1. Introduction

Intracellular fluid contains more inorganic cations than inorganic anions, creating an “anion gap” (AG). This term usually refers to the difference between the major positive and negative ions in blood plasma, but could be equally applied to the cell interior. Thus, we define the anion gap as the difference between the charges on the most abundant permeant ions: potassium, sodium, and chloride. We also add magnesium to the balance, whose total concentration (mostly in the bound form) is 15-20 mM [Romani, 2011] in mammalian cells. Thus,

AG = [K+] + [Na+] + 2[Mg2+] – [Cl-]

The frequently observed value of AG is about 0.1 M. Such a charge difference, if not neutralized by other ions, would create unsustainably strong electric forces. Therefore, one must conclude that the deficit of inorganic anions is compensated by organic anions.

In theoretical treatments, the diverse organic constituents of the cell are often called “impermeant” and treated as a single pool with average molar concentration [X-] and average valency z; the magnitudes of [X-] and z are chosen to maintain approximate isoosmolarity with external medium and electric neutrality. The nature of X- has been sufficiently characterized by metabolomic techniques [], but the exact origin of z remains uncertain; z has only been evaluated to some extent in highly specialized cells, such as erythrocytes and muscle cells [Burton, 1983; Conway, 1950; Gary-Bobo and Solomon, 1968]. It seems that most authors believe that anion gap is mainly balanced by anionic proteins, but phosphorus-containing compounds are recognized as the other possible source of negative charge [Burton, 1983; Macknight, 1987; Al-Habori, 1994; Andersen, 2013; Glykys et al, 2014; Keener and Sneyd, 2009]. In any event, this question and its implications have not been thoroughly analyzed. Indeed, the electric charge of the entire proteome is difficult to assess, and it would be challenging to create an accurate “balance sheet” of intracellular charges due to their diversity. However, intracellular phosphorus can be measured by atomic emission spectroscopy or by X-ray microanalysis, which can help to make crude estimates.

The other likely reason for the paucity of studies of impermeant ions is that they are often viewed as a static background in the events played out by the mobile inorganic ions. However, the impermeability of “impermeant” anions is relative and depends on the time scale. Cell volume regulation provides a vivid example. The typical experiment involves abruptly replacing the cell medium with a hypotonic or hypertonic solution and observing the ensuing changes in cell volume, intracellular ions, or membrane potential. Many cell types restore their volume after the initial and inevitable osmotic swelling or shrinkage, often recovering within minutes or tens of minutes. It has been shown, both theoretically and experimentally, that this rapid volume restoration is caused by the redistribution of the major permeant ions: potassium, sodium, and chloride []. Sometimes, bicarbonate [Nicholl et al, 2002; Casey, 2006; Li et al, 2021] or easily permeant organic osmolytes [Burg and Ferraris, 2008] are brought into the picture as well.

Long-term osmotic adaptation is different from the response to acute osmotic shock [Souza et al, 2000]. In particular, the constantly changing cell volume can hardly serve as a set point in growing and dividing cells, and there is evidence that cells are guided by the density of the dry mass instead (or the equivalent measure of cell water content) []. Importantly, no osmolytes can be considered impermeant during slow processes, as they gradually accumulate or are broken down and released into the environment. To understand these processes, one needs to examine the significance of the variable content and electric charge of impermeant ions.

We will start by reviewing the available experimental data to identify the major sources of impermeant anions; we will try to make a case that phosphate-based anions provide the bulk of the requisite negative charge. Moreover, their accumulation or dissipation correlates with the membrane potential. This correlation will be explained in the second, theoretical part of the paper, where we show that an increase in z (i.e., due to the synthesis of nucleic acids) is expected to hyperpolarize the cell and increase the intracellular concentration of cations, in direct agreement with experiment.

2. Phosphorus-based impermeant anions

*2a. Total cellular phosphorus roughly matches the anion gap.* A limited compilation of the available data is given in **Table 1**. Despite a spread in the data, the anion gap in mammalian cells is consistently on the order of 0.1-0.15 M with only one major outlier noted [Nkamgueu et al, 2000].

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Cell type | [K+] | [Na+] | [Cl-] | Mg2+ and other ions  (> 10 mM) | AG | P | Reference |
| Escherichia coli | 242 | 227 | 147 | Mg2+: 60  Ca2+: 12 | 466 | 302 | Heldal et al, 1985 |
| Prochlorococcus  (average over strains) | 49 | 392 | 172 | Mg2+: 371  Ca2+: 25 | 1061 | 94 | Heldal et al, 2003 |
| Synechococcus  (average over strains) | 78 | 232 | 120 | Mg2+: 104  Ca2+: 49 | 496 | 124 | Heldal et al, 2003 |
| Mouse intestine | 117 | 26 | 22 | (Mg2+: 15)2 | 151 | 141 | Butt et al, 1998 |
| Rat hepatocyte1 | 75 | 6 | 20 | Mg2+: 5 | 71 | 58 | Zierold, 2000 |
| Rabbit myocytes1 | 103 | 70 | 56 | Mg2+: 15 | 147 | 100 | Buja et al, 1983 |
| Rabbit smooth muscle1 | 120 | 33 | 55 | Mg2+: 7 | 112 | 50 | Somlyo et al, 1989 |
| Rabbit smooth muscle1 | 106 | 14 | 32 | Mg2+: 15 | 94 | 94 | Warley, 2001 |
| Rabbit cornea1 | 58 | 6 | 17 | (Mg2+: 15)2 | 77 | 44 | Alaminos et al, 2007 |
| Human prostate cancer | 67 | 8 | 12 | (Mg2+: 15)2 | 93 | 52 | Salido et al, 2001 |
| Human intestine1 | 138 | 17 | 27 | Mg2+: 15 | 158 | 154 | O'Loughlin et al, 1996 |
| Human keratinocytes1 | 77 | 7 | 35 | Mg2+: 4 | 58 | 52 | Sanchez-Quevedo et al, 2007 |
| Human fibroblasts1 | 81 | 9 | 36 | Mg2+: 4 | 62 | 61 | Sanchez-Quevedo et al, 2007 |
| Human and rat RBC | 150 | 25 | 79 | Mg2+: 3  HCO3-: 13 | 89 | 27 | Keitel et al, 1955; Tanaka et al, 2020 ;  Mouat and Manchester, 1998. |
| Human U9371 | 83 | 13 | 29 | (Mg2+: 15)2 | 97 | 62 | Arrebola et al, 2005 |
| Human HeLa1 | 64 | 66 | 63 | (Mg2+: 15)2 | 97 | 63 | Warley et al, 1983 |
| Human monocytes1 | 115 | 8 | 30 | (Mg2+: 15)2 | 122 | 115 | Skepper et al, 1999 |
| Human monocytes1 | 310 | 90 | 85 | (Mg2+: 15)2 | 345 | 260 | Nkamgueu et al, 2000 |

**Table 1.** Intracellular concentrations of the major ions and phosphorus in mmol/L in whole cells. Most measurements have been performed by atomic spectroscopy. 1Concentration were recalculated from mmol/kg dry weight data, assuming 0.3 kg dry weight/L in bacteria and 0.2 kg dry weight/L in mammalian cells. 2Total magnesium concentration was not reported and was assumed to be 15 mM [Romani, 2011].

As stated earlier, the two main hypotheses posit that the deficit of anions must be matched by proteins and/or phosphorus-containing compounds. It appears from the data in **Table 1** that the molar amounts of intracellular phosphorus in mammalian cells are equivalent to three quarters of the AG on average. Since phosphorus in biological fluids is always charged and exists as a mixture of single- and double-charged anions [Walsh, 2020], the charge compensation must be largely due to phosphorus. The important question, however, is not only which species neutralize the anion gap but whether the impermeant anion content undergoes significant variability, affecting other physiological aspects of the cell.

*2b. The main intracellular phosphorus-containing compounds.* Phosphorus is delivered to the cells as inorganic phosphate Pi (in the form of H2PO4- and HPO42-) through a sodium-coupled transporter. The main phosphate transporters in the human are type II, transporting one ion of HPO4-2 along with two or three Na+ ions, and type III, with the stoichiometry 2Na+/H2PO4- [Hernando et al, 2021]. The efflux of Pi is largely carried out by Xenotropic and Polytropic Retrovirus Receptor 1 (XPR1).

The major intracellular forms of phosphorus include nucleic acids, anionic phospholipids (phosphatidic acid, phosphatidylserine, and phosphatidylinositol), phosphorylated proteins, polyphosphate in bacteria, inorganic phosphate, nucleotides, and diverse small metabolites (such as creatine phosphate in the muscle). Of these, DNA and RNA seem to be the most variable or, at any rate, variable in the most obvious ways. But first, we will briefly review the other compounds.

*2c. Nucleotides.* Inorganic phosphate and adenine nucleotides are typically present at a concentration of several mM, with ATP4- and Pi being more abundant than ADP and AMP [Lehninger, 2000]. However, ATP is always complexed with Mg2+, making it essentially Mg-ATP2-; the remaining two charges are presumably balanced by Na+ and K+. The interconversion between ATP, ADP, and Pi is not expected to produce an immediate change in the negative charge. However, the amount of Pi that is consumed or released in the course of these reactions, and especially its maximum concentration, is tightly regulated by membrane transporters [Austin and Mayer, 2020], and therefore one can expect that ATP synthesis from ADP and Pi would slightly increase both z and [X-], and ATP hydrolysis would decrease them.

One situation when ATP varies significantly under normal conditions is the circadian rhythm, when ATP exhibits a peak during the night [Yamazaki et al, 1994; Womac et al, 2009]. During the same period, the suprachiasmatic nucleus neurons become significantly hyperpolarized [Kuhlman and McMahon, 2004]. Although this fact by itself does not prove anything (the observed correlation could be due to the simple activation of the Na+,K+ pump by ATP [Horvat et al, 2006]), it is at least compatible with the notion that accumulation of more strongly charged phosphate molecules shifts the membrane potential toward more negative values.

*2d. Polyphosphate.* Bacteria, yeast, and platelets contain large amounts of polyphosphate – a linear polymer containing hundreds or thousands of phosphate residues [Austin and Mayer, 2020]. Polyphosphate accumulates in response to amino acid starvation [Kuroda and Ohtake, 2000] and is stored in the organelles known as acidocalcisomes. The concentration of polyphosphate within acidocalcisomes can reach molar levels; their very large negative charge is compensated by magnesium, calcium, sodium, zinc, and basic amino acids [Docampo et al, 2010].

It is difficult to directly relate the membrane potential to polyphosphate, as its accumulation (which would presumably drive the membrane potential toward hyperpolarization) occurs under adverse conditions, when the membrane potential could be affected by other factors. Nevertheless, *B. subtilis* were found to be slightly hyperpolarized after a 7-day starvation compared to exponentially growing cultures [Gray et al, 2019], which, again, agrees with the notion that accumulation of phosphorus in a polymeric form favors hyperpolarization.

*2e. Phosphorylated proteins.* A third of all proteins contain covalently bound phosphate, and many have multiple phosphorylation sites [Cohen, 2000; Vlastaridis et al, 2017]. Thus, phosphorylated proteins can carry a substantial negative charge. It is less clear if this charge by itself serves any significant regulatory function, as the activity of many ion channels is affected by phosphorylation, and that can have a more direct impact on ions and membrane potential. For example, inhibition of phosphatases with okadaic acid causes an increase in the Ca2+ current [Hescheler et al, 1988], which can easily mask the effect of impermeant charges.

*2f. Phospholipids*. The overall negative membrane potential has two origins: the bulk processes, such as the pump-leak mechanism, and anionic phospholipids affixed to the plasma membrane [Honig et al, 1986; Khitrin et al, 2014; Ma et al, 2017]. Although the existence of the latter type of potential is well established, little is known about its regulation, and it will not be the focus of this paper. Phospholipids are also present in intracellular compartments, where their influence on the potential across the plasma membrane should be qualitatively similar to that of other impermeant anions. The most active synthesis of phospholipids coincides with the S phase [Jackowski, 1996].

*2g. Nucleic acids.* We can estimate the contribution of DNA to the phosphate pool and the overall negative charge. DNA or dsRNA contain 3 nmol of phosphate per mg [Barichello et al, 2010]. Since the diploid amount of human DNA per cell is 6 pg (<https://bionumbers.hms.harvard.edu/bionumber.aspx?id=111206>), the molar amount of phosphate is 18 fmol per cell. Therefore, a diploid cell with volume V mm3 will have 18/V mol/L of phosphate due to DNA. **Table 2** lists some typical amounts of cell-averaged phosphorus concentration contained in nucleic acids.

The conversion of concentrations into charge equivalents requires some caution. Although each phosphate group in nucleic acids is fully ionized with charge -1 [Lipfert et al, 2014], approximately half of that charge is neutralized by positively charged histones [Korolev et al, 2006; A. Onufriev, personal communication]. The remaining charge is compensated by potassium and sodium [Várnai and Zakrzewska, 2004; Lavery et al, 2014].

Sperm cells are an exception, as histones are largely replaced in them with protamine. As far as we know, the extent of charge compensation by protamine has not been evaluated, but its contribution must be significant because a large increase in the phosphorus content of the nucleus over that in the midpiece or the tail is not matched by a similar increase in sodium or potassium [Cottell and Harrison, 1995; Sheppard et al, 1988].

RNA makes another large pool of phosphorus, and the amount of RNA correlates with DNA and protein synthesis [Traganos et al, 1982; Crissman et al, 1985]. However, the role of RNA as an impermeant anion is harder to assess because of the existence of different types of RNA and multiple RNA-binding proteins.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Cells | Mass pg/cell | P fmol/cell | Volume,  fl/cell | [P]  mM | Reference |
| DNA | Human spermatozoa | 3 | 9 | 35 | 260 | Yeung et al, 2003 |
| Human hematopoietic stem cells | 6 | 18 | 100 | 200 | Li et al, 2015 |
| Human white blood cells | 6 | 18 | 150-300 | 100 | Segel et al, 1981; Kuse et al, 1985; Safak et al, 2017; Lee and Kim, 2013 ; Sharma et al, 2008 |
| Human HeLa | 6 | 18 | 2000 | 9 | James and Giorgio, 2000 |
| HeLa nuclei | 6 | 18 | 36 | 500 |
| *S. cerevisiae* | 0.013 | 0.05 | 100 | 0.5 | https://www.ncbi.nlm.nih.gov/genome/ |
| *E. coli* | 0.005 | 0.015 | 0.8 | 20 | Verma et al, 2019 |
| RNA | Human HeLa | 10-30 | 60 | 2000 | 30 | Qiagen1, Wu et al, 2014 |
| E. coli | 0.1 | 0.3 | 0.8 | 400 | AAT Bioquest |

**Table 2**. Cell-averaged concentrations of DNA and RNA-associated phosphorus.

1 https://www.qiagen.com/us/resources/faq?id=06a192c2-e72d-42e8-9b40-3171e1eb4cb8&lang=en

The correlation between DNA synthesis and membrane potential has been well established (reviewed by Blackiston et al, 2009]. Cells experience hyperpolarization during the S and G2 phases relative to G1 [Cone, 1969; Sachs et al, 1974; Wonderlin et al, 1995]. Moreover, hyperpolarization seems to be a requirement for the S phase initiation. Conversely, depolarization of the plasma membrane accompanies the G2/M transition.

Likewise, the correlation between with K+ channel activation and the phase of the cell cycle has been demonstrated multiple times (reviewed by Urrego et al, 2014). Since different types of K+ channels become activated and can support proliferation, one may hypothesize that their activation is secondary to the more universal event of proliferation, namely, the accumulation of phosphorus.

3. Negatively charged proteins

While databases of isoelectric points of proteins and proteomes are available [Kozlowski, 2022], less is known about the exact values of protein charges z1. There has been a large study of 30 amino acid-long segments from different organisms [Requião et al, 2017], but the published data do not allow simple extension to average z. Estimates of z can only be made with some confidence in a few cases. Proteins in *E. coli* have an average charge of 14.4 electrons/protein [Xu et al, 2013]. Assuming 2.4x106 protein molecules per cell with an average volume of 0.8 mm3 [Pedersen et al, 1978; Lababidi et al], one obtains 70 mM of equivalent charges. This can be compared to 200 mM of phosphorus measured in *E. coli* [Wade, 1952]. Even a greater predominance of phosphorus over negative proteins is found for the yeast *Saccharomyces cerevisiae*. The reported volumes of yeast cells have ranged from 40 mm3 [Pérez-Ortín et al, 2021] to 90 mm3 [Barber et al, 2020] to 300 mm3 [Zakhartsev and Reuss, 2018], but that would not affect the comparison between proteins and phosphorus. For z = -3.6 [Xu et al, 2013] and 50x106 protein molecules per *S. cerevisiae* cell (Futcher et al, 1999), one obtains 0.18 billion electron equivalents on proteins and 44 times more (7.9 billion) equivalents on phosphorus [Groombridge et al, 2013]**.** Naturally, a somewhat different picture emerges for red blood cells that lack nucleic acids. Inorganic phosphate, ATP, and 2,3-diphosphoglycerate make up around 10 mM total [Bartlett, 1980; Noorwali et al, 1982]. The net charge on a hemoglobin molecule has been estimated at 14 from amino acid composition [Scheinberg, 1957] or 5 from osmotic properties [Gros et al, 1978, Solomon et al, 1986]. At a concentration 0.34 g/ml = 5.3 mM, the concentration of electron equivalents on hemoglobin is almost an order of magnitude higher than on phosphates: approximately 75 mM or 26 mM, depending on which estimate for z is used (incidentally, the first estimate closely matches the AG value of 89 mM listed in Table 1).

1 It could be noted that the estimation of protein charge based on amino acid sequences are not always reliable [Kyne et al, 2017 and references therein].

4. Heterogeneity of the distribution of impermeant and permeant ions

Elements, including ions, are distributed within the cell highly heterogeneously [Malucelli et al, 2014]. First, we wish to point out that equilibrium ion gradients should not be detectable by fluorescent probes or ion-selective electrodes. Consider for example the fluorescent potassium indicator PBFI. Potassium binding to PBFI causes a shift in its excitation spectrum, which allows the determination of potassium concentration. However, the occupancy of PBFI by potassium is determined not necessarily by the molar concentration of the ion but by its “effective concentration” or thermodynamic activity, which is related to chemical potential. If, for example, potassium is electrostatically attracted to DNA in the nucleus, it will be at a higher concentration there compared to the cytoplasm, but its chemical potential will be the same throughout the cell (we assume for a moment that potassium can freely cross the nuclear membrane). Indeed, the equilibrium distribution of potassium between the nucleus and the cytoplasm will be determined by the condition that its chemical potential, and not necessarily its nominal concentration, is equal in both compartments; therefore, a probe that responds to potassium effective concentration is not going to show a stronger response in the nucleus.

This conclusion may be partially invalidated if the potassium distribution between the nucleus and the cytoplasm were controlled by pumps and channels, as is indeed suggested by some evidence [Garner, 2002; Jang et al, 2015; Checchetto et al, 2016]. At any rate, the results with chemical probes in the presence of strong intermolecular interactions must be interpreted with much caution. Atomic spectroscopy is a more direct way to quantify the absolute concentrations of elements.

The experimental data on sodium and potassium concentrations in the nucleus vs. cytoplasm have been variable. Moore and Morrill [1976] cite several studies, in which nuclear sodium and potassium were much elevated over those in the cytoplasm. The data of other authors have been less consistent (**Table 3**). One possible source of variability is the heterogeneous nature of the nucleus: phosphorus and potassium concentrations are significantly higher in condensed chromatin than in the nucleoplasm [Nolin et al, 2016]. The good match between the AG and phosphorus observed in many cases seems to contradict the notion that histones compensate half of the DNA negative charge (see above). However, because of the presence of variable but significant amounts of RNA in the nucleus [Piwnicka et al, 1983], one cannot attribute all the nuclear phosphorus to DNA alone.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cell type | Na | | K | | Cl | | Mg | | AG | | P | |  |
| Nuc | Cyt | Nuc | Cyt | Nuc | Cyt | Nuc | Cyt | Nuc | Cyt | Nuc | Cyt |  |
| Trypanosoma1 | 12 | 6 | 62 | 69 | 2.4 | 4 | 13 | 15 | 96 | 100 | 100 | 104 | Scott et al, 1997 |
| Leishmania1 | 47 | 50 | 86 | 92 | 55 | 51 | 10 | 13 | 99 | 117 | 135 | 149 | LeFurgey et al, 2001 |
| Chick heart1 | 16 | 21 | 172 | 179 | 34 | 38 | 14 | 17 | 181 | 196 | 100 | 93 | LeFurgey et al, 1988 |
| Rat macrophages1 | 15 | 16 | 90 | 72 | 24 | 33 | 152 | 152 | 111 | 85 | 119 | 84 | Kirk et al, 1990 |
| Rat hepatocytes1 | 22 | 20 | 52 | 60 | 12 | 27 | 8 | 8 | 78 | 69 | 71 | 64 | Cameron et al, 1980 |
| Rat hepatocytes1 | 16 | 8 | 117 | 77 | 24 | 11 | 9 | 5 | 128 | 84 | 101 | 72 | Zierold, 1997 |
| Rat hepatocytes | 20 | 14 | 162 | 149 | 23 | 27 | 14 | 10 | 187 | 156 | 160 | 127 | Bolkent and Zierold, 2002 |
| Rat mammary1 | 26 | 27 | 80 | 79 | 36 | 32 | 10 | 10 | 90 | 94 | 114 | 114 | Cameron et al, 1980 |
| Rat neurons1 | 8 | 10 | 280 | 220 | 55 | 40 | 152 | 152 | 260 | 220 | 125 | 150 | LoPachin et al, 1988 |
| Rat neurons1 | 46 | 50 | 161 | 132 | 43 | 48 | 6 | 5 | 176 | 144 | 136 | 115 | Lopachin et al, 2001 |
| Rabbit muscle1 | 35 | 34 | 130 | 118 | 51 | 56 | 8 | 7 | 130 | 110 | 119 | 50 | Somlyo et al, 1979 |
| Human leukemia1 | 20 | 32 | 116 | 128 | 34 | 40 | 152 | 152 | 132 | 150 | 108 | 128 | Arrebola et al, 2006 |
| Human HL60 | 40 | 41 | 273 | 243 | 44 | 46 | 24 | 23 | 318 | 285 | 265 | 241 | DiFranceso et al, 1998 |
| Human HeLa | 203  104 | 20 | 4703  1904 | 220 | 803  454 | 60 | 323  134 | 21 | 4743  1814 | 222 | 6503  1504 | 220 | Nolin et al, 2016 |

**Table 3.** Intracellular concentrations in mM of the major ions and phosphate in nuclei and cytoplasm. 1Concentration were recalculated from mmol/kg dry weight data assuming 0.2 kg dry weight/L in mammalian cells. 2Total magnesium concentration was not reported and was assumed to be 15 mM [Romani, 2011]. 3Condensed chromatin. 4Nucleoplasm.

3. Theory

Here we will theoretically examine the effect of large multivalent organic anions, such as DNA, on intracellular ions and membrane potential. The synthesis of molecules, such as DNA, from nucleotides mainly results in an increase in the average z. **Table 2** lists a few examples of DNA and RNA concentrations, which sometimes significantly exceed the typical ~10 mM of nucleotides and Pi. This effect is especially prominent in smaller nucleated cells, such as hematopoietic cells. In such cells, the doubling of DNA will result in almost the doubling of z.

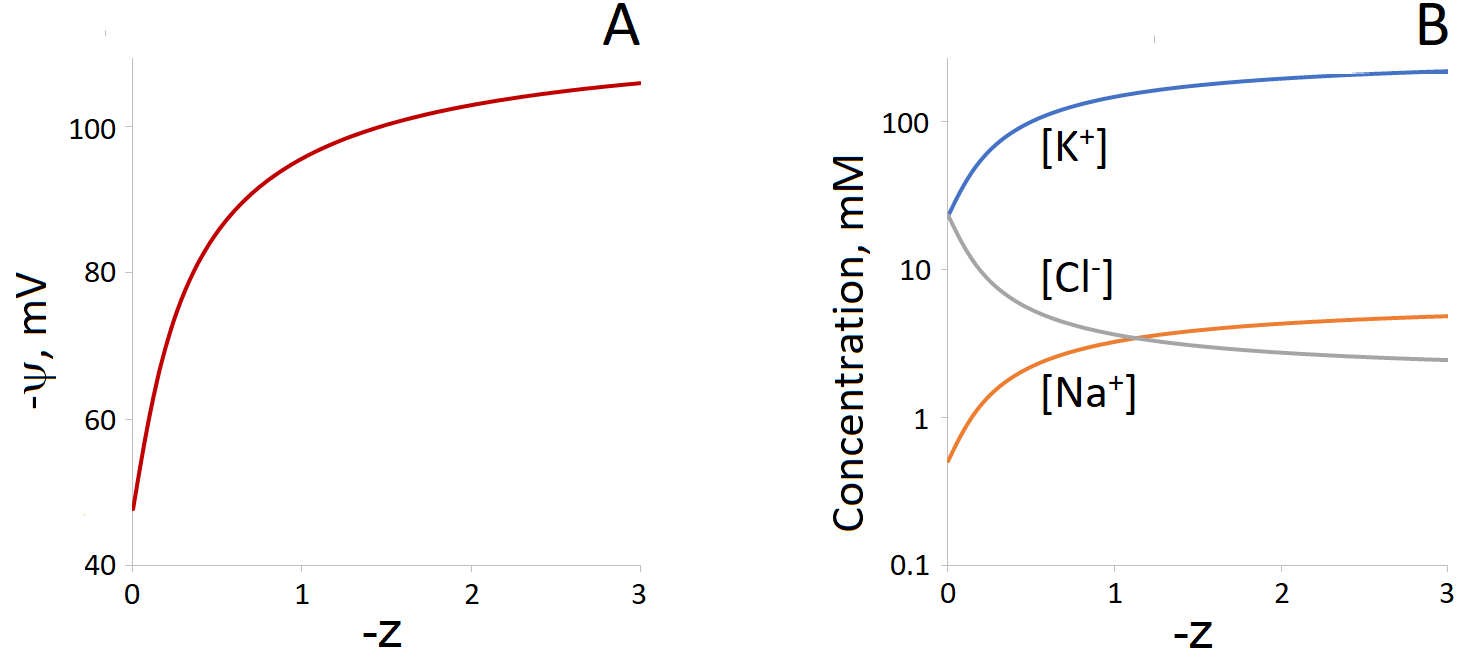
*Model 1* [Kay, 2017]. In this model (based on [Keener and Sneyd, 2009]), a spherical cell with area A and volume V is bathed in a solution with known and constant sodium, potassium, and chloride concentrations and osmolarity P. The cell interior contains variable amounts of potassium, sodium, and chloride, and a constant amount of impermeant anions X- with average valency z. Passive ion fluxes into and out of the cell are quantified through the analog of Ohm’ law, in which the driving force is represented as the difference between the membrane potential y and the equilibrium Nernst potential yeq for a given ion. In the case of potassium and sodium, the flux through the Na+,K+ pump is added. For sodium, for example, the flux equation has the form

where V is cell volume, p is the contribution of the Na+,K+ pump (assumed to be constant), and the membrane permeability for sodium is included in the coefficient q. Chloride transport is taken as entirely passive and uncoupled from other ions. These conditions provide three equations (one for each ion) for five unknowns: three intracellular ion concentrations, cell volume, and membrane potential. One of the remaining two equations needed to solve the system relates the rate of volume change to the osmolarity difference; the rate of water accumulation is proportional to membrane permeability (included in the coefficient k) and the difference between the internal and external osmolarity:

The other equation relates y to the difference between positive and negative charges:

where F is the Faraday constant and C is specific membrane capacitance.

The above set of five equations allows one to find the dependency of y and ion concentrations on z. The results for steady state are shown in Fig. 1. An increase in the negative valency z of impermeant charges produces hyperpolarization (more negative y), accumulation of potassium and sodium and a loss of chloride.



**Figure 1.** The effect of z on the membrane potential y (panel A) and the steady state values of [K+], [Na+], and [Cl-] (panel B), according to Model 1. The data are replotted from [Kay, 2017].

*Model 2.* This model has been extensively used by Vereninov and coworkers [Vereninov et al, 2007; Yurinskaya et al 2011, Yurinskaya et al, 2019; Yurinskaya and Vereninov, 2021], and the code is now available on <https://vereninov.com/cellionfluxes/>. It assumes exact electroneutrality and isoosmolarity between the internal and external solutions. Instead of Ohm’s law, the passive fluxes J are computed through the Goldman equation, such as

,

where PNa is the membrane permeability for Na+, and u is the dimensionless membrane potential:

In this model, the membrane potential is deduced not from the difference between positive and negative charges, as in Model 1, but is back-calculated from passive fluxes. The contribution of the Na+,K+ pump is represented by a linear term:

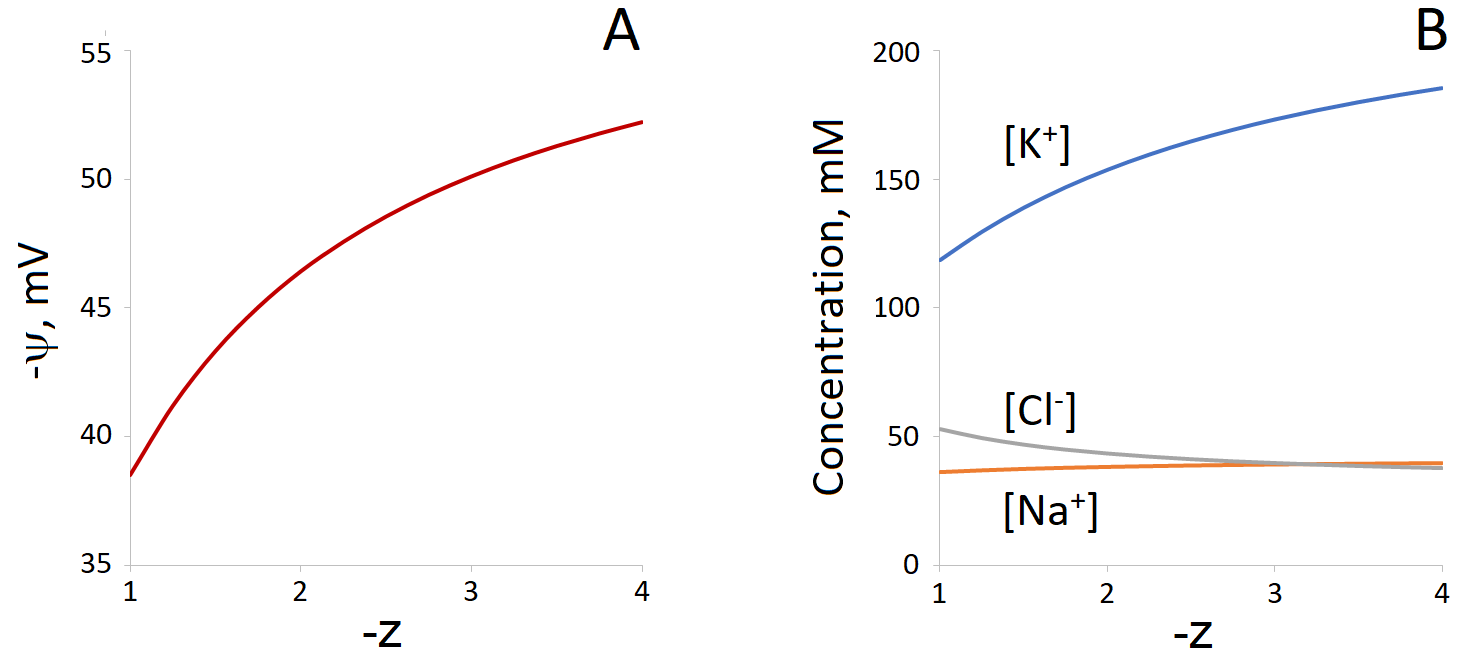
The cotransporters NC, KC, and NKCC are included as well, and their activities are expressed through the ratio of the intracellular-to-extracellular concentrations of the respective ions.

We used this model to investigate the effect of various intracellular initial concentrations on the stabilized values of y and ion concentrations. The significance of the starting concentrations is that they implicitly define both [X-] and z, which are conserved during the subsequent evolution of the system toward a stable state:

[X-] = P – [Na+] - [K+] - [Cl-]

z[X-] = [Na+]+ [K+] – [Cl-]

The results are shown in **Fig. 2**. Only the sum of cations affects the balanced state, but not the individual values of [Na+] and [K+].



**Figure 2**. The effect of z on the membrane potential y (panel A) and the steady state values of [K+], [Na+], and [Cl-] (panel B), according to Model 2. The transport parameters were taken at the default values at <https://vereninov.com/cellionfluxes/>; the osmolarity P = 310 mM was kept constant. Eliminating cotransport changed the numerical values, but not the general shapes of the curves.

TO ADD:

* DISCUSS MORE WHY K+ CHANNELS ARE INVOLVED IN THE CELL CYCLE
* MODEL 3 (DMITRIEV)
* MODEL 4 (DUSTERWALD)
* TITLE, ABSTRACT, CONCLUSION, ACKNOWLEDGEMENTS,

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