

*Historical Note*

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## The osmotic pressure of the urine—from Dutrochet to Korányi, a trans-European interdisciplinary epic

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*Perhaps we would have had no chemistry, no botany, no anatomy if doctors had not cultivated these arts.* Baron G. Cuvier, 1810

### Introduction

Understanding the physiological and pathological relevance of urinary osmolality came about through a chain of events in which botany, zoology, physical chemistry, and finally medicine used it as a practical tool. Discovered in 1824 by Henri Dutrochet (1776–1847), osmosis led Sándor<sup>1</sup> Korányi (1866–1944) to create a physiological basis of the concept of renal insufficiency. As Gustave Flaubert wrote: *'les perles ne font pas le collier, c'est le fil'*

### The discovery of osmosis

At the age of 26 in 1802 with the French revolution scarcely over, Dutrochet (Figure 1) was studying medicine in Paris. Then, although becoming a practitioner in rural Touraine, he still remained a naturalist, a faithful disciple of Lazzaro Spallanzani (1729–1799), writing in his memoirs: 'it was medicine that introduced me to natural history'. Observing plants and animalcules under the microscope, he put forward in 1824 the outlines of the cellular theory [1], then discovered osmosis, the passage of water across biological membranes [2]. In order to study this transfer, he built an osmometer with a membrane separating two compartments, the lower largely open to a reservoir filled with pure water and the upper topped by a vertical glass tube containing the experimental liquid. The maximum level attained by the column indicated the osmotic pressure. This rapid transfer of water, which he called *endosmosis*, was followed by a slower phase in the



**Fig. 1.** René Henri Joachim du Trochet (Dutrochet), who established and named the concept of osmosis. He is shown in this somewhat damaged portrait with one of his osmometers in his hand (Wellcome library, London. From reference [3]).

opposite direction, which he termed *exosmosis*, corresponding to the diffusion of solutes. The fact that endosmosis depended upon the nature of the liquid and not the membrane was demonstrated by its reversal on interchanging the solutions. In fact this biological discovery led to a hitherto unknown physical para-

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1. Korányi's first name was the Hungarian Sándor (Alexander) but since he published in German, his name is usually shown as A. (Alexander) von Korányi, as in the references cited.

meter. This was the reverse of the usual case, in which the physicist is summoned to help understand the mechanism of a biological phenomenon [3].

### Had osmosis a place in renal physiology?

Carl Ludwig (1816–1885) believed so in 1842, when he postulated that the hyperproteinaemia brought about by glomerular filtration causes in turn concentration of the urine by endosmosis into the peritubular capillaries [4], an idea soon demonstrated to be in error by Hoppe-Seyler [5].

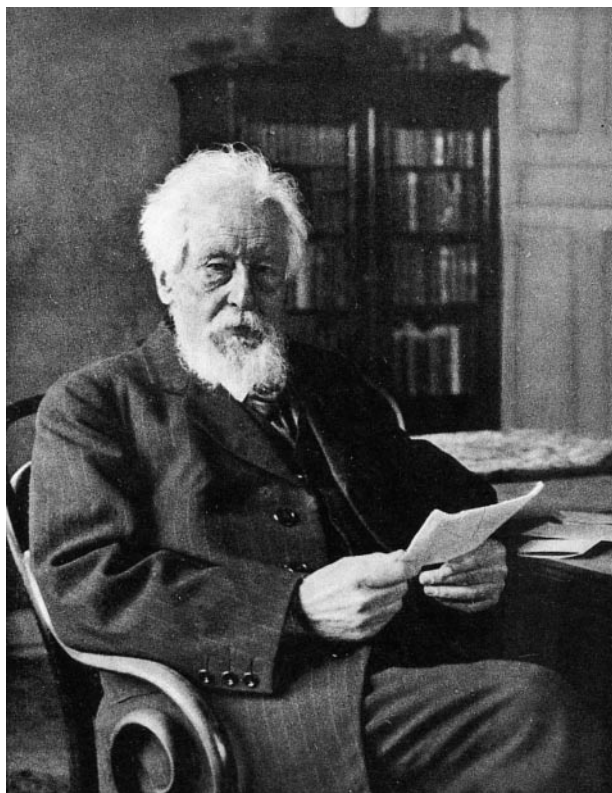
### The science of membranes

The study of artificial membranes showed Dutrochet that they had to be wettable by the solvent—in biology by water—for osmosis to operate. Shortly thereafter, in Great Britain the Scottish chemist Thomas Graham (1805–1869) described and coined the word *dialysis* (1854), the passage by diffusion of dissolved substances as a function of their concentration and molecular mass; thus he explained exosmosis. About 1865, Moritz Traube (1826–1892) botanist brother of Ludwig, the famous physician of the Charité in Berlin, used as membranes thin sheets of chemical precipitates permeable exclusively to water, but very fragile. In 1875 Wilhelm Pfeffer (1845–1920), another German botanist, improved these delicate mineral membranes and mounted them on supports, using them to study the role of osmotic pressure on the movement of fluids in plants. In this way he supplied the very tool that physical chemistry needed.

### The return to biology

Hugo de Vries (1848–1935) (Figure 2) was a Dutch botanist who by his research on hybrids rediscovered the results and laws of Mendel (then completely unknown and ignored) and also proposed a theory of mutations transmitted to offspring. In 1871 he was studying the changes induced in fragments of beetroot immersed in various solutions [6]. The sugar and the cytoplasmic pigment never entered the bath. In contrast, if the concentration of cane sugar or sodium chloride in the external liquid were raised, the cytoplasm contracted, returning to its normal volume when exposed to pure water. The impermeability of the cell wall to cane sugar and sodium chloride and the ready passage of water through it was thus established; de Vries gave the name of *plasmolysis* to this contraction of the cytoplasm, and linked it with increased osmotic pressure outside the cell.

De Vries measured the upper limit of the concentration of various solutes that did not provoke plasmolysis, and considered these to be *isotonic*<sup>2</sup> with the cytoplasm. They were not equimolecular, and de Vries



**Fig. 2.** Hugo de Vries, the Dutch botanist whose work on beetroot slices showed the effects of solute transfer into and out of cells. (Picture courtesy of the Wellcome Library, London. From Obituary notices of the Royal Society, London, Vol. 1 (1932–1935), p. 371.)

classified them according to the ratio of the concentration of molecules necessary to attain isotony compared with that of a standard. The ratio, called the *isotonic coefficient*, lay between 1 and 4.

A little later, another Dutchman, Hartog Jakob Hamburger (1859–1924) of Groningen examined the passage of water from or into red blood cells according to the osmotic pressure of the solution in which they were suspended. He examined especially the effects of dilution, leading to haemolysis as the end-point, which could easily be used to measure the phenomenon.

### De Vries inspired the physical chemists

Yet another Dutchman, Jacobus Henricus van t'Hoff (1852–1911) (Figure 3) wished to understand what the isotonic coefficient actually meant. He was one of the leaders of the nascent physical chemistry and shared with Le Bel the foundations of stereochemistry. For his researches, and especially the enunciation of the laws of chemical equilibrium, he was awarded the Nobel prize in 1901. Van t'Hoff described osmosis in terms of the fundamental laws involving pressure, volume and temperature which determined gaseous diffusion, and which were currently under investigation by physical chemists, amongst others F. Wilhelm

2. A term introduced by de Vries.



**Fig. 3.** Jacobus Henricus van t'Hoff, the Dutch chemist whose mathematical description of gaseous diffusion underlies the process of dialysis and osmosis (Bibliothèque Inter-Universitaire de Médecine, Paris).

Ostwald (1853–1932) of Leipzig<sup>3</sup> and Henry-Louis le Chatelier (1850–1936) in Paris. Thus osmosis was integrated into general physicochemical theory, and this theory is still accepted.

Three physical methods allowed theory to be tested in practice: the elevation in the boiling point, the fall in vapour pressure, and the depression of the freezing point, all in relation to that of the pure solvent. The results were in agreement: Ostwald, at the suggestion of a philosopher friend Wilhelm Wundt (1832–1920) proposed their unification as *colligative*<sup>4</sup> properties. These observations led to the studies of François M. Raoult (1830–1901)<sup>5</sup> of Grenoble on cryoscopy. He

3. Ostwald was born in Riga in Latvia and studied in Dorpat (now Tartu) in Estonia, where he qualified *privatdozent* in 1876, before becoming professor of chemistry in Riga and then leaving for Leipzig. He was a prominent member of the group which created physical chemistry, including Arrhenius, Nernst, van t'Hoff, Planck, and Raoult. These workers established that the law of mass action applied to the equilibrium and distribution of ions and dissolved salts. Ostwald was awarded the Nobel Prize in 1909.

4. *Colligative*. Concerning the union of data separately observed (W. Whewell 1837).

5. Raoult was born near Lille, and was a school teacher of physics and chemistry until 1867. Mostly he worked alone on thermo- and electrochemistry, announcing the dissociation of sodium chloride in solution. In 1878 he summarized the works of Louis de Coppet of Geneva and measured the vapour pressure and the freezing point depression of 18 non-saline salts, observations which were critical to obtaining the molecular weight (MW) of organic molecules. He

showed that this technique could be applied equally to organic matter as to electrolyte solutions, or to mixtures, and that it indicated the overall osmotic pressure of a mixture of solutions containing diverse solutes. Finally he showed that his results were consistent with the work of Svante Arrhenius (1859–1927) on ionic dissociation [7]. Because measurement of freezing point depression ( $\Delta$ ) was easy and required only small amounts of liquid for its measurement, it became the method of choice in biology.

### The basic biological studies of Heinrich Dreser

In Tübingen in 1892, Heinrich Dreser (1860–1925)<sup>6</sup> was encouraged by the work of van t'Hoff to study the physiology of water excretion. He soon established that the cryoscopic  $\Delta$  of the blood,  $-0.56^{\circ}\text{C}$ , is not altered by water intake, in contrast to that of the urine. During water restriction he showed a urinary cryoscopic  $\Delta$  of  $-2.40^{\circ}\text{C}$  in humans and  $-4.94^{\circ}\text{C}$  in the cat. During water loading, the figures obtained were  $-0.16$  to  $-0.20^{\circ}\text{C}$  and  $-0.60^{\circ}\text{C}$  respectively. The cryoscopic  $\Delta$  of a mixture of urines from 20 frogs was  $-0.24^{\circ}\text{C}$ . In a patient with diabetes insipidus, without albumin and sugar in the urine, the  $\Delta$  was only  $-0.20^{\circ}\text{C}$ , probably the first ever use of cryoscopy in a patient [8]. He pointed out that glomerular filtration alone could not explain these wide variations in osmolarity and that renal work must be necessary to achieve this. Later Jacques Winter in Paris [9] measured the osmotic  $\Delta$  of many fluids and confirmed Dreser's figures for urine.

### Korányi integrates the work of Dreser in the role of the normal and pathological kidney in maintaining the *milieu intérieur*

Sándor Korányi [10] (Figure 4) was born in Budapest in 1866, the son of a renowned medical professor, and died in 1944. Once he had qualified in Budapest in 1888, Korányi spent some time in Germany in laboratories of physical chemistry, including with Hoppe-Seyler<sup>7</sup> and

was honoured outside France, receiving a medal from the Royal Society in London in 1892 and becoming a fellow of the Chemical Society in 1896.

6. Dreser was closely involved, in 1897–1898, as chief of the pharmacology laboratories at the Bayer pharmaceutical company, with the introduction of both aspirin and heroin into clinical practice. He was not, however, the first to synthesize either compound (see Sneader W. the discovery of heroin. *Lancet* 1998; 352: 1697–1699).

7. Felix Hoppe-Seyler (1825–1895) was born in Saxony, studied in Leipzig and practised clinical medicine before becoming a biochemist, first in Rudolf Virchow's institute, then in Tübingen from 1872, and finally in Strasbourg until his death in 1895, as head of the Physiologisch—chemisches Institut. He studied the oxygen association and dissociation of haemoglobin, plasma proteins, glycogen, lecithins, and cholesterol, and was one of the founders of biochemistry, on which discipline he had a major impact.



**Fig. 3.** Sándor Korányi, the Hungarian whose careful work on the osmolarity of the urine led to a new concept of pathophysiology in renal failure. From reference [13].

Goltz<sup>8</sup> in Strasbourg. On his return to Hungary in 1890, a particular patient had a great influence on his thinking. Korányi had to decide, at the request of a urological colleague, whether a kidney, of which the ureter had been cut and attached to the skin, could be safely removed. As the concentration of several urinary solutes on that side was clearly lower than that of the bladder urine, Korányi counselled nephrectomy, which was carried out with excellent long-term results [11].

Strengthened by this experience, he decided after having read the work of Dreser to examine renal function using the cryoscopic  $\Delta$  of blood and urine during water deprivation and loading. He soon confirmed the results of Dreser and Winter. Since proteinuria did not modify the cryoscopic  $\Delta$  he set out to study chronic, presumed to be bilateral, nephropathies. He observed that the cryoscopic  $\Delta$  of the blood increased with the progression of clinical nephropathy in parallel with the increase in urea concentration, and

8. Friedrich Goltz (1834–1902) was a surgeon before becoming a physiologist. He studied in Königsberg (now Kaliningrad) in Hermann von Helmholtz's laboratory. His main experimental work was on the central nervous system, in which he achieved delicate surgical resections of the cerebral cortex with survival of the experimental animals. In 1884 Charles Sherrington (Nobel Prize 1932) spent a semester in Goltz's laboratory to learn his techniques.

right throughout the evolution of the disease the difference between the  $\Delta$  of the urine during water restriction and loading diminished, both figures tending towards that of the blood plasma. At the end it was *hypo-* or better *iso-sthenuria*, terms which Korányi introduced [12,13].

Even more important perhaps was the concept of *funktionelle Nierendiagnostik* which Korányi promulgated in the spirit of Claude Bernard, of whom he considered himself a disciple. The kidney maintained the *milieu intérieur*, and in particular its osmotic state. The urinary osmolarity<sup>9</sup> in hydropenia measured the physiological capacity of the kidney to conserve water as a global test, capable of revealing and following *both evident and clinically latent renal insufficiency*, the latter state being almost unknown before Korányi [14].

The same year, 1897, in Paris, Charles Achard (1860–1944) and Joseph Castaigne (1871–1951) used the excretion by the kidney of methylene blue to measure its permeability [15]. The index they used was the percentage of dye, injected subcutaneously, which appeared in the urine within the next 24 h.

*Both urinary osmolarity and the excretion of methylene blue measured the defect in overall renal function.*

### Urinary osmolarity is quickly forgotten

Instead of the measurement of osmolarity, the measurement of the urinary specific gravity championed by Franz Volhard (1876–1950) in Germany [16] was quickly substituted, even though it was devoid of physiological meaning, and totally inaccurate in the presence of significant proteinuria. However the urine specific gravity could be measured at the bedside of the patient, without recourse to a laboratory, and was strongly promoted by the school of clinical nephrology, which held sway until 1940. Besides, even if the clinicians had known what the specific gravity might mean, they were ignorant of almost all that the osmolarity signified. It took the work of the successful school of American physiology in the inter-war years for urinary osmolarity to be accepted as a quantum of physiological data that could enlighten and guide the clinician. A late triumph for the naturalist Dutrochet who, it must not be forgotten, was also a doctor.

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### Further reading

Some of the topics touched on in this essay are dealt with in more detail in the following papers:

Richet G. Edema and uremia from 1827 to 1905: the

9. Osmolarity (=osmoles/l of water) is used here rather than osmolality (=osmoles/kg of water) since there is little difference for urine, although in plasma osmolality is 7% less than osmolarity because of the high protein concentration.

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15. Achard C, Castaigne J. Diagnostic de la perméabilité rénale. *Bull Mém Soc Méd Hôp Paris* 1897; 14: 637, 831, 1128
16. Szám I, Kenéz J, Kaiser W. Beziehungen zwischen Volhard und Korányi. *Med Welt* 1973; 24: 43–44