based on Working Tables. This system was financed by governments, universities, scientific institutions from all over the world.

There the investigators were granted working facilities, living biological material from the Mediterranean sea and an enthusiastic environment for research that was nurtured personally by Anton Dohrn.

"The winter of 1889-90 he spent in Naples carrying on experiments on heteromorphosis and the deep migration of animals (in the latter in collaboration with Groom [4] and it was there that he became interested in America through his contact with Henry B Ward and W. W. Norman" [1].

We can say that Osterhout [1] failed to illustrate the fact that, at the Zoological Station of Naples, the

Figure 5.
Anton Dohrn (Archives Stazione Zoologica di Napoli Anton Dohrn)

Table 1. Scientists at the Zoological Station of Naples in th period October 10, 1889 to April 21, 1991

Working Tables	119
Students	2/119
Investigators	93/119
Professors	24/119

1. A. Meyer, Münster; 2. L. v. Graff, Graz; 3. C. Groben, Vienna; 4. L. Savastano, Naples; 5. A. Kowalesti, Odessa; 6. F. Vejdowsky, Prague; 7. A. Della Valle, Modena; 8. C. Emery, Bologna; 9. H. Ludwig, Bonn; 10. S.E. Apathy, Klausenburg; 11. O. Büschli, Heidelberg; 12. S. Exener, Vienna; 13. J. van Rees, Amsterdam; 14. P. Knoll, Prague;15. M.G. von Koch, Darmstadt; 16. O. Nüsslin, Karlsruhe; 17. H. Hambron, Lipsia; 18. F. Zschokke, Basel; 19. F. Hoppe-SDeyler, Strassburg; 20. W. Skìchimkewitch, Pertersburg; 21. M. Holl, Graz; 22. W. His, Lipsia; 23. F. Rückert, Munich, 24. A. Hansen, Darmstadt

(Compiled from data made available from the Archives of the Stazione Zoological Anton Dohrn of Naples)

physiologist, using the methods of a marine biologist, was turned into a biophysicist.

Naples was the place where Loeb's interest in marine biology was nurtured. So the studies at station Dorhn are the roots for research which granted him, later in life, an international reputation (he was even on the list of potential recipients of the Noble Price, however he was not awarded it). Indeed the atmosphere at the newly founded zoological Station of Naples was stimulating. There was time for exchange between scientists and great visitors which made the place unique. Anton Dohrn (Figure 5) was there to help nurture the creativity of those young international scientists. However, there were also renowned university professors.

During the stay of Jacques Loeb at the Zoological Station in Naples a total of 119 working tables were occupied, 2 by students, 93 by young investigators from all over the world, and 24 from full university professors. Among them were Felix Hoppe Seyler (1825-1895), the founder of biochemistry, and Wilhelm His Sr (1831-1904), the Swiss anatomist professor in Lipsia. So in the years 1889-1891 at the Station the ratio between young investigators and academicians with a track curriculum was around 4 to 1. It should be noticed that there were only 3 Italian professors, one of them being from Naples (Table 1).

Aims

The goal of this study is to illustrate the contributions of Jacques Loeb to the understanding ion movements in the living organisms.

Results

Defining a general law of mineral equilibrium for preservation of life

In a study of 1906, Loeb studied the stimulating and the inhibitory effects of magnesium and calcium upon the rhythmical contractions of a jellyfish (*Polyorchis*). He used as a model either the animals living in the Bay of San Francisco, or the isolated centre of this medusa that was deprived of its margin containing the sense organisms and the central nervous system [5] [6].

He showed that

1. "The spontaneous normal swimming motions of *Polyorchis* occurs only in presence of magnesium and that, their occurrence in sea water is due to the presence of magnesium in rather high concentration. [...] The effect of magnesium can be inhibited by the addition of an equivalent amount of calcium or of potassium"
2. "A pure solution of calcium chloride will cause the isolated centre to beat rhythmically, whereas it does not beat in normal sea-water. [...] The calcium acts in the same way when added to a sodium chloride solution" [...]. It requires much less barium chloride than calcium chloride to call forth immediately rhythmical contractions of the isolated centre in a sugar solution. [...] Strontium chloride also calls forth the rhythmical contractions of the centre when added to sea-water, sodium chloride solutions, sugar solution and distilled water. [...] It was practically impossible to produce rhythmical contractions by magnesium chloride"
3. "The decalcifying salts cause rhythmical contracts"
4. "Acids cause the isolated centre of *Polyorchis* to beat, while alkalies have the opposite effect". [...] Acid has the same stimulating effect when added to sea-water, only much more acid must be used than in sodium chloride, because of the presence of bicarbonates and magnesium chloride"
5. "Sea-water is considerably less injurious than the addition of an equivalent amount of calcium"

The role of salt for preservation of life

Loeb did seminal experiments on this topic and some intriguing results were published in *Science* in 1911 [4] and in the *Journal Biological Chemistry* in 1914 [7].

In *Science* he started pointing out that

1. "Less is known of the role of salts than on the role of the three main food-stuffs, namely carbohydrates, fats and proteins. As far as the latter are concerned, we know at least that through oxidation they are capable of furnishing heat and other forms of energy. The neutral salts, however, are not oxydizable. Yet it seems to be

a fact that no animal can live on an ash-free diet for any length of time, although no one can say why this should be so".

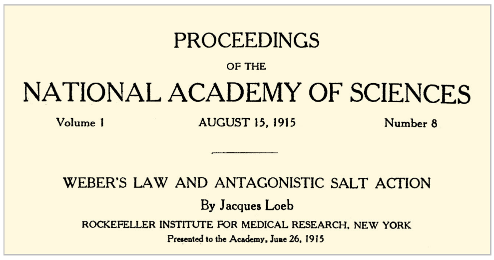
2. "The cells of our body live longest in a liquid which contains 3 salts, NaCl, KCl and $CaCl_2$ in a definite proportion, namely 100 molecule of NaCl, 2.2 molecules KCl and 1.5 molecules of $CaCl_2$. [...] NaCl, KCl and $CaCl_2$ exist in our blood in the same relative proportions as in the ocean. [...] KCl and $CaCl_2$ are only necessary to prevent the harmful effects that NaCl produces if it is alone in solution and if the concentration is > 1/8 M. [...] NaCl and KCl alone cause abnormal contractions of the heart which are rendered normal by the addition of $CaCl_2$. [...] A mixture of NaCl + $CaCl_2$ also causes abnormal contraction of the heart, but they are rendered normal by the addition of KCl".
3. "We are dealing, in other words, with a case of antagonistic salt action; an antagonism between NaCl in one hand and KCl and NaCl on the other. The discovery of the antagonistic salt action was made by Ringer who found that there is a certain antagonism between K and Ca in the action of the heart. Biedermann had found that alkaline solutions cause twitchings in the muscle and Ringer found that the addition of Ca inhibited these twitchings" [...]. "If we put the eggs of *Fundulus* immediately after fertilization into a pure sodium chloride solution which is isotonic with sea-water, they usually die without forming an embryo. If however only a trace of a calcium salt or of any other salt with a bivalent metal is added to the M/2 NaCl solution, the toxicity of the solution is diminished or even abolished. [...] Not only the bivalent metals are able to render the sodium chloride solution harmless, but the reverse is also the case, namely that NaCl is required to render the solution of many of the bivalent metals, harmless. [...]
   The antagonistic action of salts consists in a modification of the egg membrane by a combined action of two salts, whereby the membrane becomes less impermeable for both salts".

With pride he added that:

"In a series of papers beginning in 1900 [8] - Figure 6 - I have shown that:

(*a*) It is necessary for the normal functions of living organs and organism that the ration of the concentration of antagonistic ions (Na+K/Mg+Ca) of the surrounding solution be kept within certain limits. If the value of the quotient becomes either too high or too low, life phenomena become abnormal and finally impossible [9]".

(*b*) The salts to be considered as antagonist in this sense are in the first place those of univalent and bivalent metals and that therefore the most important critical quotient will generally be C_{Na+K}/C_{Mg+Ca}



PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 1 AUGUST 15, 1915 Number 8

WEBER'S LAW AND ANTAGONISTIC SALT ACTION
By Jacques Loeb
ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK
Presented to the Academy, June 26, 1915

Figure 6.
Loeb's paper on Weber's law and antagonistic salt action (Archives Stazione Zoologica di Napoli Anton Dohrn).

(*c*) There is also an antagonism between the salts of bivalent ions such as Sr and Mg and Ca. This was the genesis of Loeb's General Law of Mineral Equilibrium [9] (Figure 7).

"Life phenomena as a rule take place in a medium whose composition and concentration undergoes little or no variation, such as sea water or blood serum and the majority of the organisms cannot stand any wide variation from this fixed standard C_{Na+K} can be considered only the lower and upper limit for C_{Mg+Ca} can be determined for this value". He then tried to assess the possible lower and upper limit of C_{Mg+Ca} change when C_{Na+K} varies, and showed that it occurs in accordance with Weber's law [10].

The physiologically balanced salt solutions

He demonstrate since 1900 that water of the sea as well as blood are physiologically balanced by a mixture of "96 cc. 5/8 N NaCl + 2 cc. 10/8 N $CaCl_2$ + 2 cc. 5/8 N KCl". Years later he defended his primacy in a letter to *Journal Biological Chemistry* [11].

The effect of acids

Loeb had a great continuous interest on the effects of acids [12] [13] [14] [15]. In 1911 [6], Loeb in *Science* reported about the first time upon experiments he had made with Mr. Wasteneys on the toxic action of acids upon *Fundulus* and showed that "the toxic action of acids can be annihilated by salts". They showed that "not only butyric acid, could be rendered harmless by neutral salts, e.g., HCl by NaCl", They also showed

1st Loeb's Equation

$$\frac{[K^+] + [Na^+]}{[Ca^{++}] + [Mg^{++}]}$$

Figure 7.
Loeb's 1st equation

that $CaCl_2$ was "8 to 11 times as great and powerful as the action of NaCl".

It is of interest to stress that at that time Loeb had developed the idea that "each cell may be considered a chemical factory. Its work depends on the diffusion of substances into the cell is restricted if its composition is not made of fats". "The antagonism between acids and salts suggests the idea that the surface film of cells consists exclusively or essentially of certain proteins".

Loeb published data on the addition of acid to 4 days old eggs of *Fundulus*in distilled water and to eggs in salt solution. He added acetic acid (M/500) in order to measure the time to heart standstill of the embryo in the egg [16].

"It was found that the time in which the hearts stopped beating was much shorter when acid was added to distilled water than when it was added to salt solutions" [...]. "We will show that the protective action of salts is a distinct function of the nature and valency of the anion". In fact "the organic anions and the bivalent one being much more efficient than inorganic univalent anions Cl, Br, I and NO_3. The antagonistic action of salts with bivalent cations is very much greater than that of the univalent cations, $CaCl_2$, and to some extent $MgCl_2$ are much stronger antagonists to acid than were the chlorides of monovalent metals".

"The presence of acids also retards the diffusion of calcium into the egg". "To kill the embryo the acid must diffuse through the membrane of the egg and the question arises whether the salt in the outside solution inhibits or retards this diffusion or whether it diffuses with the acid into the egg and prevents the injurious action of the acid upon the embryo inside the membrane".

Loeb concluded

1. It is shown that salts inhibit the toxic action of acids upon the embryo of *Fundulus.*
2. This inhibitory action of salts is a function of the anion as well as the cation. Rhodanates, acetates, sulphates and tartrates inhibit very strongly, chlorides, bromides and nitrates much less, and iodides least of all. The bivalent cations Ca and Sr and to a smaller degree Mg also inhibit more strongly that the univalent cations.
3. The antagonistic action of the anions retarded the rate of diffusion of the acid through the membrane [16].

A general salt effect can be demonstrated also for the diffusion of acid through the membrane of *Fundulus egg*. The concentration of neutral salt required for the salt effect is considerably smaller in the case of diffusion of an acid than in the case of diffusion of potassium salt. Very weak acid solutions can supply the general salt effect. When the concentration of neutral salt added to the acid is a little higher, the diffusion of acid is retarded or inhibited (antagonistic salt action).

From Osterhout [1] we learn that "Loeb found that to a certain extent the behavior of potassium in entering the cells paralleled that of the acid. He observed that weak acids and bases appear to penetrate much more rapidly than strong ones, indicating that the protoplasm is not readily permeable to ions [...]. Later studies demonstrated that "The importance of hydrogen ions lies in the fact that in alkaline solutions the protein acts like an anion but in sufficient acid solutions it behaves like a cation. At the isoelectric point the two actions are approximately equal" [17].

This caused the inclusions of hydrogen ion in equation 1 which became the final equation (Figure 8):

"His program dealt with the fundamental properties of protoplasma as affected by ions. He used as a model the *Fundulus* whose eggs develop equally well in distilled water and/or sea water. Loeb was surprised to find that on adding to distilled water as much sodium chloride as contained in sea water the eggs could not develop, in other words the sodium chloride is toxic and it was evident that the other salt found in sea water must somehow overcome this toxicity. The announcement of this fact was received with genuine astonishment" [1].

Final Loeb's Equation

$$\frac{[K^+] + [Na^+]}{[H^+] + [Ca^{++}] + [Mg^{++}]}$$

Figure 8.
Loeb's final equation

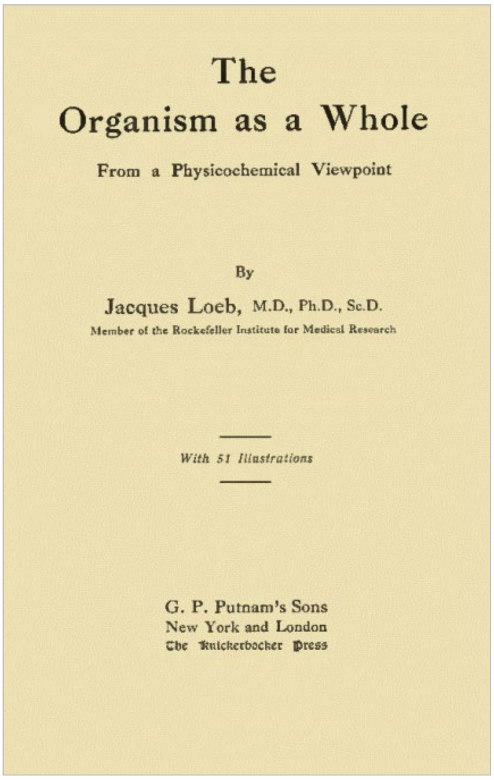
He found that the addition of all sorts of salts with bivalent or trivalent cations in the right proportion could more or less completely remove the toxicity due to salts with monovalent cations. He spoke of this as antagonistic salt action and called solutions as sea water, in which the toxicity is suppressed by the admixture of salt in the proper proportions, as physiologically balance solution [1].

In order to have antagonistic salt action toxic salts must be present in sufficient concentration to produce injurious effects and these injurious effects must be overcome by other salts which have a protective action [1].

Clinical applications of Loeb's equations

Loeb's equations are a dissipated heredity since they can still help in finding a rationale for understanding the effects of hyperkalemia, hyperkalemia with acidosis, hypocalcemia, hypocalcemia with alkalosis, and hypocalcemia with hypomagnesaemia [16] [17].

It should be noticed that all experiments were performed in the whole animal, as we learn [18] from his 1916 book *The Organism as a Whole* (Figure 9).



The
Organism as a Whole
From a Physicochemical Viewpoint

By
Jacques Loeb, M.D., Ph.D., Sc.D.
Member of the Rockefeller Institute for Medical Research

With 51 Illustrations

G. P. Putnam's Sons
New York and London
The Knickerbocker Press

Figure 9.
The Organism as a Whole (1916).

Acknowledgements

We wish to express heartfelt thanks to the people of the Archives of the Stazione Zoologica Anton Dohrn of Naples for continuous and expert assistance. They are capable, aware of their mission, work with enthusiasm and team spirit and keep the documents for posterity.

References

[1] Osterhout WJV. Biographical memoir of Jacques Loeb (1859-1924). Biographical memories XIII (IV Memmoir), National Academy of Sciences. 1930

[2] Andersen OS. A brief history of the journal of general physiology. The Journal of general physiology 2005 Jan; 125 (1): 3-12 (full text)

[3] Kobelt N. Jacques Loeb: bibliography. The Journal of general physiology 1928 Sep 15; 8 (1): LXI-LXXXVIII

[4] Groom TT, Loeb J. Der Heliotropismus der Nauplien von Balanus perforatus und die periodischen Tiefen wanderungen pelagischer Tiere. Biologisches Centralblatt 1890-1891; 10: 160-177

[5] Loeb J. The Stimulating and the inhibitory effects of magnesium and calcium upon the rhythmical contractions of a jellyfish (Polyorchis). J Biol Chem 1895-1906; I: 427-436

[6] Loeb J. The role of salts in the preservation of life. Science (New York, N.Y.) 1911 Nov 17; 34 (881): 653-65

[7] Loeb J. Is the antagonistic action of salt due to oppositely charged ions? J Biol Chem 1914; XIX: 431-443

[8] Loeb J. Weber's Law and Antagonistic Salt Action. Proceedings of the National Academy of Sciences of the United States of America 1915 Aug; 1 (8): 439-44

[9] Loeb J. The dynamic of living matter. New York 1906

[10] Loeb J. On the artificial production of normal larvae from unfertilized eggs of the sea urchin (Arbacia). Am J Physiol, 1899-1900, III, 434-471

[11] Loeb G. The origin of the conception of physiologically balanced salt solutions. J Biol Chem 1918; XXXIV: 503-504

[12] Loeb J. Chemische Konstitution und physiologische Wirksamkeit der Säuren Biochem Zeitschr 1909; 15: 254-271

[13] Loeb J. Über die Hemmung der Giftwirkung von Hydroxylionen auf das befruchtete Seegeleimittels Sauerstoffmangel. Biochem Zeitschr 1910; 26: 289-292

[14] Loeb J. Über die Hemmung der zerstörende neuštraler Salzlösungen auf das befruchtete Eimittels Cyankalium. Biochem Zeitschr 1910; 27: 304-310

[15] Loeb J. Über den Einfluss der Konzentration der Hydroxylionen in einer Chlornatriumlösung auf die relative entgiffendeWirkung von Kalium and Calcium. Biochem Zeitschr 1910; 28: 176-180

[16] Sgambato F, Sgambato E, Fucci A. La formula di Loeb: una ricca eredità dissipata. Emergency Care Journ, 2006; IV: 13-20

[17] Sgambato F, Prozzo S, Sgambato E. L'ABC dell'equilibrio acido-base "umanizzato" senza logaritmi. Diaconia Grafica, S. Maria a Vico, Caserta, 2015, pp. 211-220

[18] Loeb J. The organism as a whole, from a physico-chemical viewpoint. 1916, G.B. Putnam's Sons, New York, London