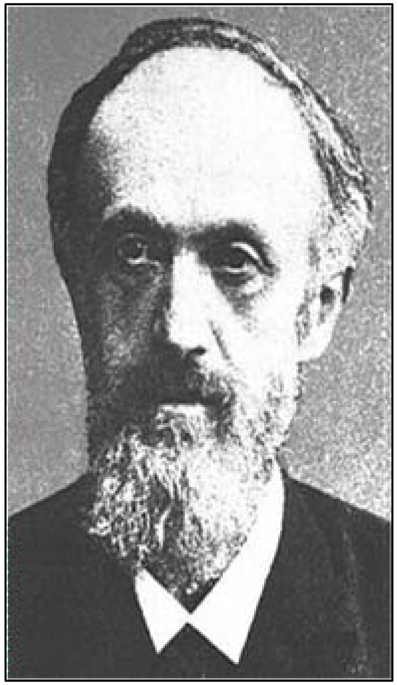
Sorption (bulk phase) theory of solute distribution between the living cell and medium:

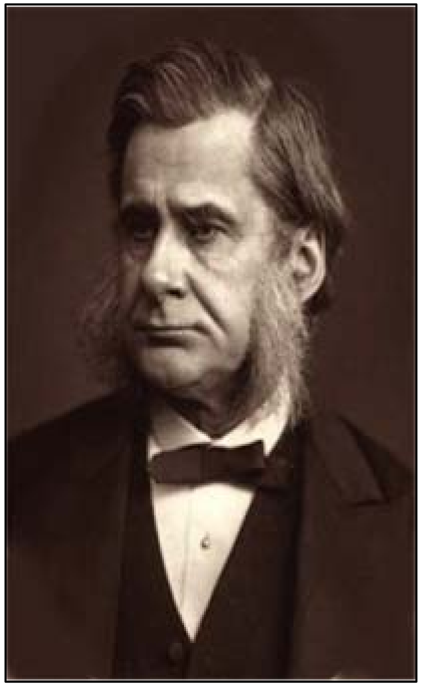
Huxley, Bernard, Nasonov, Troshin, Ling, Pollack

V. V. Matveev
Institute of Cytology, Russian Academy of Sciences
http://vladimirMATVEEV.ru

The ancestors of the two trends in representations of the physical nature of the living cell



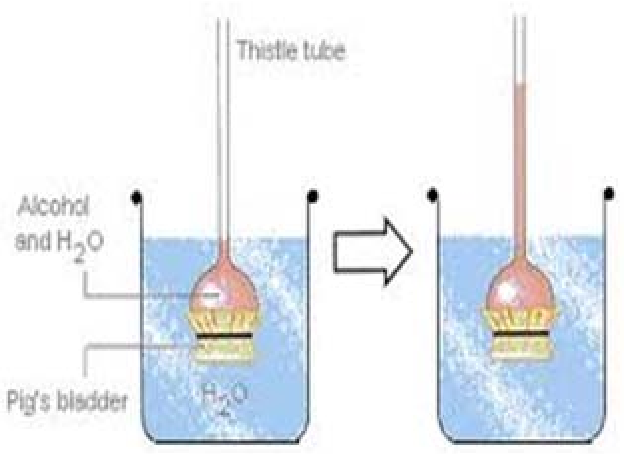
Moritz Traube
(1826-1894)



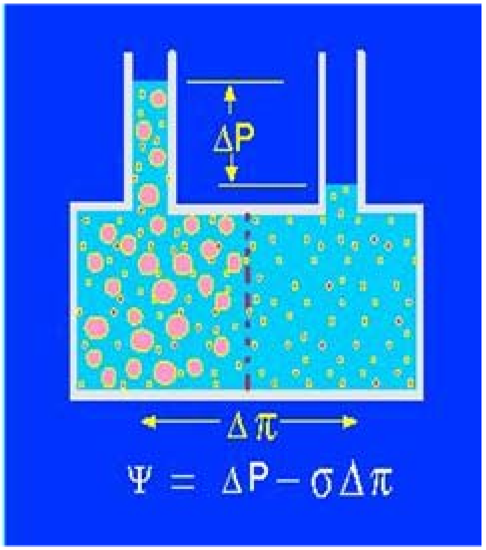
Thomas Henry Huxley
(1825–1895)

The living cell is a bag containing diluted solution of constituents separated by a semipermeable membrane

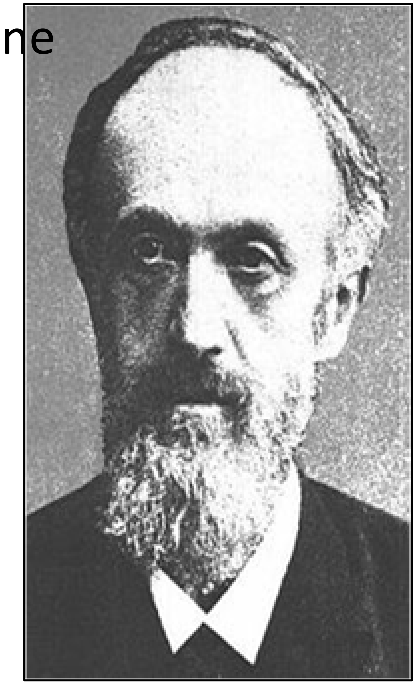
Artificial, copper ferrocyanide, membrane
(potassium ferrocyanide + copper sulfate)



Living-tissue membrane

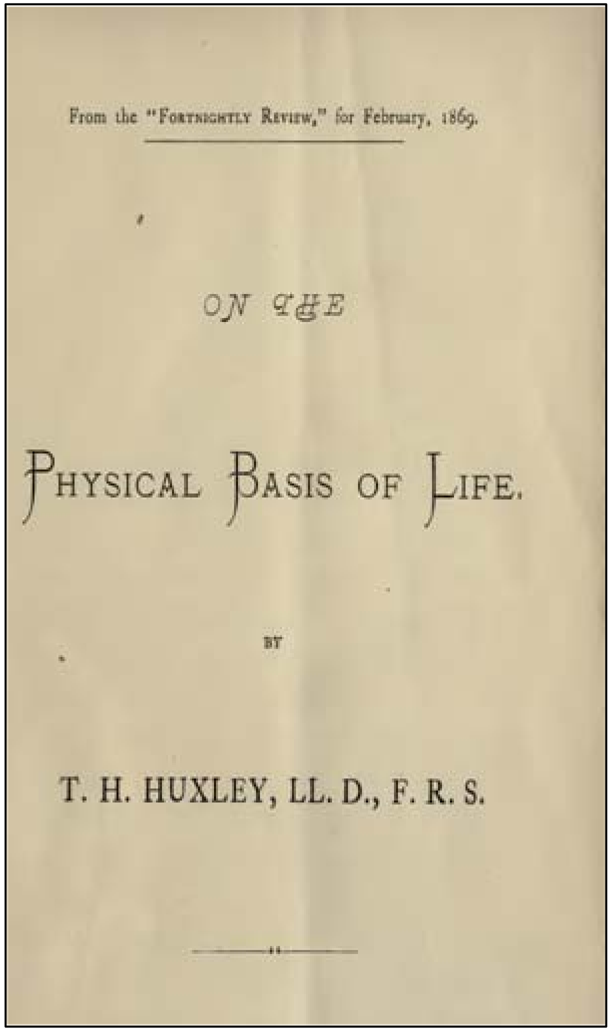


1864



Moritz Traube
(1826-1894)

The living cell is a bulk phase, protoplasm, the physical basis of life



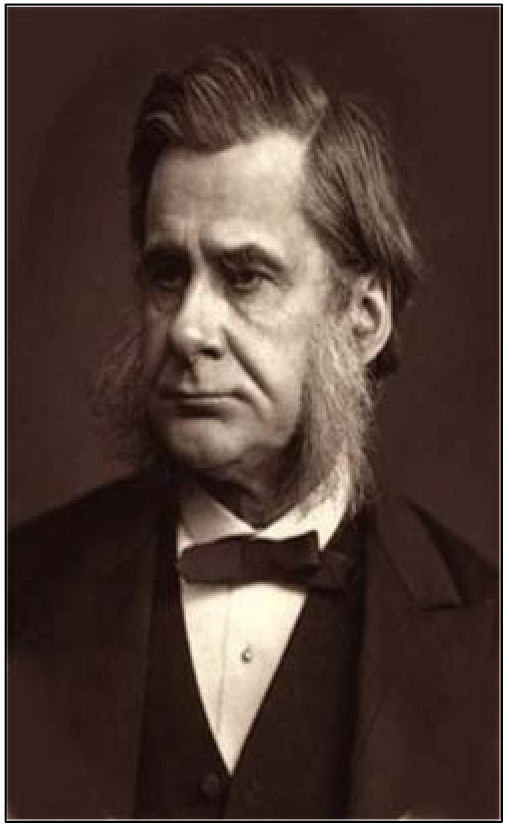
From the "Fortnightly Review," for February, 1869.

ON THE

PHYSICAL BASIS OF LIFE.

BY

T. H. HUXLEY, LL. D., F. R. S.



Thomas Henry Huxley
(1825–1895)

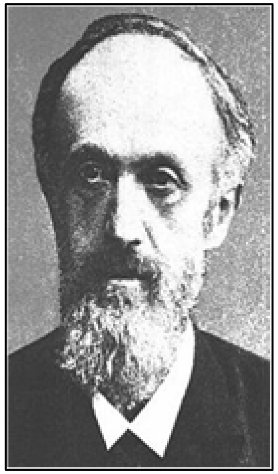
The basic principles of protoplasmic theory

- Protoplasm is the physical basis of life, the cell is only its geometrical form (physical principle instead of morphological one).
- Protein is the key component of protoplasm that determinates all features of the living state.
- Protein is the connecting link for all relationships and interactions in the living cell.
- Changes in protein state (reversible coagulation) is the substantional basis for cell responses to external stimuli (protein as an acceptor of stimuli).

Huxley T.H. On the Physical Basis of Life.
The Fortnightly Review, 1869, N.S., 5 (5): 129-145.

The evolution of the two research programs in cell physiology

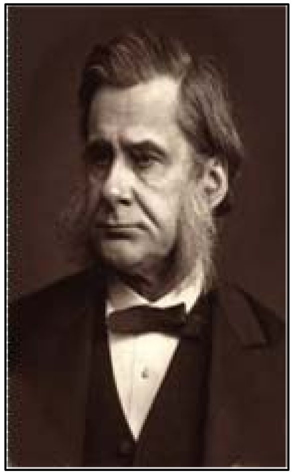
1864



Moritz Traube

The living cell is covered by lipid membrane

1868



Thomas Henry Huxley

Coagulation of protoplasm determinates cell narcosis

Charles Ernest Overton
1865-1933

- **Lipid theory** of narcosis → Rejected
- **Membrane** physiology → Remains generally accepted

Claude Bernard
1813-1878

- **Protein theory** of narcosis → Generally accepted after oblivion
- **Bulk phase** physiology → Remains marginal
  - **East**: Nasonov, Troshin
  - **West**: Ling, Pollack

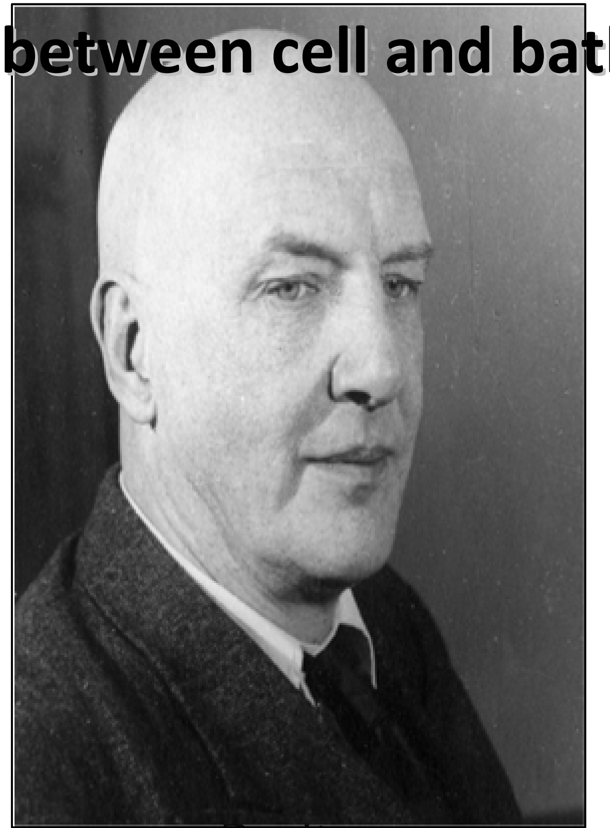
Perouansky M. The quest for a unified model of anesthetic action: a century in Claude Bernard's shadow. Anesthesiology. 2012, 117(3):465-474.

Protoplasmic theory was used to explain the mechanism of action of medicines, general anesthetics for the first time

Bernard (1813-1878)

Coagulation of protoplasm is an universal mechanism of cell narcosis (1878)

Protoplasmic theory was used to explain mechanism of all varieties of cell activity, narcosis and distribution of solutes between cell and bathing solution for the first time



Dmitry Nasonov (1895-1957)

Coagulation of protoplasm is the universal mechanism of cell excitation, damage and narcosis.

Coagulation of protoplasm takes place due to denaturation of proteins.

Sorption properties of proteins are the key factor in determining distribution of solutes between a cell and bathing solution.

Nasonov D.N. and Aleksandrov V.Ya. The reaction of living matter to external stimuli. Moscow-Leningrad, Academy of Sciences of the USSR, 1940

Similarities between coagulation of protoplasm and denaturation of proteins

Nasonov D.N. and Aleksandrov V.Ya.
The reaction of living matter to external stimuli.
Moscow-Leningrad, Academy of Sciences of the USSR, 1940

Рис. 20. Ультрамикроскопическая картина сувойки (*Vorticella sp.*) после прекращения наркоза. Мерцательные реснички возобновляют движение. Свечение макронуклеуса и цитоплазмы исчезает (из Макарова, 1935).

Рис. 21. Ультрамикроскопическая картина сувойки (*Vorticella sp.*) после действия необратимой дозы наркотика. Цитоплазма и ядро грубо коагулированы (из Макарова, 1935).

ром нейтрального красного. Далее, сперматозоиды отмывались от окружаю-

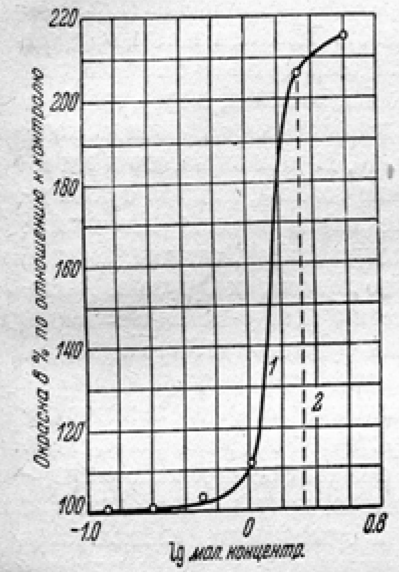


Рис. 43. Окраска 0.005% раствором нейтральрота сперматозоидов лягушки, предварительно обработанных этиловым спиртом. *1*—кривая адсорбции красителя, *2* — предельная наркотическая концентрация (из Раевской и Трошина, 1937).

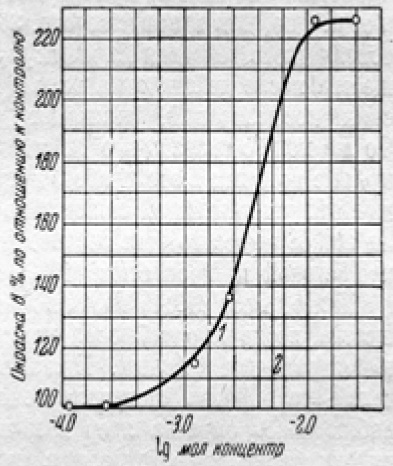
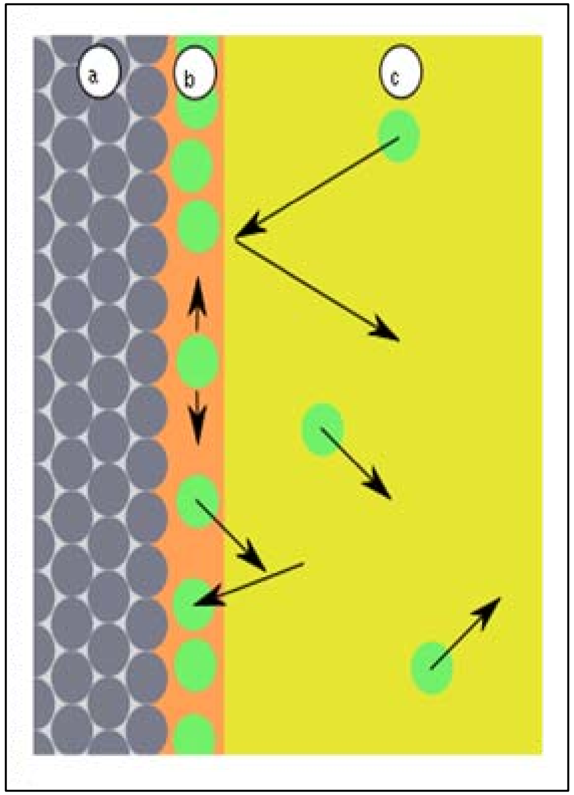


Рис. 44. Окраска 0.005% раствором нейтральрота сперматозоидов лягушки, предварительно обработанных хинином. *1* — кривая адсорбции краски, *2* — предельная наркотическая концентрация (из Раевской и Трошина, 1937).

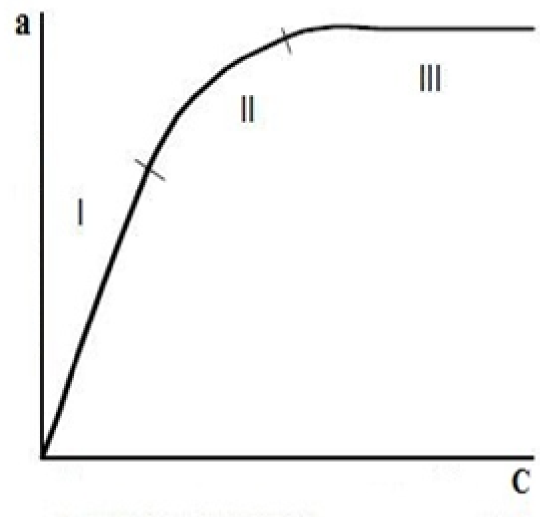
Protoplasm coagulation (infusorium) by ethanol:
aggregation and turbidity increase.

In strengthening damage by ethanol the cells' ability to bind vital dye increases. That is adsorption mechanism of dye distribution between cell and bathing solution.

Langmuir's (1914-1918) theory of isothermal monolayer adsorption



a - adsorbent
b - adsorbate
c - adsorptiv



Basics of the theory:

1) Adsorption is localized in adsorption sites.
2) The active sites are considered independent (i.e., the sites do not influence one another) and identical in its physical properties.
3) Each active site binds only one molecule (atom) of adsorbate and as a result monolayer adsorption takes place.
4) The adsorption/desorption process is in dynamic equilibrium (therefore it is necessary to study the adsorption under condition of diffusion equilibrium).

Protoplasmic theory was used to explain mechanism of equilibrium solute distribution between cell and bathing solution for the first time

Afanasy Troshin (1912-1985)

Sorption theory of cell permeability:

Solute distribution between the cell and bathing solution is determined by adsorption properties of cellular structures.

Intracellular water is different from bulk one: its solvency reduced.

General equation for solute distribution:

$$C_c = C_e K \left(1 + \frac{A_\infty}{C_e K + a}\right)$$

The equilibrium distribution of organic solutes between cell/coacervate and bathing solution (Troshin, 1956)

$$C_c = C_s K \left(1 + \frac{A_\infty}{C_s K + a}\right)$$

Coacervate / galactose

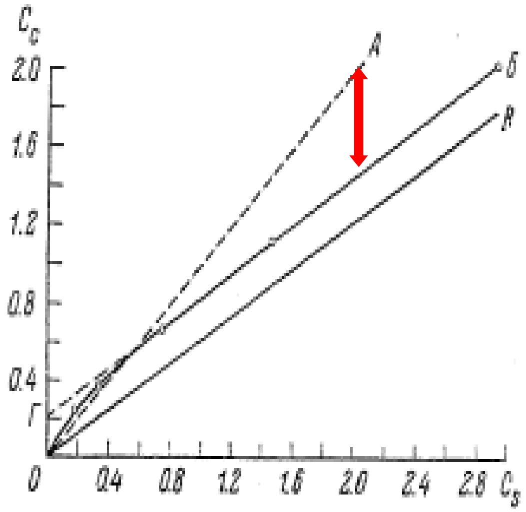


Рис. 20. Зависимость концентрации галактозы в коацервате (C_c, в г на 100 мл воды коацервата) от ее концентрации в равновесной жидкости (C_s, в %).

Muscle / alanine

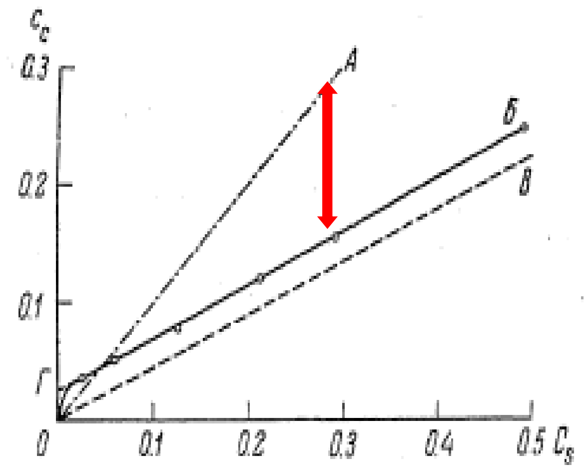


Рис. 51. Зависимость концентрации аланина в мышечных волокнах (C_c) от его концентрации в равновесной среде (C_s).

Yeast / lactose

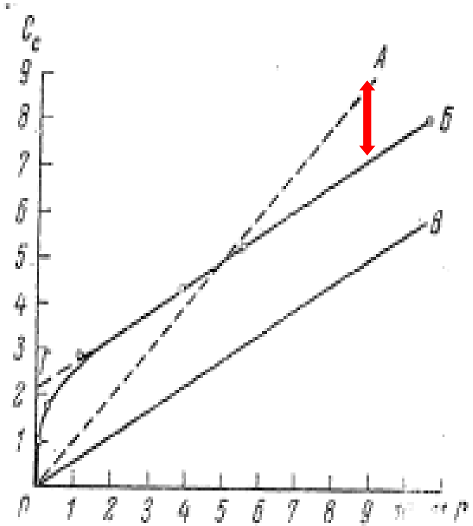


Рис. 42. Зависимость концентрации лактозы в дрожжах (C_c, в г на 100 мл внутри клеточной воды) от ее концентрации в среде (C_s, в %).

C_c – concentration in cell/coacervate water; C_s – concentration in bathing solution.
Dotted line A indicates equality $C_c = C_s$

Red arrows indicate decreased dissolving power of cell water

Equilibrium distribution of K+, Na+ and Cl- between the cell and bathing solution

$$C_c = C_s K\left(1 + \frac{A_\infty}{C_s K + a}\right)$$

Muscle / K^+

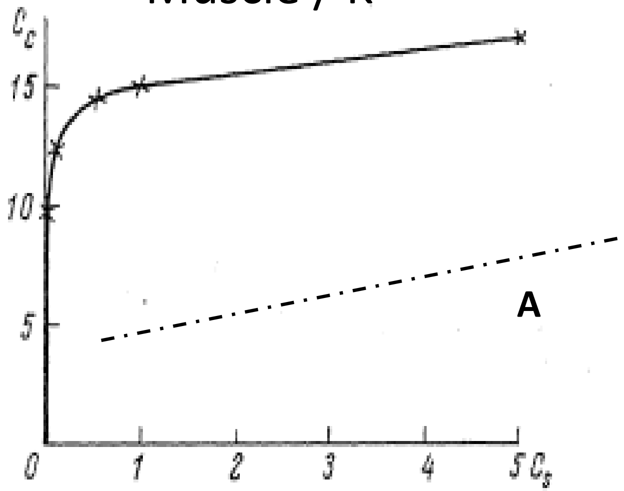


Рис. 92. Зависимость концентрации ионов калия в ретракторах голотурии (C_c, в мм. на 100 г мышц) от концентрации их в морской воде (C_s, в мм. на 100 см³). [Из Стейнбаха (Steinbach, 1937)].

Muscle / Na^+, Cl^-

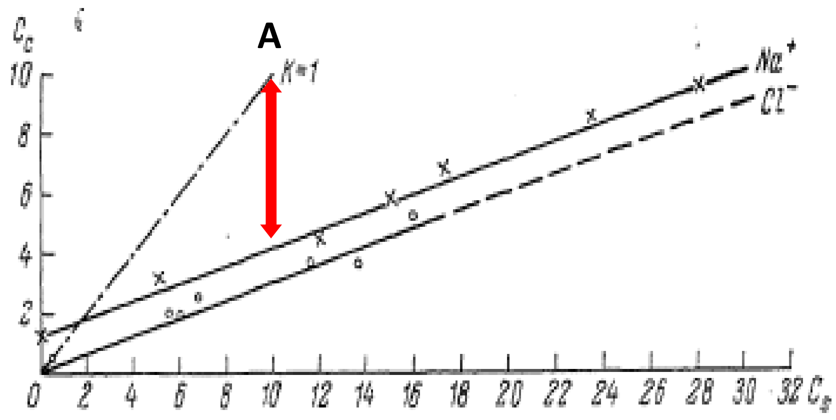


Рис. 81. Зависимость концентрации ионов натрия и хлора в мышечных волокнах (C_c, в мэкв% в воде мышечных волокон) от концентрации их в среде (C_s в мэкв%). [По Фенну, Кобб и Маршу (Fenn, Cobb and Marsh, 1934/1935)].

C_c – concentration in cell/coacervate water; C_s – concentration in bathing solution.
Dotted line A indicates equality $C_c = C_s$

Red arrow indicates decreased dissolving power of cell water

Adsorption isotherms for solutes binding by cell structures / coacervates (A.S. Troshin, 1956)

Erythrocyte / galactose

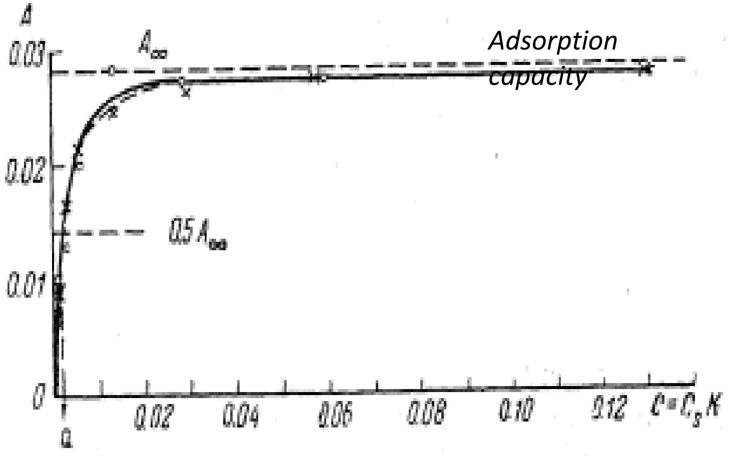


Рис. 30. Изотерма адсорбции галактозы эритроцитами кролика.

По оси абсцисс отложены концентрации галактозы в воде эритроцитов (в %), по оси ординат — количество адсорбированной галактозы (в г на 55 г сухого остатка или 100 мл клеточной воды).

Coacervate / galactose

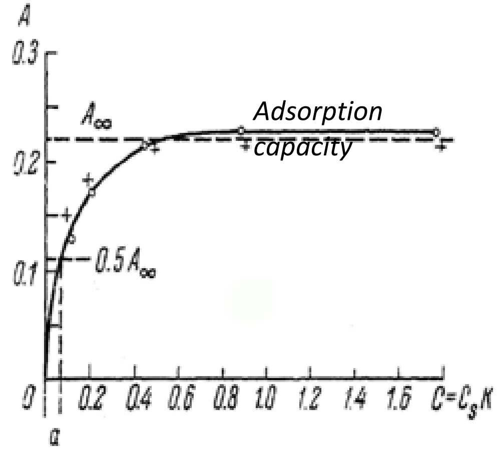


Рис. 22. Изотерма адсорбции галактозы коллоидами коацервата.

По оси абсцисс отложены концентрации галактозы, растворенной в воде коацервата ($C = C_s K$, в %), а по оси ординат — количество адсорбированной галактозы в коацервате (A, в г).

The data obtained are in satisfactory agreement with Langmuir's adsorption model

Advantages and disadvantages of Troshin's sorption theory

Troshin's equation satisfactorily describes the equilibrium solute distribution between the cell and bathing solution. That is important evidence in favor of the sorption mechanism of this distribution.

$$C_o = C_e K\left(1 + \frac{A_\infty}{C_e K + a}\right)$$

Troshin's theory did not explain the following:

1. What is the nature of binding sites for K^+?
2. Why the binding sites selectively bind K^+ in the presence of Na^+?
3. Why intracellular water is a poor solvent in compare with bulk water?
4. Why adsorption capacity of cellular structures depends on available metabolic power (ATP role)?

Current state of the protoplasmic theory: in search of the physical basis of life on the basis of the latest achievements of science

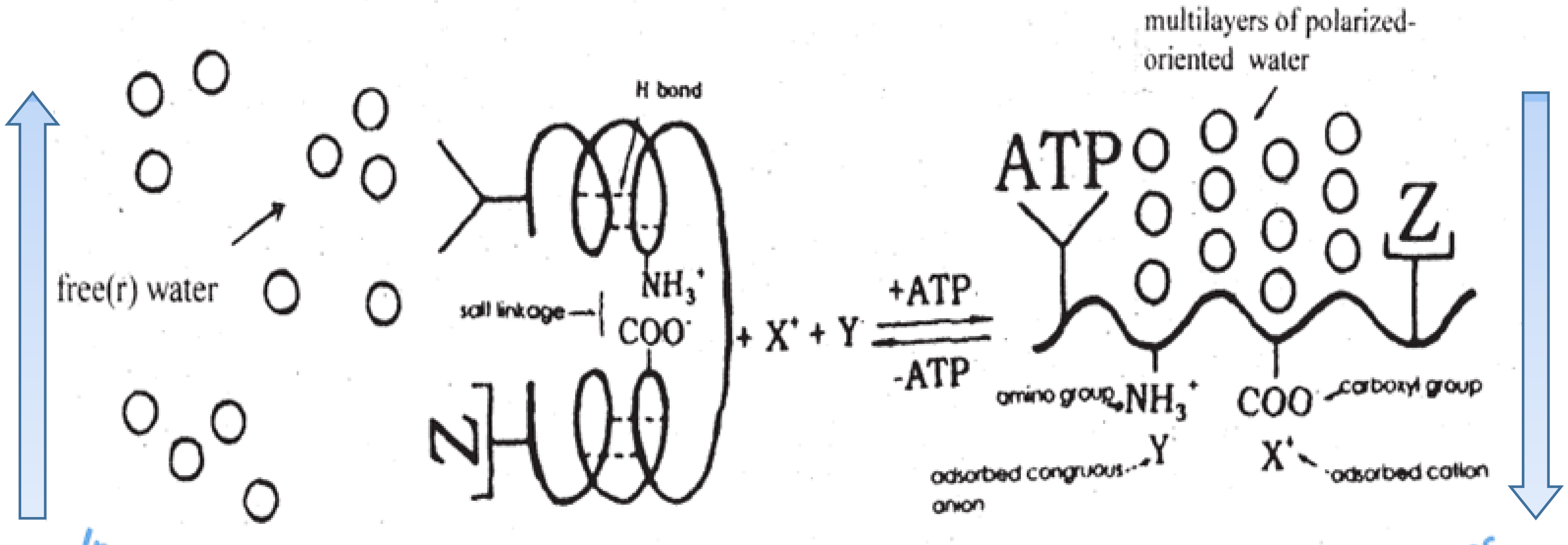
Gilbert Nin Ling

The creator of modern sorption theory explaining the fundamental physical properties of the living cells:

selective permeability;
selective accumulation of substances;
osmotic resistance;
electric potentials.

The cornerstone of the Ling's theory (as well as of earlier protoplasmic theories) is a ***protein*** which sorption properties create particular physical nature of the living state.

Key principles of Ling's theory



Increases

Decreases

ATP is an electron acceptor

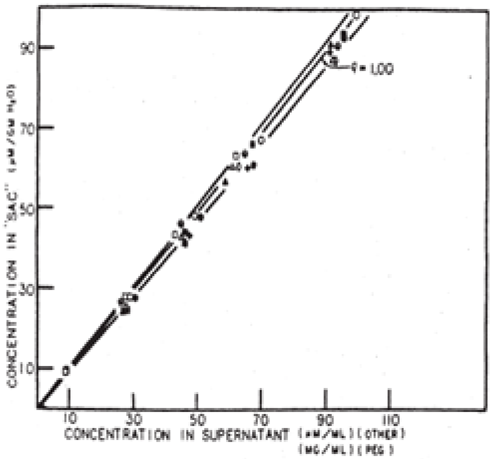
Electron density at the functional groups

Change in electron density on

(1) functional groups of the peptide bond determines their affinity to water and to other peptide groups in the same polypeptide backbone;

(2) carboxyl groups determines their affinity for K^+ or Na^+.

Equilibrium solute distribution between dialysis bags filled by solution of bovine hemoglobin and bathing solution

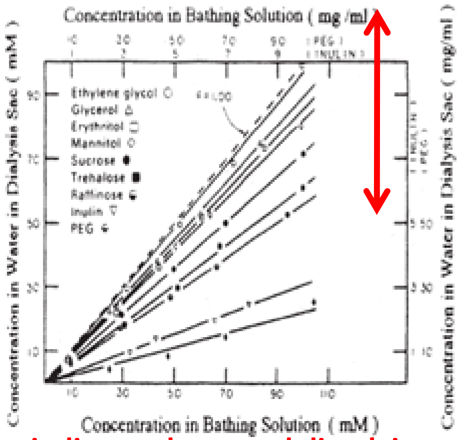


Native hemoglobin (globular), 39%

A

Free water

Ling GN and Hu W. Studies on the physical state of water in living cells and model systems. X. The dependence of the equilibrium distribution coefficient of a solute in polarized water on the molecular weights of the solute: experimental confirmation of the "size rule" in model studies. Physiol Chem Phys Med NMR. 1988; 20(4): 293-307.



Denatured hemoglobin (unfolded), 20%

B

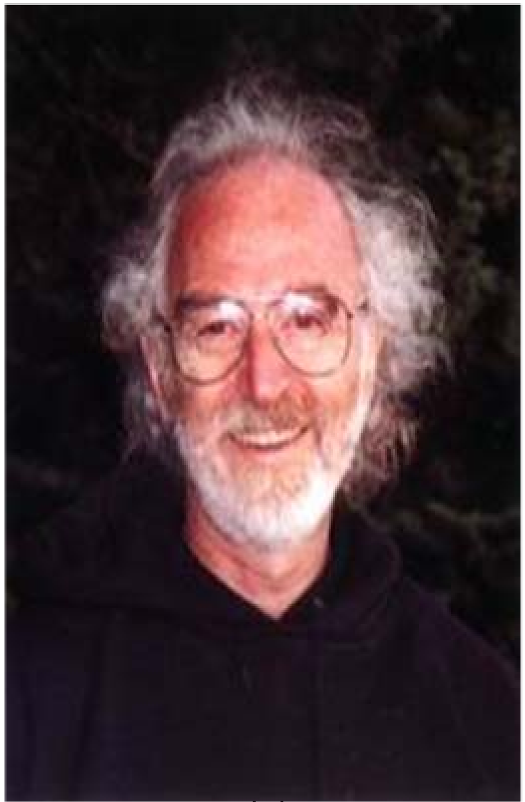
Adsorbed water

Red arrow indicates decreased dissolving power of the bag water

FIGURE 22. A. Equilibrium distribution of various solutes in a neutral solution of bovine hemoglobin (39%) after a 5-day incubation at 25°C. Solution contained 0.4 M. NaCl. Ethylene glycol •, glycerol △, erythritol □, D-xylose +, sorbitol ▲, mannitol ◇, trehalose ■, raffinose ○. B. The equilibrium distribution of various solutes in an alkaline solution containing 20% bovine hemoglobin and 0.4 M. NaOH. Incubation time and temperature same as in A. (from Ling and Hu 1988)

Enhanced sorption theory: cellular structures adsorb not only solutes, but a water too

Experimental confirmation of the key point of protoplasmic theory: proteins adsorb water and as a result its solvent power decreases



Gerald Pollack

Has made extraordinary contribution to study of physical nature of multilayer adsorption of water by living cellular structures and by hydrophilic polymers modeling the sorption properties of proteins.

Exclusion zones of macroscopic dimensions discovered and investigated by Pollack

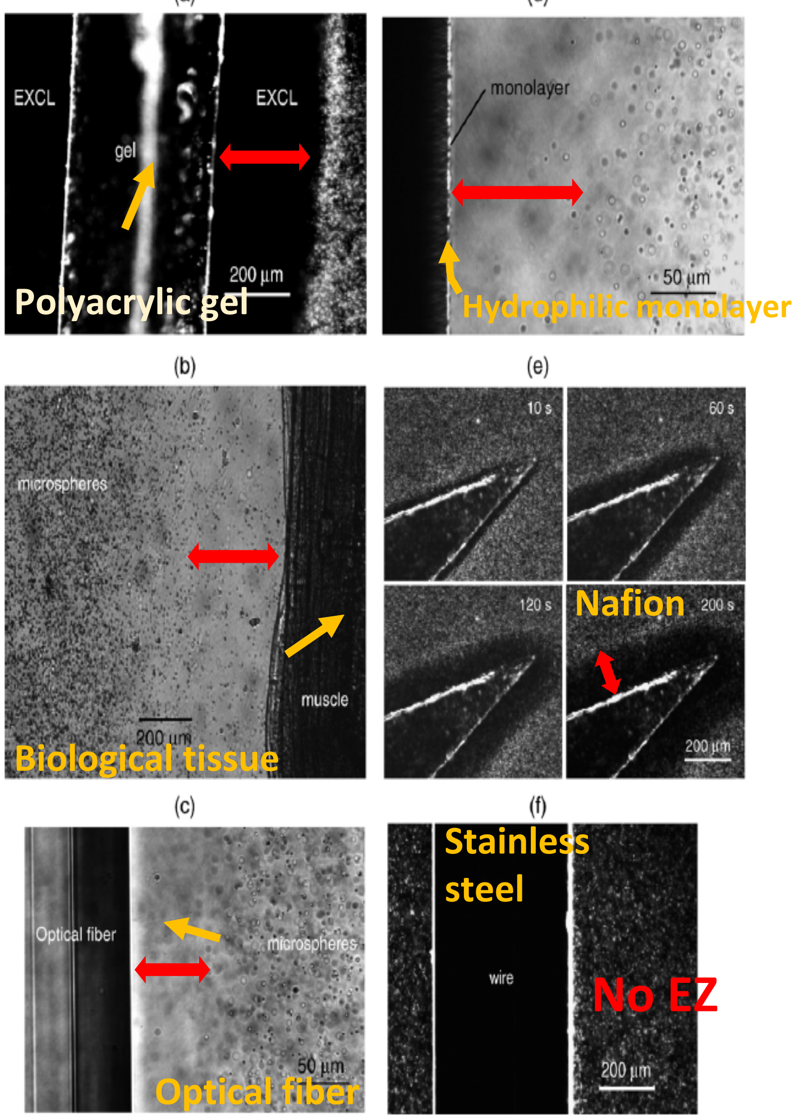


Fig. 1. Examples of solute exclusion from various interfaces. (a) Solute exclusion (EXCL) in the vicinity of polyacrylic acid gel. The gel was placed on a coverslip, superfused with a suspension of 1-µm carboxylate-coated microspheres, and observed in an inverted microscope (Zeiss Axiovert I35) equipped with a 20x objective. Image obtained 20 min. after superfusion. Microspheres (seen on right edge) undergo active thermal motion. (b) Microsphere exclusion in the vicinity of biological tissue. In eight specimens examined under similar conditions, the size of the exclusion zone, measured on video images, was found to be 360±50 µm. (c) Optical fiber FS-SC-7324, Thorlabs, Newton, NJ (left) inserted into a microsphere suspension. Microspheres translate toward right. 20x objective, 2 µm carboxylate microspheres. (d) Hydrophilic monolayer, containing COOH groups. (e) Nafion-117 film, spear shaped, 170-µm thick, was sandwiched between two glass cover slips, much larger than the film. A carboxylated microsphere suspension 2-µm diameter was infiltrated around the sandwiched film. Dark zones are microsphere-free. Numbers in upper right indicate time after infiltration, in seconds. Only the first several minutes are shown. (f) Stainless steel wire sandwiched between two glass slides and exposed to microsphere suspension.

Zheng JM, Chin WC, Khijniak E, Khijniak E Jr, Pollack GH. Surfaces and interfacial water: evidence that hydrophilic surfaces have long-range impact. Adv Colloid Interface Sci. 2006, 127(1):19-27.

Red arrows indicate exclusion zone due to reduced solvent power of adsorbed water

The history of exclusion zone water

Troshi n → Ling → Pollac k

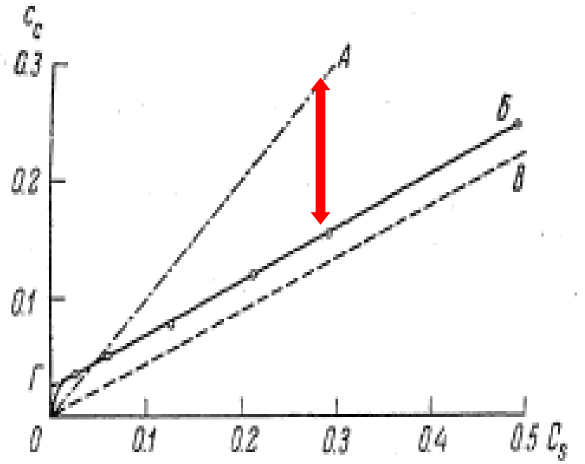


Рис. 51. Зависимость концентрации аланина в мышечных волокнах (C_c) от его концентрации в равновесной среде (C_s).

Red arrows indicate exclusion zone due to reduced solvent power of adsorbed water

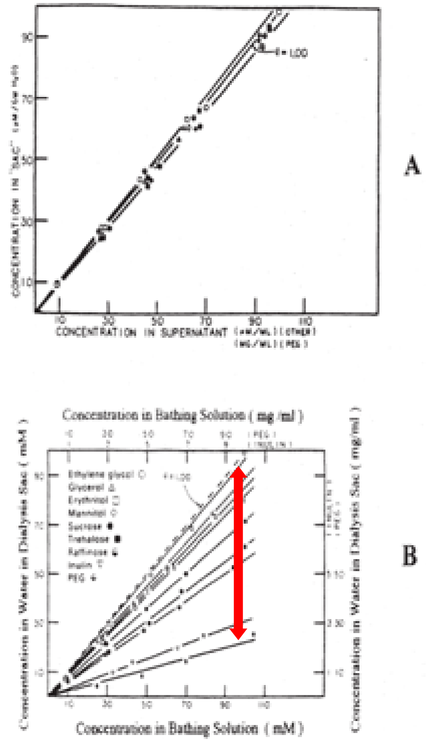


FIGURE 22. A. Equilibrium distribution of various solutes in a neutral solution of bovine hemoglobin (39%) after a 5-day incubation at 25°C. Solution contained 0.4 M. NaCl. Ethylene glycol •, glycerol △, erythritol □, D-xylose +, sorbitol ▲, mannitol ◇, trehalose ■, raffinose ○. B.The equilibrium distribution of various solutes in an alkaline solution containing 20% bovine hemoglobin and 0.4 M. NaOH. Incubation time and temperature same as in A. (from Ling and Hu 1988)

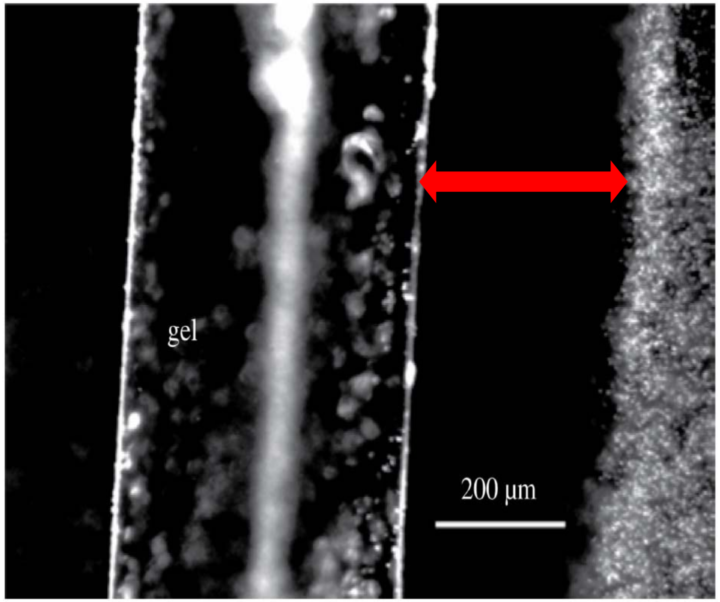


Figure 1.
Solute exclusion in the vicinity of polyacrylic-acid gel. The gel was placed on a coverslip, superfused with a suspension of 1-µm carboxylate-coated microspheres and observed in an inverted microscope. Image was obtained 20 min after superfusion. (Fuzzy vertical line within gel is optical artifact.) Microspheres move away from gel, leaving regions (black) on either side of gel that are devoid of them. Microspheres seen on right edge of figure.

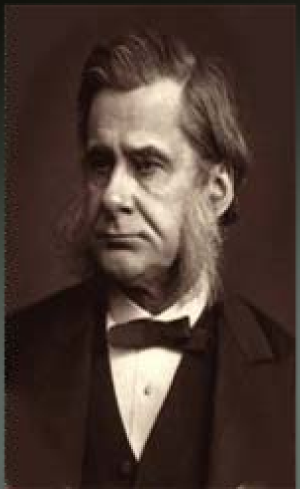
The methodology of protoplasmic theory

- Protein and its sorption properties should be in focus of the research program «The Physical Basis of Life".
- Effects of physical factor on the living cell can be understood only if proteins and their sorption properties are in focus.

The huge fiasco of the lipid theory of anesthesia proves fallaciousness of any deviation from the protoplasmic theory. Study of water with ignoring of its connection with protein also fraught with failure.

Past Lives Imply Future Lives

- Wayne (Wirs)



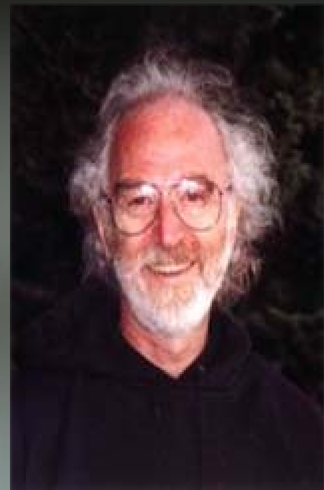
Thomas Huxley

Claude Bernard

Dmitry Nasonov

Afanasy Troshin

Gilbert Ling



Gerald Pollack