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Personal Comments on Ringer Solutions in Comparison with Body Fluid

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Prologue

Many organs work with each other to maintain the homeostasis of ionic composition of extracellular fluid (ECF) or body fluid, which is signified "milieu interieur" by Claude Bernard (1878). Kidneys play an important role in the homeostasis of body fluid, and the renal function was one of my teaching subjects for pre-clinical students. Cells *in situ* are soaked in ECF, so it is best but impossible to carry out experiments in vitro using blood or serum of the animal, thus we must use artificial saline solutions. I myself had been engaged in the research of smooth muscles *in vitro*, and about three liters of Krebs solution were wasted a day.

From physiologic NaCl solution to Ringer solutions

We still hear of physiologic NaCl solution, but excised tissues lose their proper functions quickly in this solution; intracellular K decreases and Na increases, because a Na-K pump can not be operated. In addition, cell membranes begin to leak ions in the absence of divalent cations. The first reported use of the artificial saline solution was by Sydney Ringer for perfusing an isolated frog heart to pulsate (1883). Ringer prepared perfusing solutions by adding presumed salts (NaCl, KCl, $NaHCO_3$) to distilled water in vain (the ionic composition of frog serum was not exactly known in those days). One day, his assistant added the salts to tap water, when his boss took holiday , and a frog heart beat vigorously. Ringer considered the result deeply, and he reached the conclusion that Ca contained in tap water was effective, thus they succeeded. They were lucky, because they used a frog heart as a bioassay system. They would have failed, if they used skeletal muscles, to find the important role of the Ca ion in 'excitation-contraction coupling' which was later coined by Sandow (1952). "Ringer" is now a common term meaning an artificial saline solution. Ca was highlighted 70 years later by the finding of Ca-receptive proteins for triggering contraction; troponin C in skeletal and cardiac muscles (1952) and calmodulin in smooth muscles (1970).

Ever since the introduction of frog Ringer, artificial saline solutions for perfusing mammalian tissues *in vitro* were designed and modified by Locke (1901), Tyrode (1910), Krebs-Henseleit (1932) and others. ECF and matrix contain ions, hormones and bioactive substances, some of which are yet unknown. We add or reduce native or foreign ions and substances to and from the artificial saline solution, a control solution, and we observe cellular responses; bioelectricity, contraction, secretion etc. For long-term experiments concerning such things as cell growth and differentiation, solutions of more complex composition are required. Oncotic pressure is needed for perfusing organs *in vitro* via blood vessels, and for dialysis therapy for renal diseases.

More than 90% of the osmolarity of ECF is held by NaCl. Kidneys work to keep the Na concentration and water volume of body

fluid constant. Previously, red blood cells have been a favorite material for investigating the movement of Na and other ions across cell membrane, because changes in water inflow and outflow accompanied with those of the ionic permeability and electrochemical gradient are easily detected by shrinkage or swelling of red cells under an optical microscope. More recently, important roles of Na ions in ECF have been elucidated as a current carrier in generating action potential, postsynaptic potential and endplate potential in neuronal axon, dendrite and neuron, and neuromuscular junction, respectively; Hodgkin, Huxley, Katz and Eccles were awarded by Nobel Prize in 1963 for their contribution to this field. An unexpected - yet reasonable - role of Na is that the electrochemical gradient of Na across the cell membrane provides a force to drive the antiport mechanisms of intracellular cations (Na/H, Na/Ca exchanges). For this reason, smooth muscles undergo a contracture and an intracellular acidosis in low Na solution. The Na/Mg exchange is now under precise investigation. So, it is interesting to recognize that Na ions in ECF contribute to the cellular functions in multiple ways. In other words, nature obeys an economic principle so as to utilize the same thing for many purposes.

Ca influx due to voltage-operated or receptor-operated Ca channels causes a contraction in cardiac and smooth muscle, and a secretion from glands. The latter is called stimulus-secretion coupling; the former (muscular contraction) is called excitation-contraction coupling. Bones in vertebrates constitute a skeleton composed of solid Ca crystal, hydroxyapatite, and a fraction of bone Ca serves as a pool of Ca which is exchangeable with serum Ca by the biological action of osteocytes. This is done under the influence of parathyroid hormone, calcitonin and active vitamin D. Reabsorption of Ca in renal tubules is one of the ways by which serum Ca concentration is kept constant at about 2.5mM. Serum Mg concentration is reported to be about 1.2mM in mammals, and about 50mM in marine invertebrates. Compared to the Ca regulation, very little is known about the regulation of serum Mg concentration. It has been suggested that parathyroid hormone and mineralocorticoid are related to the Mg homeostasis. Serum Mg is reported to be 1.9mM in pregnant women, and 1.4mM in non-pregnant women. This suggests that physiologic change in serum Mg underlies for the maintenance of pregnancy. One may imagine that serum Mg is somehow regulated by ovarian hormones.

Mg is a necessary cofactor for many enzyme reactions which occur at not only extracellular but also intracellular phases. The general action of Mg on excitable membranes is to antagonize Ca, for example Mg injections to pregnant women cause a tocolysis. It was found in our laboratory that plateau potential and contraction of rat myometria *in vitro* were depressed by 1mM Mg. Another action of Mg is the promotion of receptor-ligand binding, which is known for β-adrenergic agents, angiotensin, vasopressin, oxytocin, relaxin etc. The original Locke-Ringer does not contain Mg. In other mammalian Ringer solution such as Tyrode or Krebs-Henseleit solutions, about 1mM Mg is contained, and the concentration is different depending on the researcher's taste. Care must be used about Mg, when the experimental results are compared.

A small amount of Mn is contained in a healthy body, and it's function is not well understood. Mn is known to be a potent inorganic Ca antagonist; *in vitro* study indicates that plateau potential in cardiac muscles and spike potential in smooth muscles are depressed by the application of 0.2-1mM Mn. In addition, Mn (and Cd) penetrates cell membrane, and intracellular polyvalent cations modify cellular function probably by affecting intracellular enzymes. It was found in our laboratory that the inhibitory effect on myometrial contraction caused by forskolin was enhanced, whereas the effect caused by porcine relaxin, a polypeptide hormone mainly produced by ovaries, was attenuated, when the tissue was pretreated with 0.6mM Mn. When a myometrial tissue is pretreated with Cd, action potentials are generated but contractions do not follow.

Investigations on how cellular functions are affected by foreign ions are useful in knowing the physiological role of native ions.

Life originated in the sea, and native ions are those that used to compose the sea water. It seems that cell membranes have been so designed during evolution, that foreign polyvalent cations loaded intracellularly are difficult to extrude. Therefore, foreign cations which are accumulated in cells cause dysfunctions of the body.

Epilogue

Physiology deals with subjects from cells *in vitro* to the whole body and ecology. Body fluid *in vivo* and Ringer solutions *in vitro* are central in the investigation of biological functions. Salts in body fluid are provided by drinks, foods and seasonings. Therefore, body fluid is deeply related to the earth and the surrounding atmosphere. As far as salts are concerned, certain salts should be avoided, and others should be taken. It is a pity that we know so little about what we swallow. Shokuen and Ajishio contain NaCl more than 99%, and 90% NaCl + 10% Na glutamate, respectively. On the other hand, 1.3g Mg and 0.4g Ca are contained in one liter sea water. This kind of natural salt made from sea water was used for cooking, for making soya sauce, miso, tsukemono, etc. long before 45 years ago in Japan. As a result, compositions of body fluid and bones have probably changed in modern Japanese people.