Nernst–Planck equation, ie the chemically and the electrically-driven fluxes.[6] Indeed, assuming the constant-field and the electroneutrality conditions and that membrane thickness is negligible, then it can be demonstrated that the membrane potential from Nernst–Planck equation is equivalent to $2RT/F$ times the parameter α (Eqn 7). In other words, the force exerted by an electric field generated by a microscopic deviation of electroneutrality (as described by the algorithm presented here) corresponds to that exerted by the presence of a membrane potential (as described by Nernst–Planck formulation) under electroneutrality conditions. Moreover, assuming probe concentration has reached the steady-state, it can be demonstrated from Eqns 1, 6 and 7 that the Nernst potential of the probe can take the form of the GHK equation. Similar arguments can be applied to the other situations considered.[4]

```
100 REM *******************************************************
110 REM *          GENERATION OF MEMBRANE POTENTIALS          *
120 REM *******************************************************
130     DIM C(8,2):REM Conc(specie,membrane side), in mM
140     DIM P(8),Z(8),DN(8):REM Permb,charge, conc.increments
150 REM
160 REM **************** DATA INPUT *************************
170     NI = 6:          REM Number of ionic species
180     BETA = 1.05:  REM Na,K-ATPase pump parameter
190 REM ** Data of the probe
200  C1(1, 1) = .1:  C1(1, 2) = .1:    Z(1) = 1: P(1) = 10
210  C1(2, 1) = .1:  C1(2, 2) = .1:    Z(2) = -1:P(2) = 10
220 REM ** Data of Na+, Cl-, K+ and X-
230  C1(3, 1) = 14:  C1(3, 2) = 141:  Z(3) = 1: P(3) = 0.012
240  C1(4, 1) = 7.5: C1(4, 2) = 106.5:Z(4) = -1:P(4) = 0.13
250  C1(5, 1) = 139: C1(5, 2) = 6:    Z(5) = 1: P(5) = 0.3
260  C1(6, 1) = 146: C1(6, 2) = 40:   Z(6) = -1:P(6) = 0
270 REM ** Input iterations and time interval
280     INPUT "Number of total iterations =", NK
290     INPUT "Time interval per iteration=", DT
300 REM ** Calculation denominator of parameter alpha
310     SUMPI = 0
320     FOR I = 1 TO NI
330  SUMPI = SUMPI + Z(I) ^ 2 * P(I) * (C1(I, 1) + C1(I, 2))
340     NEXT I
350 REM
360 REM ********************* MAIN LOOP *********************
370     FOR K = 1 TO NK
380 REM ** Computation difference of charge generated
390     DQ = 0
400     FOR I = 1 TO NI
410         DQ = DQ + Z(I) * (C1(I, 2) - C1(I, 1))
420     NEXT I
430 REM ** Computation of parameter alpha
440     ALFA = - DQ / (2 * SUMPI)
450 REM ** 'Neutralizing' step
460     FOR I = 1 TO NI
470       DN(I) = ALFA * Z(I) * P(I) * (C1(I, 1) + C1(I, 2))
480       C2(I, 2) = C1(I, 2) + DN(I)
490       C2(I, 1) = C1(I, 1) - DN(I)
500     NEXT I
510 REM ** Computation Nernst potential of the probe, in mV
520     VNERNST = 25.6 / Z(1) * LOG(C2(1, 2) / C2(1, 1))
530 REM ** 'Free diffusion' step
540     FOR I = 1 TO NI
550         DN(I) = -DT * P(I) * (C2(I, 2) - C2(I, 1))
560         C1(I, 2) = C2(I, 2) + DN(I)
570         C1(I, 1) = C2(I, 1) - DN(I)
580     NEXT I
590 REM ** Action of Na,K-ATPase pump
600         DN(3) = 3 * BETA * DT
610         DN(5) = -2 * BETA * DT
620         C1(3, 2) = C1(3, 2) + DN(3)
630         C1(3, 1) = C1(3, 1) - DN(3)
640         C1(5, 2) = C1(5, 2) + DN(5)
650         C1(5, 1) = C1(5, 1) - DN(5)
660 REM ** Output of results
670     PRINT , C1(3, 1), C1(4, 1), C1(5, 1)
680     PRINT VNERNST, C2(3, 1), C2(4, 1), C2(5, 1)
690 REM ** End loop
700     NEXT K
710     END
```

Conclusion

The program presented in this paper can be very useful in explaining the physicochemical phenomena underlying the generation of membrane potentials in excitable cells. Its simplicity allows students to understand the nature of these processes through a direct, hands-on approach. Also, the simulated voltage and concentration kinetics agree well with those predicted by more complicated thermodynamic models.

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The Windogradsky Column: A Simple and Inexpensive Approach to Teach Environmental Biochemistry

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Introduction

One of the issues in biochemistry poorly understood by students of biological sciences is related to the importance of autotrophic, litotrophic, organotrophic and heterotrophic metabolisms in the cycle of the elements. This in turn relates to the role of microorganisms in the dynamics of the transformation of organic matter and how these processes form the basis of life in the ecosphere.[1,2]

We propose a simple and inexpensive experiment that will provide an exciting source of information about the main biological processes which may occur in nature during the eutrophication of lakes and ponds. The observation and interpretation of the modifications that take place during the eutrophication of a water column constitutes an illustrative approach for understanding environmental biochemistry and the relationship between the different types of metabolism mentioned above.

Winogradsky's Column

In 1888 Sergei Winogradsky (1856–1953) introduced an original method to enrich microbial populations.[3] The method allowed him to discover an amazing variety of biochemical processes, many of which were hitherto unknown. This method disclosed how different microbial groups adapt themselves to defined ecological niches and participate in nature's dynamics.

This 'method' required a simple glass column containing an aquatic environment supplemented with a few chemical compounds. These very simple compounds are then transformed by microorganisms over a relatively long period of time. This provides a gradient of H_2S, CO_2, and organic materials that allows other microorganisms to grow. By this means, it is possible to create on the lab scale, a model that mimics the environmental conditions of microbial populations in nature. The experiment allows the students to observe the development of a complete ecosystem and to isolate the different microbial species that participate in these processes. Students carrying out the experiment will be able to understand the cycles of the elements, assigning to specific groups of microorganisms the origin of the transformations they observe.

Making a Winogradsky Column

Variations of a procedure to prepare the water column can be found in practically any of microbiology text.[4] A simple and successful one is made as follows. The inoculum (100–200 g of superficial and subsuperficial sediment of a pond or marsh) is prepared by mixing the mud with 4 g of each of the following salts: $CaCO_3$, $CaSO_4$ and $CaHPO_4$. The addition of $CaCO_3$ and $CaHPO_4$ enhances the microbial development. This preparation

is used to fill a glass cylinder (2–4 cm inside diameter, height 40–50 cm). Water is added to 3 cm below the top of the cylinder and the suspension is left for 2–3 days. Finally, fine divided filter paper is added until the surface of the sediment is covered by 3–5 cm of paper. Care must be taken to avoid air bubbles entrapped within the column. It is recommended to cover the column for a week with aluminium foil to protect it from light. During this period the first group of organisms start to grow and they generate an ascendent flux of H_2S and CO_2 (ie heterotrophic anaerobic respiration). This ascendent flux will create a positive gradient of oxygen towards the top of the cylinder. The column may then be placed under daylight illumination or a 60 watt lamp (wave length 720–1000 nm). It is also recommended that the top of the cylinder be covered to prevent significant evaporation of water. Water must be added periodically and carefully in order to avoid disturbing the steady-state fluxes of gases and to disperse air into the column.

Macroscopic and microscopic observations of samples of water at different heights of the cylinder can be performed in order to follow the whole process from the beginning. After two or more months, the column will present a typical development, allowing the students to observe the process of eutrophication.

The description of the main chemical reactions taking place and the biochemical pathways used by the microorganisms (settled at fixed positions of the column, see Fig 1) during this process should be the focus of theoretical developments and discussions.

Transformation of organic matter through the sulfur cycle in the Winogradsky column

The sulfur cycle[5,6] (Fig 2) constitutes an interesting model for understanding the relationships between different biochemical processes and the dynamics of an ecosystem. Participating in this cycle are microorganisms with photosynthetic or chemotrophic metabolisms which obtain carbon autotrophically or heterotrophically and use organic or inorganic compounds as a source of reducing power (see Scheme 1).

Fig 1 shows, that it is possible to assign biochemical reactions and groups of microorganisms to each portion of the column in agreement with the physicochemical and ecological environment.

Chemolitotrophic metabolisms

In nature, sulfur is transformed by bioprocesses carried out by animals, plants and microorganisms, and can therefore, be found in various oxidation states. Sulfide ($S^=$), sulfur (S^0) and sulfate ($SO_4^=$) are the main species involved in ecological processes.[6,7] Plants and microorganisms reduce $SO_4^=$ and incorporate it into several organic compounds. This process is called assimilatory reduction of $SO_4^=$ and can be positioned in the vertical plane of the metabolic cube between the autotrophic and the heterotrophic phases[5] shown in Fig 2. In region 1 of the cube, the process of oxidation of sulfur compounds under autotrophic conditions is positioned within the aerobic region that corresponds to litotrophic metabolism. Gliding bacteria of the genus

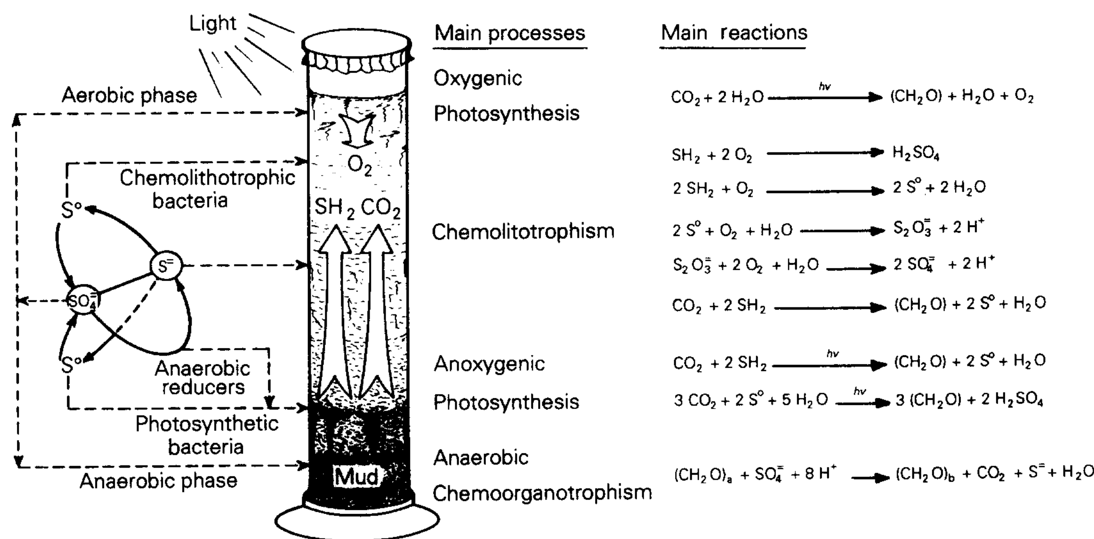


Figure 1 Simplified picture of the main biochemical processes that take place in the Winogradsky column. The major microbial groups participating in these processes are also indicated

Carbon Source	Source of energy and electron donors: Light – Organic	Light – Inorganic	Chemical reactions – Organic	Chemical reactions – Inorganic
Carbon Dioxide	Photoorganotrophic-autotrophy **POAT**	Photolitotrophic-autotrophy **PLAT**	Chemoorganotrophic-autotrophy **COAT**	Chemolitotrophic-autotrophy **CLAT**
Organic Compounds	Photoorganotrophic-heterotrophy **POHT**	Photolitotrophic-heterotrophy **PLHT**	Chemoorganotrophic-heterotrophy **COHT**	Chemolitotrophic-heterotrophy **CLHT**

(CLAT and CLHT → Myxotrophy)

Scheme 1

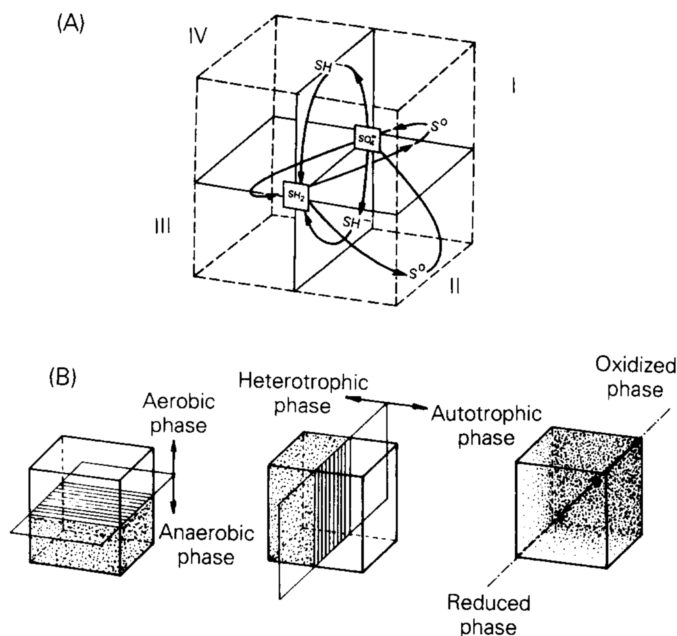
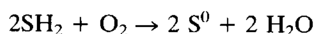


Figure 2 Three dimensional arrangement of the sulfur cycle (A) and division of the cube into trophic and environmental regions (B)

Beggiatoa and *Thiothrix*[1] are located within this region. These bacteria grow mainly in a region of the water column where H_2S and O_2 are present (Fig 1). These microorganisms have a chemolitotrophic metabolism[8,9] (**CLT**), and have developed the capacity to consume CO_2 for biosynthesis, while generating ATP and NADPH by the oxidation of reduced sulfur compounds. The capacity to reduce CO_2, allows us to locate these microorganisms in the autotrophic subspace of the cube (Fig 2).

One of the first transformations carried out by these microorganisms is



$$2SH_2 + O_2 \rightarrow 2\ S^0 + 2\ H_2O$$

The biochemical process involved in the oxidation of sulfur compounds are depicted in Figure 3. S^0 may be accumulated intracellularly or it can be exported to the medium.[1] Members of the genus *Beggiatoa* and *Thiothrix* isolated from the water column and cultured in selective media allow us to observe this phenomenon. It is possible to distinguish *Beggiatoa* from *Thiothrix* microscopically since the former are long single cells, whereas the latter are clustered in a rosette-like structure.[10,11] In both cases the intracellular accumulation of S^0 is observable during the first week of cultivation. When the cultures get older, the content of sulfur granules diminishes, disappearing completely after a month, indicating the consumption of the accumulated S^0. It is also interesting to observe under the microscope the photophobic behavior displayed by *Beggiatoa* (ie it moves away from the incident light). It has been suggested that this photophobic behavior was developed to protect these organisms from light, since they do not contain carotenoids to perform that function.[10]

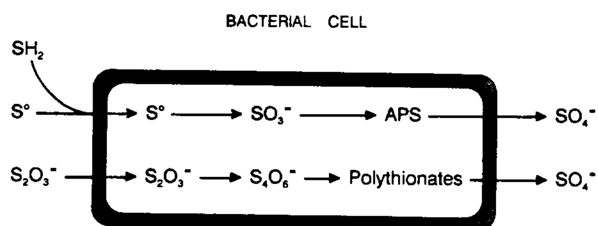
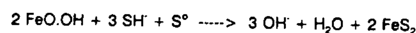


Figure 3 Simplified scheme of the biochemical reactions carried out by microorganisms showing CLT metabolism during the oxidation of sulfur compounds

Thiobacillus can also accumulate S^0 or export it to the medium,[1,2] and in both cases S^0 may be oxidized to $SO_4^=$ through intermediates like $SO_3^=$, $S_2O_3^=$, $S_4O_6^=$ and polythionates. This process implies the overall transference of $8e^-$ and the liberation of an energy equivalent to 184 Kcal/mol. This energy is accumulated, partially as ATP.[1,2]

The contribution of these CLT bacteria to the recycling of sulfur is difficult to establish since sulfur compounds (H_2S mainly), are oxidized spontaneously in the presence of O_2 without bacterial mediation. Nevertheless, this process would not exist during the oxidation of ore sulfur (lixiviation) where spontaneous oxidation is practically null (Fig 4). These reactions constitute the energetic bases of the metabolism of *Thiobacillus*, *Beggiatoa* and *Thiothrix*. They can grow in chemolitoautotrophic (CLAT) conditions, contributing to the fixation of CO_2 in stratified sediments where H_2S exists.

Although *Beggiatoa* and *Thiobacillus* can grow well under lithotrophic conditions, in most cases they are not able to use CO_2 as the only carbon source. Organisms that can use inorganic compounds or light as a source of energy and cannot use CO_2 as the unique source of carbon are called 'myxotrophic'. Accordingly, *Beggiatoa* and *Thiobacillus* could be included not only in region I of the trophic cube (Fig 2), but also in region IV, displaying a chemolitomixotrophic (CLMT) metabolism. They should also be located on the vertical semiplane within the aerobic phase.



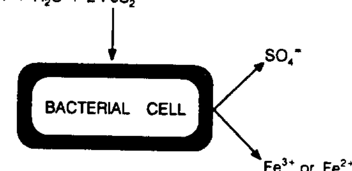


Figure 4 Simplified scheme of the sulfur oxidation and lixiviation carried out by bacteria displaying chemolithoautotrophic metabolism (CLAT)

Phototrophic metabolisms

Phototrophic organisms found in region II of the cube (Fig 2), are anaerobic bacteria that require light and reduced inorganic compounds.[12] This group of microorganisms is clearly observed in the water column after two months, since a colored pattern develops above the sediment. Pink, purple, violet, orange and green are colors observed, from the top to the bottom of the column. By biochemical and microbiological techniques,[13–15] it is possible to show that some blue-green algae (*Cyanobacteria*), purple non-sulfur bacteria (*Rhodospirillaceae*), purple sulfur bacteria (*Chromateaceae*) and green sulfur bacteria (*Chlorobeaceae*) may be found in this aquatic system, occupying specific niches according to their metabolic features and the chemical environment (Fig 1).

In our experiments, we were able to detect the presence of these groups of bacteria by simple observation of the color patterns. The correlation of colors and groups was confirmed later when samples from different levels of the sediment were taken and analyzed. Green non-sulfur bacteria (*Chloroflexaceae*) were not present, most likely due to their thermophilic characteristics.[10]

In the aerobic and microaerophilic regions of the water column, *Cyanobacteria* carry out photosynthesis, by using H_2O

or H_2S as the source of electrons to generate O_2 or $SO_4^=$ respectively (Fig 1). For this reason *Cyanobacteria* can be classified as a photolithoautotrophic microorganism (PLAT). These bacteria can be located either in region I of the cube (Fig 2) carrying out oxygenic photosynthesis (with no participation in the sulfur cycle), or in region II (anoxygenic photosynthesis) using $S^=$ as the electron donor. In the latter case *Cyanobacteria* carry out a process different than those mentioned in CLT metabolism, but involving the same reactants and products.

Purple sulfur and non-sulfur bacteria are autotrophic facultative microorganisms that can be included either in region II as PLAT microorganisms, or in region III of the cube (Fig 2) carrying out the same transformation of S^0 under photolithotrophic-heterotrophic conditions (PLHT, not shown in Fig 1). Both types of bacteria (sulfur and non-sulfur) may grow in photoorganotrophic-heterotrophic conditions (POHT) using organic compounds as a source of electrons (in photosynthesis) and cellular carbon. In this case these metabolisms would be located in region III of the cube, without participating in the Sulfur Cycle (Fig 2).

Microorganisms belonging to the group of *Rhodospirillaceae* (specifically *Rhodospirillum* and *Rhodopseudomonas*) may also grow aerobically in the dark displaying a chemoorganoheterotrophic metabolism (COHT). This particular group of organisms that exhibit enormous metabolic diversity, could also be located in region IV of the cube with no participation in the Sulfur Cycle (Fig 2).

Chlorobium species, are strict phototrophic bacteria and can grow using a PLAT or photolithomyxotrophic (PLMT) metabolism. That is, they use either $S^=$ or S^0 as electron donors during photosynthesis, and use CO_2 assimilation together with some organic compounds (mainly acetate) as cellular carbon sources. In this case, *Chlorobium* would occupy simultaneous subspaces II and III of the trophic cube, or the vertical semiplane that corresponds to the anaerobic regions (Fig 2). All of these photosynthetic bacteria, have pigments that enable them to utilize light of long wavelengths, which is not normally used by the oxygenic photosynthetic organisms. Bacterial photosynthesis does not take place in natural aquatic habitats below 20 meters deep, since light becomes insufficient. Moreover, the sulfur compounds would inhibit the development of microorganisms (ie *Chlorobium*, most tolerant group, 4–8 mM; *Chromatium*, 0.8–4 mM; *Rhodospirillaceae*, 0.4–2 mM).[7]

Due to the high sulfur tolerance of the green sulfur bacteria, they are located in 'sulfureta' (their natural habitat), usually underneath the levels where the purple sulfur bacteria are located. The latter is clearly observed in the Winogradsky column (Fig 1). In this system, when a sulfuretum is established, there is not a quantitative requirement of sulfur to maintain an active development of CLT and PLT metabolisms participating in the Sulfur Cycle. In fact, $SO_4^=$ produced mainly in subspaces I and II of the cube (Fig 2), is reduced again by anaerobic respiration (region III of the cube) by bacteria belonging to the genus *Desulfovibrio* and *Desulfotomaculum*,[1,2] closing the cycle of sulfur utilization (Fig 2).

The interrelationships among these sulfate-reducing, purple and green bacteria is quite precise. The green and purple bacteria provide preformed carbon nutrient to the sulfate-reducers for their COHT metabolisms (region III of the cube), and the sulfate-reducers, supply H_2S to the PLAT and CLAT metabolisms (Fig 2). The initial source of CO_2 in this complex biological chain is the $CaCO_3$ added at the beginning of the experiment.

Although prokaryotes may play a minor role in the assimilatory reduction of CO_2, the contrary happens with the mineralization of the organic matter. In anaerobic environments, sulfate-reducing bacteria have a major role. For instance, in marine sediments they catalyze the recycle of 50% of the organic matter.[7] In this process, a large quantity of sulfur is formed and accumulated combined with heavy metals (ie sulfur), whereas only a minor fraction is transferred to the atmosphere. The major part is afterwards reoxidized by other bacterial groups displaying CLAT or CLT metabolisms.

The students can easily observe this phenomenon in a water column that was protected from light in order to avoid the development of photosynthetic organisms, since a black sediment is formed in the column as result of the precipitation of sulfides. The dark sediment covering the paper, the bubble formation (H_2S) and the anaerobic conditions established in the column, indicate that $S^=$ is formed and accumulated during the development of active sulfate-reducing metabolisms, as consequence of the elimination of the anaerobic photosynthetic processes provided by photosynthetic bacteria.

Conclusions

Our experience in using this scheme yielded optimum results by interesting the students in the biochemical processes that take place in nature and in the complex interrelationships between environmental conditions and particular groups of organisms. The students were able to verify that, in unusual habitats and under extreme physicochemical conditions, life is still possible and that the metabolisms that those organisms use for living are also important for recycling materials, often our own residues. The biochemical pathways developed by these organisms for obtaining energy (ATP and reducing power) and carbon source become the most efficient mechanisms for overcoming adverse physicochemical conditions established in such habitats.

The students were also able to appreciate that, in each step or any cycle of the elements, a group of organisms is always involved. This means that a particular organism and its metabolic products may serve as an essential nutrient for neighbors. If microbial enrichments are carried out, each culture may represent single, double or multiple steps of the sulfur or carbon cycle (ie when CLT were found, they were shown to participate in two cycles). Furthermore, a detailed discussion on the composition of the media used for each microorganism[16] was very helpful for understanding the main metabolic features displayed by different groups of bacteria. These results were used for confirming the theoretical approach developed by the students when the sulfur cycle was explained from the Winogradsky column.

The presence of both prokaryotes and eukaryotes (algae and protozoa), was also interesting, because the students were able to observe the biological, physiological and morphological evolution within the water column, from the autotrophic, litotrophic and anaerobic way of life, towards the aerobic and heterotrophic. A rudimentary biological chain could also be built up by using their experimental observations.

Acknowledgements

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A Computer Display of the Free Energy Changes of ATP Hydrolysis

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Introduction

In living organisms, the most important molecule both for capturing and transferring free energy is adenosine triphosphate (ATP). The hydrolysis of ATP yields chemical energy and couples with the various endergonic processes including biosynthesis of macromolecules, active transport across membranes, and cell motility.

In order to understand some aspects of these energy coupling reactions, it is necessary to know the value of the free energy changes for the hydrolysis of ATP to ADP and inorganic phosphate (Pi). However, several factors in cellular systems can influence the magnitude of the free energy of ATP hydrolysis. For example, Mg^{2+}, a common requirement for several types of ATP-linked energy reactions, forms complexes with the ionic species ATP^{4-}, $HATP^{3-}$, ADP^{3-}, $HADP^{2-}$, $H_2PO_4^-$, and HPO_4^{2-}, all being involved in the hydrolysis of ATP. Since the affinity of Mg^{2+} for these species differs and since each species occurs to a different extent depending upon the pH, it is quite clear that both Mg^{2+} and pH can alter the free energy of ATP hydrolysis.

Alberty first calculated the free energy changes of ATP hydrolysis at any given pH and Mg^{2+} ion concentration.[1] Along these lines we have extended the calculations and compared the ATP hydrolysis reaction as a function of pH and the concentration of various kinds of metal ions, including Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}, Li^+, Na^+, and K^+ ions.[2,3]

In this paper, we present a program for displaying the free energy changes for the hydrolysis of ATP as a contour diagram on a personal computer.

Theoretical background

In the hydrolysis of ATP to ADP and Pi at physiological pH, each reactant exists in more than one ionized form. When a divalent metal ion M^{2+} is present, the hydrolysis should therefore be expressed as,

$$\Sigma ATP + H_2O \rightarrow \Sigma ADP + \Sigma Pi \quad (1)$$

where

$$\Sigma ATP = ATP^{4-} + HATP^{3-} + MATP^{2-} + MHATP^{1-} \quad (2)$$

$$\Sigma ADP = ADP^{3-} + HADP^{2-} + MADP^{1-} + MHADP^0 \quad (3)$$

$$\Sigma Pi = HPO_4^{2-} + H_2PO_4^- + MHPO_4^0. \quad (4)$$

For this reaction, the observed equilibrium constant, K_{obs}, is defined in terms of the molar concentrations:

$$K_{obs} = [\Sigma ADP] \times [\Sigma Pi]/[\Sigma ATP] \quad (5)$$

As described previously,[2,3] this is given finally by the following equation as a function of the free $[H^+]$ and metal ion concentrations involving the various constants:

$$K_{obs} = [HADP^{2-}] \times [HPO_4^{2-}]/[ATP^{4-}] \times (1 + [M^{2+}]/K_dMHADP + K_1ADP/[H^+] \times (1 + [M^{2+}]/K_dMADP)) \times (1 + [M^{2+}]/K_dMP + [H^+]/K_2P)/(1 + [M^{2+}]/K_dMATP + [H^+]/K_1ATP \times (1 + [M^{2+}]/K_dMHATP)) \quad (6)$$

where

$$K_dMHATP = [M^{2+}][HATP^{3-}]/[MHATP^{1-}] \quad (7)$$

$$K_dMATP = [M^{2+}][ATP^{4-}]/[MATP^{2-}] \quad (8)$$

$$K_dMHADP = [M^{2+}][HADP^{2-}]/[MHADP^0] \quad (9)$$

$$K_dMADP = [M^{2+}][ADP^{3-}]/[MADP^{1-}] \quad (10)$$

$$K_dMP = [M^{2+}][HPO_4^{2-}]/[MHPO_4^0] \quad (11)$$

$$K_1ATP = [H^+][ATP^{4-}]/[HATP^{3-}] \quad (12)$$

$$K_1ADP = [H^+][ADP^{3-}]/[HADP^{2-}] \quad (13)$$

$$K_2P = [H^+][HPO_4^{2-}]/[H_2PO_4^-] \quad (14)$$

By inserting literature values[4,5] for the required constants in Eqn 6, the standard free energy change, $\Delta G^{o\prime}$, for the hydrolysis of ATP is then calculated from:

$$\Delta G^{o\prime} = -RTln(K_{obs}) \quad (15)$$

A computer program to present the free energy map for the hydrolysis of ATP

In previous papers, a digital computer was used to print out the calculated values as an array above the pH-pM^{2+} plane, and the desired contour lines were then drawn in by hand.[1–3] However, manually drawing the contour lines was very laborious so that a more up-to-date procedure was sought.

We present here a personal computer program, which can directly display the free energy map of ATP on a screen by inputting the required values from keyboard. Its complete program listing is shown in the **Appendix**. In this program, it is necessary to input the following two items: (1) the name of the metal ion, and (2) the required metal-complex dissociation constants (K_d) in the form of $pK_d(= -\log(K_d))$. For example, if we are interested in the effect of Mg^{2+} ion, the literature values