Microbiology Course Material

Semester – II (CC3)

CC3: Biochemistry
Unit 1: Bioenergetics

By

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❖ Coupled reactions

➢ Coupling

Standard Free Energy Value of Chemical Reactions are Additive. A noteworthy thermodynamic fact is that the overall free energy change for a series of reactions is equal to the sum of the free energy changes of the individual steps. Consider the two consecutive reactions where each reaction has its own equilibrium constant and a characteristic standard free energy change, $\Delta G1°'$ and $\Delta G2°'$.

$A \rightarrow B \ \Delta G1°' = -8$ kcal/mol

$B \rightarrow C \ \Delta G2°' = +5$ kcal/mol

Since the two reactions are sequential, the intermediate product B cancels out and the overall reaction with its standard free energy change, $\Delta Gs°'$ may be written as : $A \rightarrow C \ \Delta Gs°' = \Delta G1°' + \Delta G2°' = -8 + (+5) = -3$ kcal/mol

Under standard conditions, A can be spontaneously converted into B because ΔG is negative. However, the conversion of B into C, under standard conditions is thermodynamically not feasible. But as the free energy changes are additive, the conversion of A into C has a $\Delta G°'$ value of –3 kcal/mol, which obviously means that A can be converted into C spontaneously under standard conditions. Thus, the above two sequential reactions are coupled by the intermediate product, B. In other words, **a thermodynamically unfavourable reaction can be driven by a thermodynamically favourable reaction**. Two sequential steps from glycogen breakdown in muscles will illustrate this fact more clearly.

Glucose 1-phosphate + enzyme Phosphoglucomutase → Glucose 6-phosphate $\Delta G1^{\circ\prime} = -\ 1.74$ kcal/mol Glucose 6-phosphate

Glucose phosphate +enzyme isomerase → Fructose 6-phosphate

$\Delta G2^{\circ\prime} = +\ 0.40$ kcal/mol

On adding the two reactions, we get :

Glucose 1-phosphate → Fructose 6-phosphate This has a standard free energy change value, $\Delta Gs^{\circ\prime} = \Delta G1^{\circ\prime} + \Delta G2^{\circ\prime} = -1.74 + (+\ 0.40) = -1.36$ kcal/mol

Because $\Delta Gs^{\circ\prime}$ is negative, glucose 1-phosphate is converted into fructose 6-phosphate in the muscles.

Adenosine tri phosphate (ATP) plays a central role in the transference of free energy from the exergonic (= energy-yielding) to the endergonic (= energy-requiring) processes in the cells. During breakdown of energy-rich foodstuffs or fuel molecules, some of the free energy is harnessed to make ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi), a process that requires input of free energy. ATP then donates much of its chemical energy to energy-requiring processes (biosynthesis, transport etc.) by undergoing a breakdown to ADP and Pi.

ATP hydrolysis is coupled with an endergonic reaction to make it exergonic and make the reaction possible under the condition.

ATP AS UNIVERSAL CURRENCY OF FREE ENERGY IN BIOLOGICAL SYSTEMS

The living objects require a continuous supply of free energy mainly for the following 4 purposes:

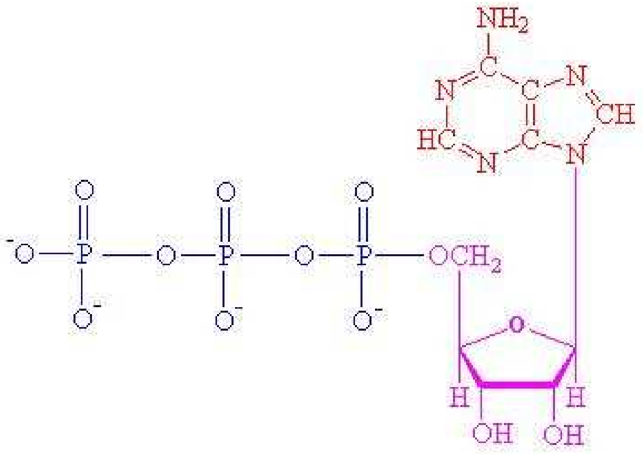
(a) to synthesize macromolecules from simpler and smaller precursors,

(b) to transport molecules and ions across membranes against gradients,

(c) to perform mechanical work, as in the muscle contraction, and

(d) to ensure fidelity of information transfer.

The free energy in these processes is derived from the environment. The phototrophs obtain this energy by trapping light energy from the sun. On the other hand, the chemotrophs obtain it by the oxidation of foodstuffs. This free energy (derived from light or from the oxidation of foodstuffs) is partly transformed into a special form before it is used for biosynthesis, transport, motion and fidelity. This special carrier of free energy is adenosine triphosphate (ATP).



Adenosine tri phosphate

Adeosine triphosphate (ATP) and its successive hydrolysis products, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are nucleotide, consisting of an adenine, a ribose and a 3, 2 or 1 phosphate group(s) respectively. ATP, ADP and AMP occur not only in cell cytosol but also in mitochondria and the nucleus. In normal respiring cells, ATP makes up about 75% or more of the sum of all 3 adenine ribonucleotides.

ATP serves as the principal immediate donor of free energy in biological systems rather than as a storage form of energy. **In a typical cell, an ATP molecule is consumed within a minute of its formation. The turnover of ATP is very high**. For instance, a resting human consumes about 40 kg ATP in a day. During strenuous labour, the ATP is consumed at the rate of even 0.5 kg per minute. **The endergonic processes such as biosynthesis, active transport etc., can occur only if ATP is continuously regenerated from ADP.** Phototrophs harvest the free energy in light to regenerate ATP whereas chemotrophs form ATP by the oxidation of foodstuffs.

When ADP is hydrolyzed to AMP and inorganic phosphate, the $\Delta G°'$ value of this reaction is the same as that of the reaction, ATP → ADP + Pi, that is –7.3 kcal/mol. Thus, the two terminal phosphate groups of ATP (β and γ) are both high energy groups. On the contrary, the $\Delta G°'$ value of hydrolysis of AMP to yield adenosine and phosphate is much lower, that is only –3.4 kcal/mol. Thus, the phosphate group of AMP (i.e., the a phosphate group of ATP) is in the low energy class. The hydrolysis of ATP to yield AMP plus PPi proceeds with $\Delta G°'$ –7.7 kcal/mol, slightly greater than the $\Delta G°'$ for the hydrolysis of the terminal or γ phosphate bond. $ATP + H_2O \rightarrow AMP + PPi$ The inorganic pyrophosphate is subsequently hydrolyzed by the enzyme pyrophosphatase to yield 2 moles of inorganic orthophosphate. The $\Delta G°'$ value of this reaction is –6.9 kcal/mol.

$$\mathbf{PPi + H_2O \rightarrow 2Pi}$$

The anhydride linkages in ATP and other high energy phosphates are giving the energy but not any ester linkage of phosphates . But thioester linkages are of high energy .

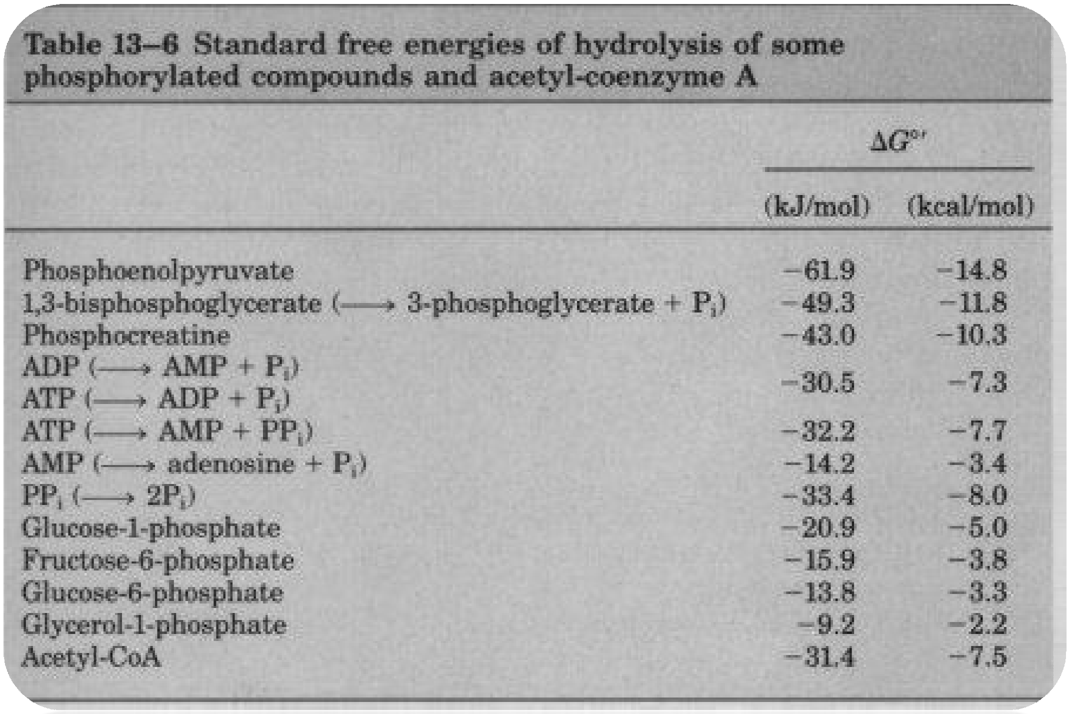
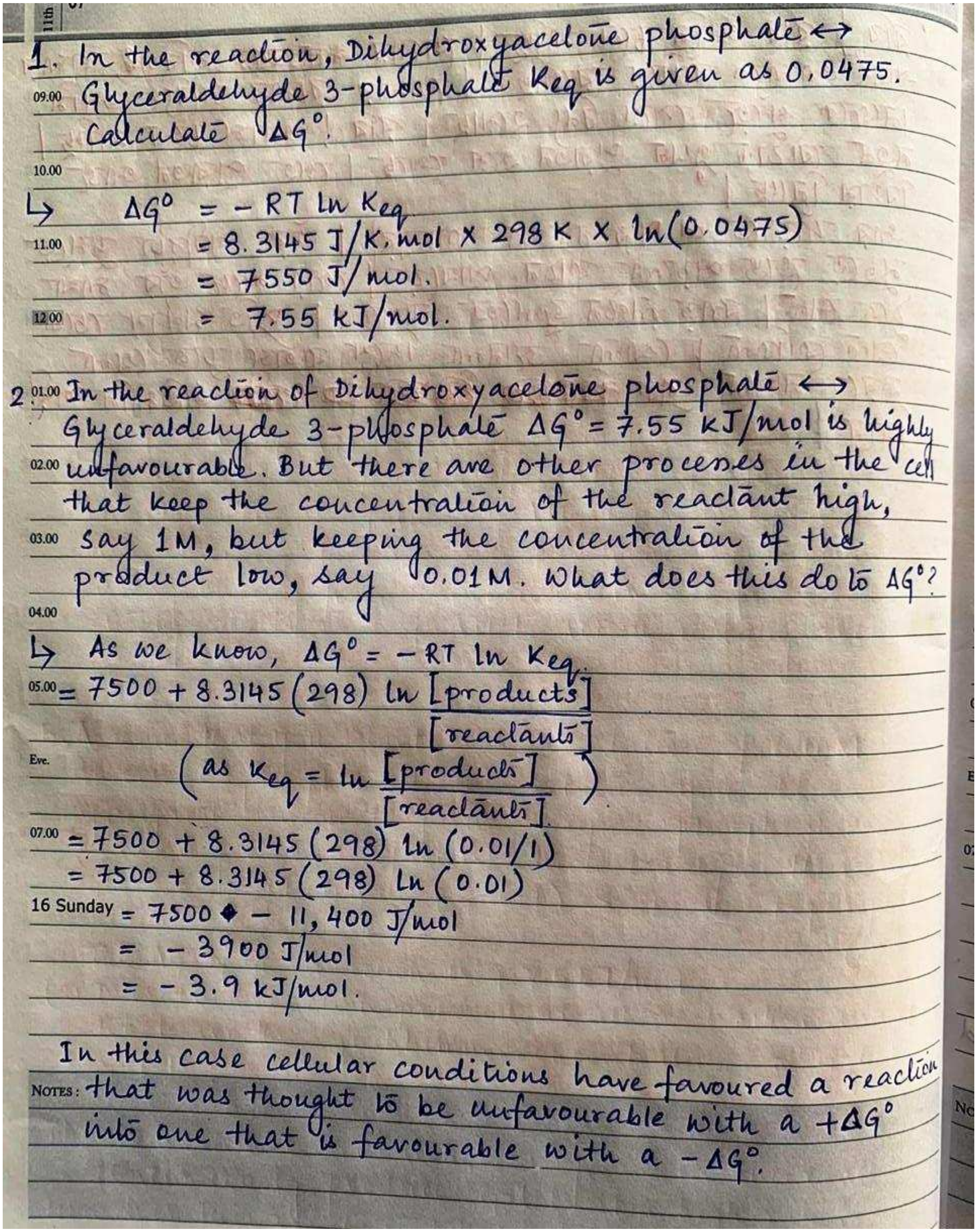


Table 13–6 Standard free energies of hydrolysis of some phosphorylated compounds and acetyl-coenzyme A

	$\Delta G^{\circ\prime}$ (kJ/mol)	$\Delta G^{\circ\prime}$ (kcal/mol)
Phosphoenolpyruvate	−61.9	−14.8
1,3-bisphosphoglycerate ($\longrightarrow$ 3-phosphoglycerate + P_i)	−49.3	−11.8
Phosphocreatine	−43.0	−10.3
ADP ($\longrightarrow$ AMP + P_i)	−30.5	−7.3
ATP ($\longrightarrow$ ADP + P_i)		
ATP ($\longrightarrow$ AMP + PP_i)	−32.2	−7.7
AMP ($\longrightarrow$ adenosine + P_i)	−14.2	−3.4
PP_i ($\longrightarrow$ $2P_i$)	−33.4	−8.0
Glucose-1-phosphate	−20.9	−5.0
Fructose-6-phosphate	−15.9	−3.8
Glucose-6-phosphate	−13.8	−3.3
Glycerol-1-phosphate	−9.2	−2.2
Acetyl-CoA	−31.4	−7.5

❖ Some Solved Questions



11th

1. In the reaction, Dihydroxyacetone phosphate ↔
09.00 Glyceraldehyde 3-phosphate Keq is given as 0,0475.
Calculate ΔG°.

10.00

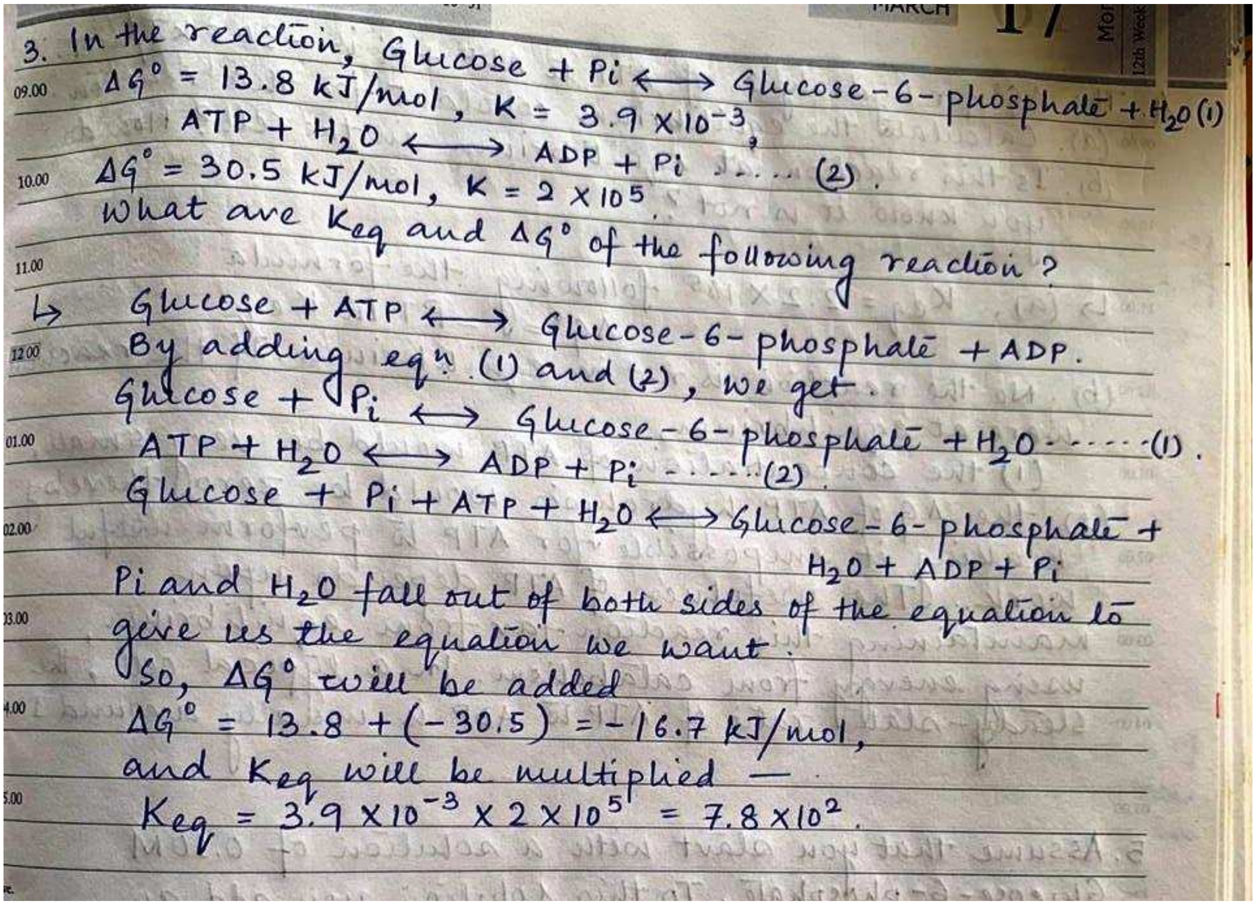
↳ $\Delta G^{\circ} = -RT \ln K_{eq}$
11.00 $= 8.3145\ \mathrm{J/K.mol} \times 298\ \mathrm{K} \times \ln(0.0475)$
$= 7550\ \mathrm{J/mol}$.
12.00 $= 7.55\ \mathrm{kJ/mol}$.

2. 01.00 In the reaction of Dihydroxyacetone phosphate ↔
Glyceraldehyde 3-phosphate $\Delta G^{\circ} = 7.55\ \mathrm{kJ/mol}$ is highly
02.00 unfavourable. But there are other processes in the cell
that keep the concentration of the reactant high,
03.00 say 1M, but keeping the concentration of the
product low, say 0.01M. What does this do to ΔG°?

04.00

↳ As we know, $\Delta G^{\circ} = -RT \ln K_{eq}$.
05.00 $= 7500 + 8.3145(298) \ln \frac{[\text{products}]}{[\text{reactants}]}$
Eve. $\left(\text{as } K_{eq} = \ln \frac{[\text{products}]}{[\text{reactants}]}\right)$
07.00 $= 7500 + 8.3145(298) \ln(0.01/1)$
$= 7500 + 8.3145(298)\ \mathrm{Ln}(0.01)$
16 Sunday $= 7500 - 11,400\ \mathrm{J/mol}$
$= -3900\ \mathrm{J/mol}$
$= -3.9\ \mathrm{kJ/mol}$.

In this case cellular conditions have favoured a reaction
NOTES: that was thought to be unfavourable with a +ΔG°
into one that is favourable with a −ΔG°.



3. In the reaction, Glucose + $P_i \longleftrightarrow$ Glucose-6-phosphate + H_2O (1)

$\Delta G^\circ = 13.8$ kJ/mol, $K = 3.9 \times 10^{-3}$,

ATP + $H_2O \longleftrightarrow$ ADP + P_i …… (2).

$\Delta G^\circ = 30.5$ kJ/mol, $K = 2 \times 10^{5}$.

What are K_{eq} and ΔG° of the following reaction?

↳ Glucose + ATP $\longleftrightarrow$ Glucose-6-phosphate + ADP.

By adding eqn (1) and (2), we get.

Glucose + $P_i \longleftrightarrow$ Glucose-6-phosphate + H_2O …… (1).

ATP + $H_2O \longleftrightarrow$ ADP + P_i …… (2)

Glucose + P_i + ATP + $H_2O \longleftrightarrow$ Glucose-6-phosphate + H_2O + ADP + P_i

P_i and H_2O fall out of both sides of the equation to give us the equation we want.

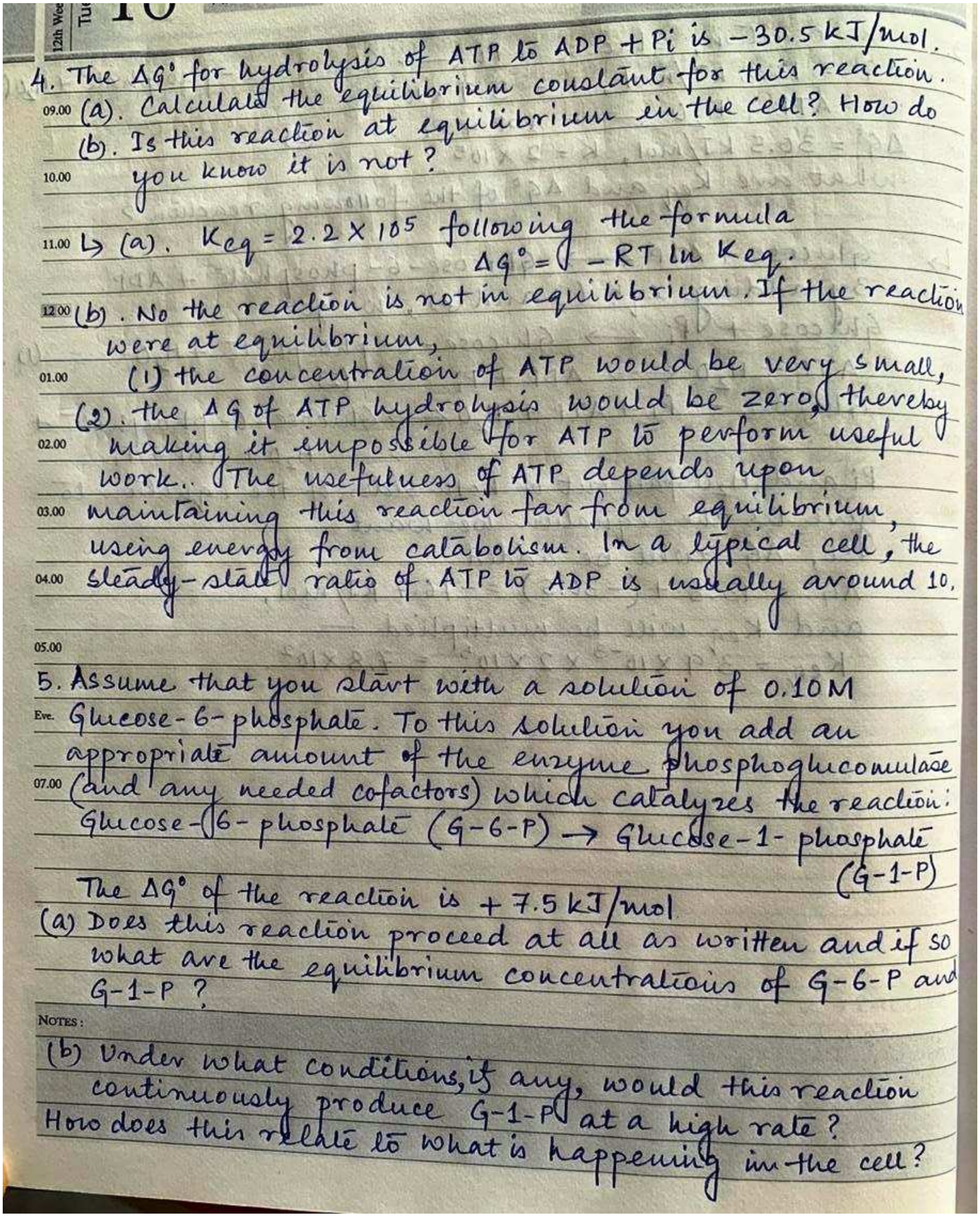
So, ΔG° will be added

$$\Delta G^\circ = 13.8 + (-30.5) = -16.7 \text{ kJ/mol},$$

and K_{eq} will be multiplied —

$$K_{eq} = 3.9 \times 10^{-3} \times 2 \times 10^{5} = 7.8 \times 10^{2}.$$

For any queries or confusion regarding understanding the solved answers, please **contact Dr. Priyadarshini Mallick**, Head of Department, Department of Microbiology, Asutosh College through WhatsApp Number: **9674006269**



4. The ΔG° for hydrolysis of ATP to ADP + Pi is -30.5 kJ/mol.
(a). Calculate the equilibrium constant for this reaction.
(b). Is this reaction at equilibrium in the cell? How do you know it is not?

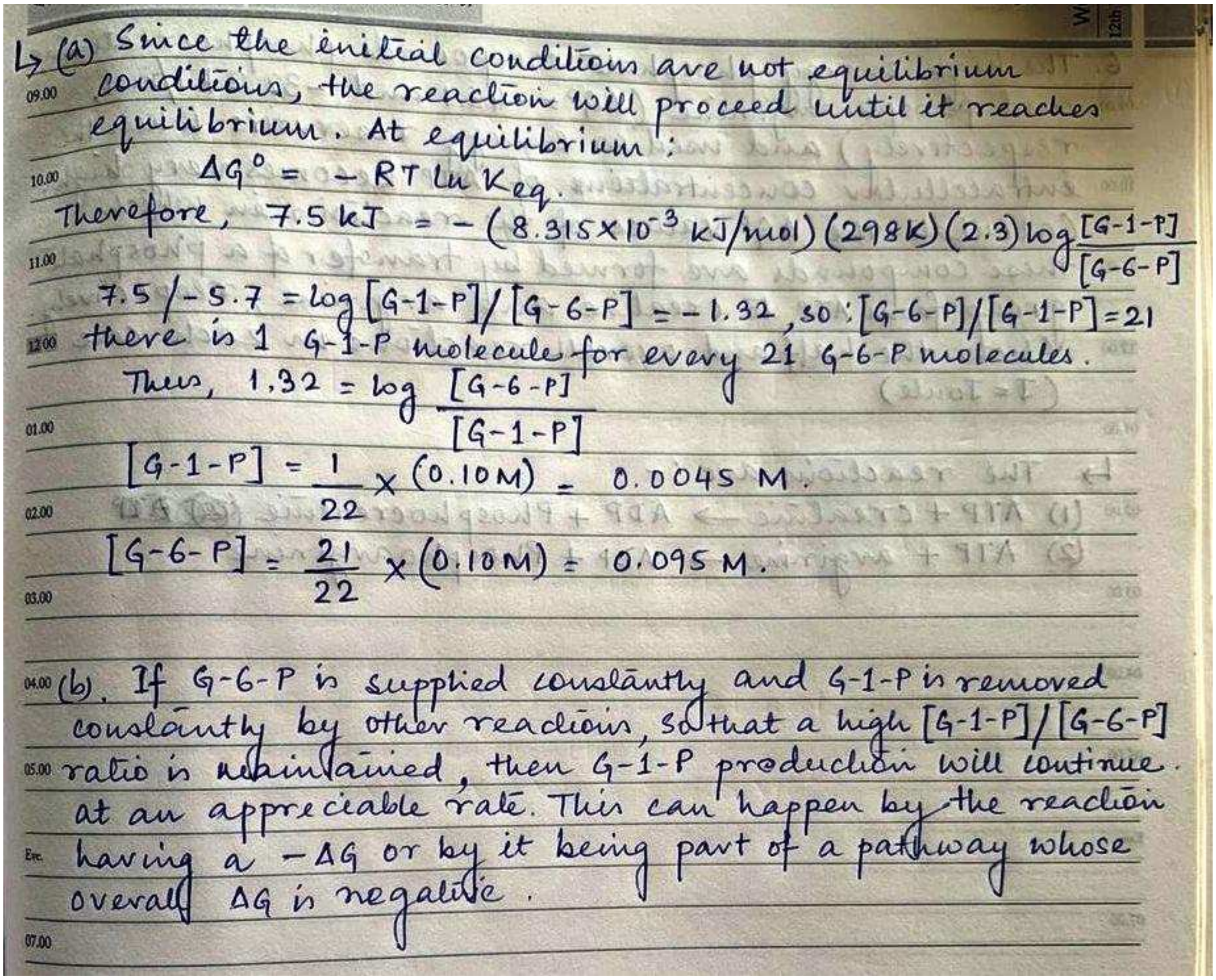
↳ (a). $K_{eq} = 2.2 \times 10^5$ following the formula
$\Delta G^\circ = -RT \ln K_{eq}$.

(b). No the reaction is not in equilibrium. If the reaction were at equilibrium,
(1) the concentration of ATP would be very small,
(2). the ΔG of ATP hydrolysis would be zero, thereby making it impossible for ATP to perform useful work. The usefulness of ATP depends upon maintaining this reaction far from equilibrium, using energy from catabolism. In a typical cell, the steady-state ratio of ATP to ADP is usually around 10.

5. Assume that you start with a solution of 0.10 M Glucose-6-phosphate. To this solution you add an appropriate amount of the enzyme phosphoglucomutase (and any needed cofactors) which catalyzes the reaction:
Glucose-6-phosphate (G-6-P) → Glucose-1-phosphate (G-1-P)

The ΔG° of the reaction is $+7.5$ kJ/mol
(a) Does this reaction proceed at all as written and if so what are the equilibrium concentrations of G-6-P and G-1-P?

(b) Under what conditions, if any, would this reaction continuously produce G-1-P at a high rate? How does this relate to what is happening in the cell?



↳ (a) Since the initial conditions are not equilibrium conditions, the reaction will proceed until it reaches equilibrium. At equilibrium:

$$\Delta G^{0} = -RT \ln K_{eq}.$$

Therefore, $7.5\ kJ = -(8.315\times10^{-3}\ kJ/mol)(298K)(2.3)\log\frac{[G\text{-}1\text{-}P]}{[G\text{-}6\text{-}P]}$

$7.5/-5.7 = \log[G\text{-}1\text{-}P]/[G\text{-}6\text{-}P] = -1.32$, so: $[G\text{-}6\text{-}P]/[G\text{-}1\text{-}P] = 21$

there is 1 G-1-P molecule for every 21 G-6-P molecules.

Thus, $1{,}32 = \log\frac{[G\text{-}6\text{-}P]}{[G\text{-}1\text{-}P]}$

$$[G\text{-}1\text{-}P] = \frac{1}{22}\times(0.10M) = 0.0045\ M.$$

$$[G\text{-}6\text{-}P] = \frac{21}{22}\times(0.10M) = 0.095\ M.$$

(b). If G-6-P is supplied constantly and G-1-P is removed constantly by other reactions, so that a high [G-1-P]/[G-6-P] ratio is maintained, then G-1-P production will continue at an appreciable rate. This can happen by the reaction having a $-\Delta G$ or by it being part of a pathway whose overall ΔG is negative.

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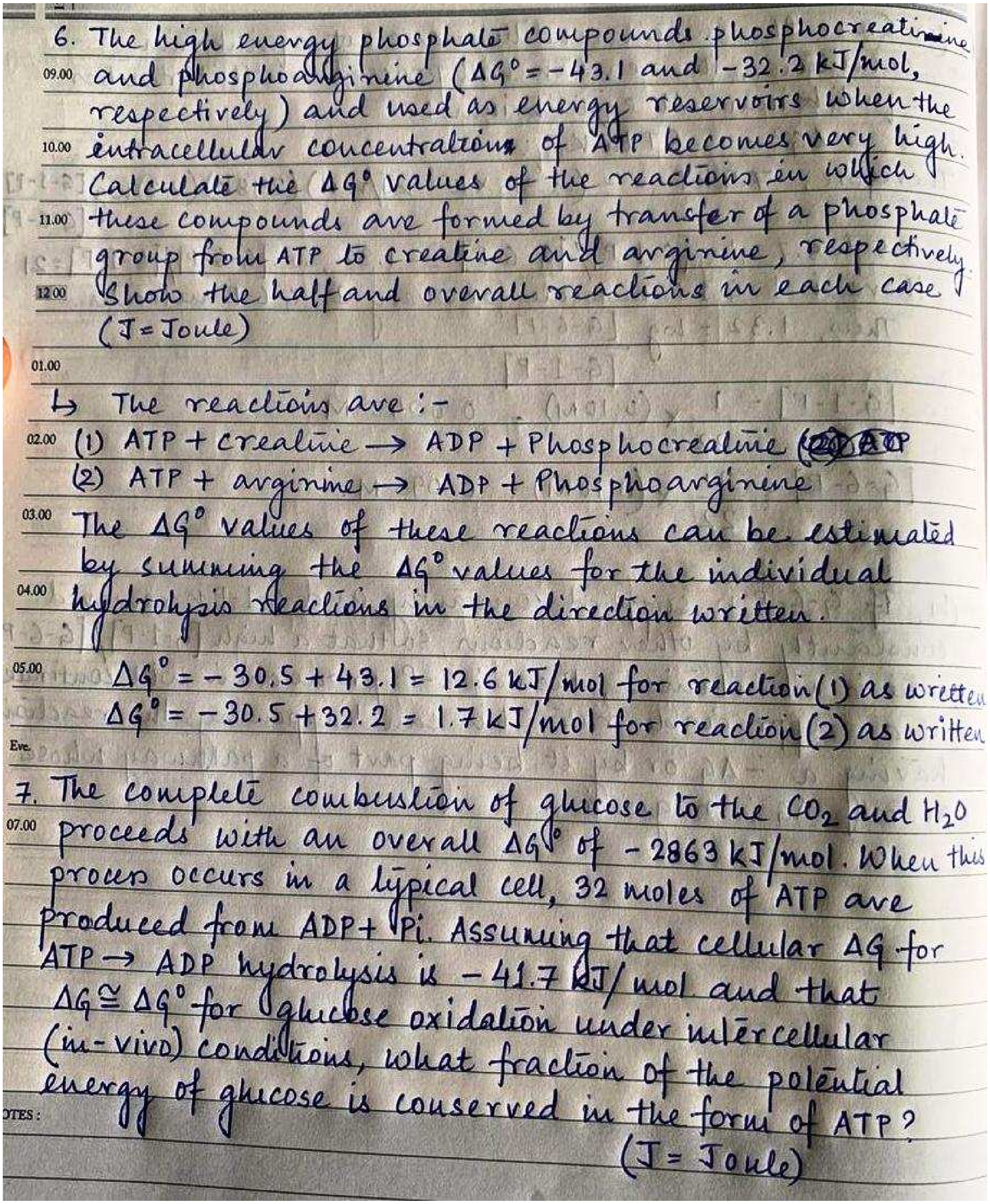


6. The high energy phosphate compounds phosphocreatine and phosphoarginine ($\Delta G^\circ = -43.1$ and -32.2 kJ/mol, respectively) and used as energy reservoirs when the intracellular concentrations of ATP becomes very high. Calculate the ΔG° values of the reactions in which these compounds are formed by transfer of a phosphate group from ATP to creatine and arginine, respectively. Show the half and overall reactions in each case. (J = Joule)

↳ The reactions are :-

(1) ATP + creatine $\rightarrow$ ADP + Phosphocreatine

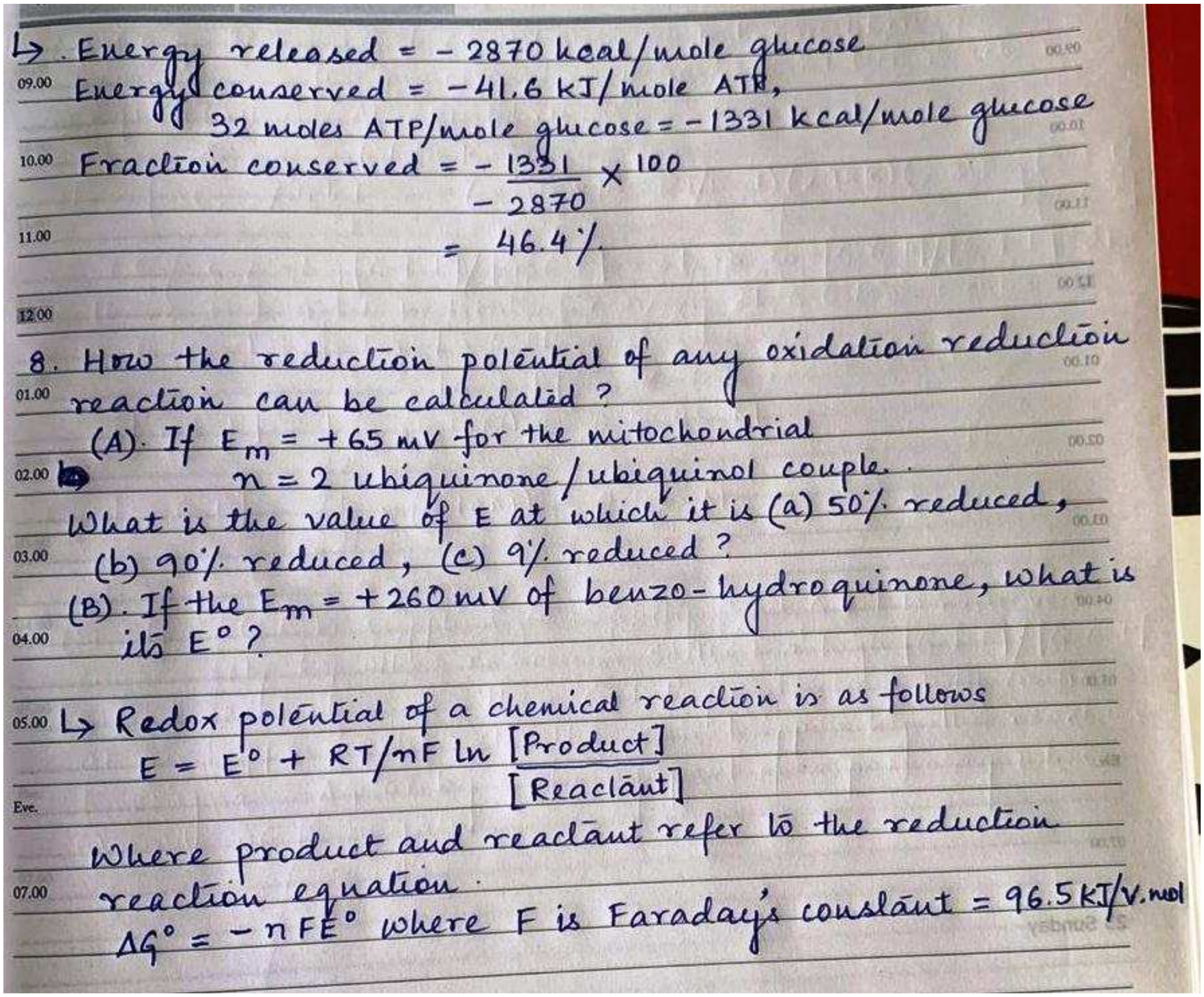
(2) ATP + arginine $\rightarrow$ ADP + Phosphoarginine

The ΔG° values of these reactions can be estimated by summing the ΔG° values for the individual hydrolysis reactions in the direction written.

$\Delta G^\circ = -30.5 + 43.1 = 12.6$ kJ/mol for reaction (1) as written

$\Delta G^\circ = -30.5 + 32.2 = 1.7$ kJ/mol for reaction (2) as written

7. The complete combustion of glucose to the CO_2 and H_2O proceeds with an overall ΔG° of -2863 kJ/mol. When this process occurs in a typical cell, 32 moles of ATP are produced from ADP + Pi. Assuming that cellular ΔG for ATP $\rightarrow$ ADP hydrolysis is -41.7 kJ/mol and that $\Delta G \cong \Delta G^\circ$ for glucose oxidation under intercellular (in-vivo) conditions, what fraction of the potential energy of glucose is conserved in the form of ATP? (J = Joule)



↳ Energy released = −2870 kcal/mole glucose

Energy conserved = −41.6 kJ/mole ATP,

32 moles ATP/mole glucose = −1331 kcal/mole glucose

$$\text{Fraction conserved} = \frac{-1331}{-2870} \times 100$$

$$= 46.4\%$$

8. How the reduction potential of any oxidation reduction reaction can be calculated?

(A). If E_m = +65 mV for the mitochondrial $n = 2$ ubiquinone/ubiquinol couple.

What is the value of E at which it is (a) 50% reduced, (b) 90% reduced, (c) 9% reduced?

(B). If the E_m = +260 mV of benzo-hydroquinone, what is its E°?

↳ Redox potential of a chemical reaction is as follows

$$E = E^\circ + RT/nF \ln \frac{[\text{Product}]}{[\text{Reactant}]}$$

Where product and reactant refer to the reduction reaction equation.

$\Delta G^\circ = -nFE^\circ$ where F is Faraday's constant = 96.5 kJ/V.mol

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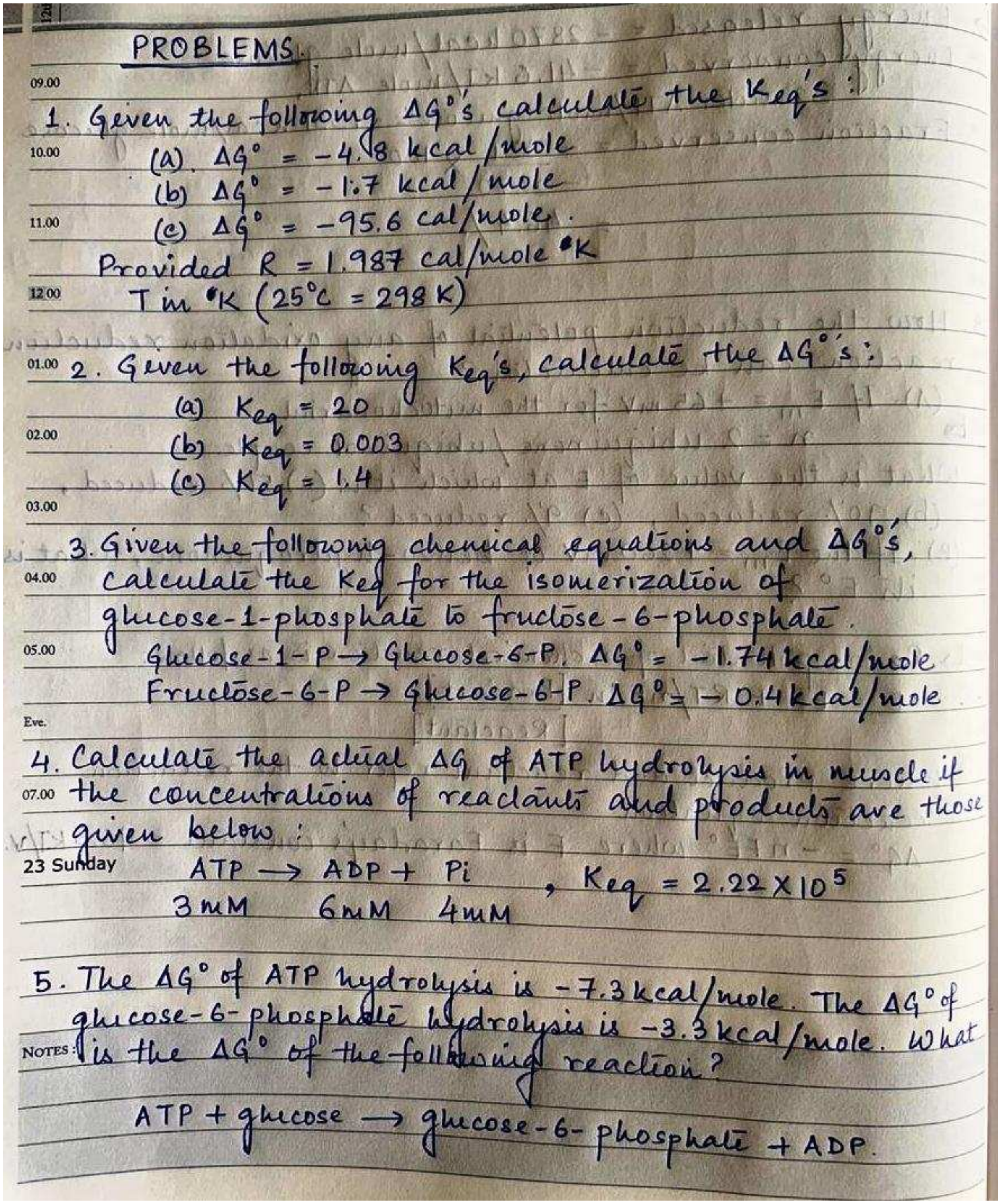
❖ Solve yourselves



PROBLEMS

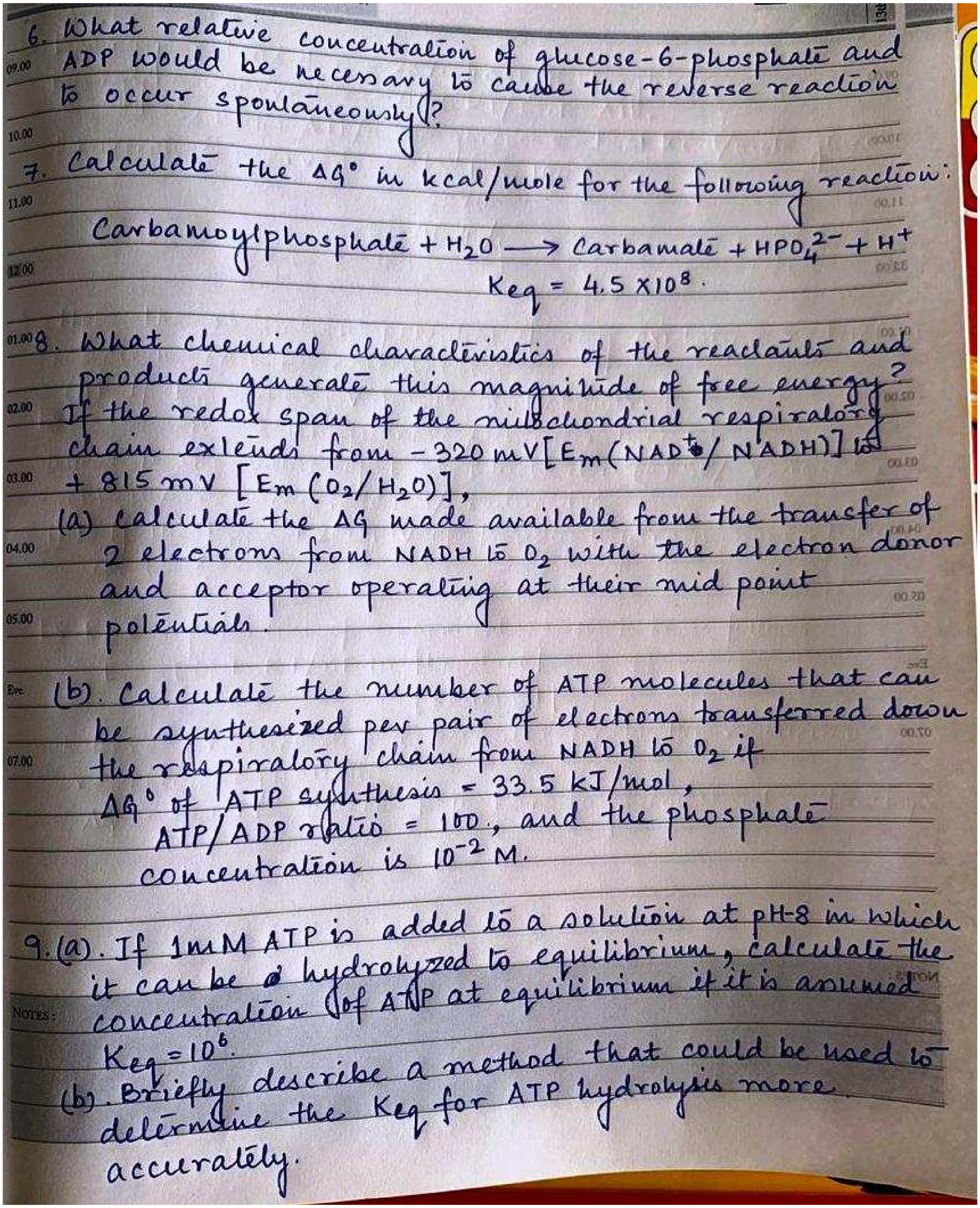
1. Given the following ΔG°'s calculate the K_{eq}'s:
(a) $\Delta G^\circ = -4.8$ kcal/mole
(b) $\Delta G^\circ = -1.7$ kcal/mole
(c) $\Delta G^\circ = -95.6$ cal/mole.
Provided $R = 1.987$ cal/mole °K
T in °K (25°C = 298 K)

2. Given the following K_{eq}'s, calculate the ΔG°'s:
(a) $K_{eq} = 20$
(b) $K_{eq} = 0.003$
(c) $K_{eq} = 1.4$

3. Given the following chemical equations and ΔG°'s, calculate the K_{eq} for the isomerization of glucose-1-phosphate to fructose-6-phosphate.
Glucose-1-P → Glucose-6-P, $\Delta G^\circ = -1.74$ kcal/mole
Fructose-6-P → Glucose-6-P, $\Delta G^\circ = -0.4$ kcal/mole

4. Calculate the actual ΔG of ATP hydrolysis in muscle if the concentrations of reactants and products are those given below:
ATP → ADP + Pi , $K_{eq} = 2.22 \times 10^5$
3 mM 6 mM 4 mM

5. The ΔG° of ATP hydrolysis is -7.3 kcal/mole. The ΔG° of glucose-6-phosphate hydrolysis is -3.3 kcal/mole. What is the ΔG° of the following reaction?
ATP + glucose → glucose-6-phosphate + ADP.



6. What relative concentration of glucose-6-phosphate and ADP would be necessary to cause the reverse reaction to occur spontaneously?

7. Calculate the ΔG° in kcal/mole for the following reaction:

Carbamoylphosphate + $H_2O \longrightarrow$ Carbamate + $HPO_4^{2-} + H^+$

$K_{eq} = 4.5 \times 10^8$.

8. What chemical characteristics of the reactants and products generate this magnitude of free energy?

If the redox span of the mitochondrial respiratory chain extends from -320 mV [$E_m(NAD^+/NADH)$] to $+815$ mV [$E_m(O_2/H_2O)$],

(a) calculate the ΔG made available from the transfer of 2 electrons from NADH to O_2 with the electron donor and acceptor operating at their mid point potentials.

(b). Calculate the number of ATP molecules that can be synthesized per pair of electrons transferred down the respiratory chain from NADH to O_2 if
ΔG° of ATP synthesis = 33.5 kJ/mol,
ATP/ADP ratio = 100, and the phosphate concentration is 10^{-2} M.

9.(a). If 1mM ATP is added to a solution at pH-8 in which it can be hydrolyzed to equilibrium, calculate the concentration of ATP at equilibrium if it is assumed $K_{eq} = 10^6$.

(b). Briefly describe a method that could be used to determine the K_{eq} for ATP hydrolysis more accurately.