Fructose-6-P + Pi → acetyl-P + erythrose-4-P
Erythrose-4-P + fructose-6-P → sedoheptulose-7-P + glyceraldehyde-3-P → ribose-5-P + xylulose-5-P
Ribose-5-P → xylulose-5-P
2 Xylulose-5-P + 2 Pi → 2 acetyl-P + glyceraldehyde-3-P
2 Glyceraldehyde-3-P → fructose-1:6-di-P → fructose-6-P + Pi

Sum: Fructose-6-P + 2 Pi → 3 acetyl-P
3 Acetyl-P + 3 adenosine diphosphate → 3 adenosine triphosphate + 3 acetate

Thus, with the aid of acetokinase, three molecules of adenosine triphosphate would be formed, representing a net formation of two molecules of adenosine triphosphate from two of adenosine diphosphate per mole of glucose, an efficiency comparable to that of glycolysis. The acetyl phosphate may alternatively be metabolized via the citric acid cycle[1].

This work was supported by a grant (*RG* 4290) from the National Institutes of Health, U.S. Public Health Service, Bethesda, Md.

M. Schramm
E. Racker

Division of Nutrition and Physiology,
The Public Health Research Institute
of the City of New York, Inc.,
New York 9, N.Y.
April 11.

[1] Schramm, M., Gromet, Z., and Hestrin, S., *Biochem. J.* (in the press).
[2] Schramm, M., and Hestrin, S., *J. Gen. Microbiol.*, **11**, 123 (1954).
[3] Nossal, P. M., *Aust. J. Exp. Biol. Med.*, **31**, 583 (1953).
[4] Heath, E. C., Hurwitz, J., and Horecker, B. L., *J. Amer. Chem. Soc.*, **78**, 5449 (1956). Horecker, B. L., Heath, E. C., Hurwitz, J., and Ginsburg, A., *Fed. Proc.*, **16**, 198 (1957).
[5] Schramm, M., Gromet, Z., and Hestrin, S., *Nature*, **179**, 28 (1957).
[6] Berger, L., Slein, M. W., Colowick, S. P., and Cori, C. F., *J. Gen. Physiol.*, **29**, 379 (1946).
[7] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, **66**, 375 (1925).
[8] Lowry, O. H., and Lopez, J. A., *J. Biol. Chem.*, **162**, 421 (1946).
[9] Lipmann, F., and Tuttle, C. L., *J. Biol. Chem.*, **159**, 21 (1945).
[10] Rose, A. I., Grunberg-Manago, M., Korey, S. R., and Ochoa, S., *J. Biol. Chem.*, **211**, 73 (1954).
[11] Srere, P. A., Kornberg, H. L., and Racker, E., *Fed. Proc.*, **14**, 285 (1955).
[12] Stadtman, E. R., and Barker, H. A., *J. Biol. Chem.*, **184**, 769 (1950).

The Free Energy of Hydrolysis of Adenosine Triphosphoric Acid

During the past decade, the value of the energy of adenosine triphosphate breakdown has several times been subject to revision. The more precise, though indirect, calculations[1] resulted in lower values (7–9 kcal./mol.) than the earlier results (11–13 kcal./mol.).

We have tried to determine the free energy of hydrolysis of adenosine triphosphate by establishing the equilibrium constant of the hexokinase reaction, using as a tracer the radioactive isotope of phosphorus:

Glucose-6-phosphate* + ADP ⇌ glucose + ATP*

The reaction mixture consisted of adenosine diphosphate, glucose-6-phosphate, labelled with radioactive phosphorus (specific activity 150–460 counts/min./μgm.), magnesium ions, yeast hexokinase and glycine buffer (*p*H 7·25).

Hexokinase prepared according to the Meyerhof procedure contained no admixture of phosphoglucomutase or myokinase. The adenosine diphosphate was free of any apparent traces of the triphosphate. The mixture was incubated in a water-bath at 37° C. for 3–6 hr. After inactivation of the enzyme by trichloracetic acid, the triphosphate synthesized was determined by its radioactivity. A strictly measured amount of non-radioactive adenosine triphosphate was added to the mixture as a carrier. The usual procedures were employed for the isolation of adenosine triphosphate and for purifying it of any trace of radioactive glucose-6-phosphate. As a criterion of the purity of the adenosine triphosphate, control experiments were performed in exactly the same conditions, but with hexokinase previously inactivated by boiling.

The amount of radioactive adenosine triphosphate was calculated from the quantity of triphosphate added as carrier and the specific activity of the portion of it isolated. The concentration of the glucose in equilibrium was considered to be equal to the concentration of adenosine triphosphate. The equilibrium constant of the hexokinase reaction (K) and the change of the free energy were calculated by the equation:

$$K = \frac{[\text{glucose-6-phosphate}]\,[\text{ADP}]}{[\text{glucose}]\,[\text{ATP}]};\quad \Delta F^0 = -RT\ln K$$

The hydrolytic breakdown of adenosine triphosphate can be represented as the sum of the hexokinase reaction and the hydrolysis of glucose-6-phosphate:

$$\text{glucose} + \text{ATP} \rightleftharpoons \text{glucose-6-phosphate} + \text{ADP};\ \Delta F^0{}_1 \quad (1)$$

$$\frac{\text{glucose-6-phosphate} + H_2O \rightleftharpoons \text{glucose} + \text{phosphate}}{(1+2)\,\text{ATP} + H_2O \rightleftharpoons \text{ADP} + \text{phosphate};\ \Delta F^0{}_{1+2}};\quad \Delta F^0{}_2 \quad (2)$$

Then the energy of hydrolysis of adenosine triphosphate can be determined as the sum of $\Delta F^0{}_1$ and $\Delta F^0{}_2$:

$\Delta F^0{}_{1+2} = \Delta F^0{}_1 + \Delta F^0{}_2$, where $\Delta F^0{}_2$ is $-2{\cdot}45$ kcal. per mol. (ref. 2).

The results of experiments are given in Table 1. The mean value of the free-energy change of the hexokinase reaction, according to the results in Table 1, is $-3{\cdot}2$ kcal. per mole, and the mean free energy of hydrolysis of adenosine triphosphate is $-5{\cdot}6$ kcal. per mole. There are reasons for considering the lower values of the equilibrium constant of hexokinase reaction as the most reliable.

Table 1. Equilibrium Constant Data of Hexokinase Reaction and ΔF^0 Values

Exp. No.	ADP (m.moles)	Glucose-6-phosphate (m.moles)	Specific activity of glucose-6-phosphate (counts/min./μgm.)	Duration of incubation (hr.)	Amount of ATP carrier (mgm.)	Amount of synthesized ATP (m.moles)	Equilibrium constant of hexokinase reaction (K)	ΔF^0 of hexokinase reaction (kcal./mole)	ΔF^0 of ATP hydrolysis (kcal./mole)
1	0·126	0·185	238	5·5	16·45	0·0040	1·380	−4·4	−6·85
2	0·124	0·193	147	3·0	16·63	0·0080	0·335	−3·5	−5·95
3	0·135	0·132	305	5·5	12·71	0·0087	0·204	−3·2	−5·65
4	0·124	0·193	147	5·0	16·63	0·0107	0·180	−3·2	−5·65
5	0·138	0·145	248	3·0	19·50	0·0120	0·116	−2·9	−5·35
6	0·118	0·193	461	2·5	11·80	0·0130	0·112	−2·9	−5·35
7	0·109	0·142	268	6·0	14·31	0·0135	0·073	−2·6	−5·05

The order of values obtained is in accordance with the new results[3] for the heat of hydrolysis of adenosine triphosphate, which is given as − 4·7 kcal. per mole, instead of the previously accepted value of − 12 kcal. per mole. It is nearly equal to the heat of hydrolysis of pyrophosphate and trimetaphosphate (− 5·8 and − 6·2 kcal. per mole of phosphate anhydride bond).

Under physiological conditions, the phosphate bond-energy of adenosine triphosphate will change, depending on concentrations of adenosine tri- and di-phosphate and inorganic phosphate, and will be considerably higher. Thus, if we accept the concentration of phosphoric acid as 0·01 *M* (which is usually the case in tissues) and that of adenosine triphosphate as the same as that of the diphosphate, it would be − 8·4 kcal.

Hence, in spite of the lower free-energy of adenosine triphosphate hydrolysis under standard conditions, this compound does not lose its biological significance as a universal accumulator of chemical energy.

G. E. Vladimirov
V. G. Vlassova
A. Y. Kolotilova
S. N. Lyzlova
N. S. Panteleyeva

Leningrad State University.
March 4.

[1] Vladimirov, G. E., and Guinodman, L. M., *Biokhimya*, **18**, 490 (1953). Vladimirov, G. E., *Utchenye zapiski* (of Leningrade State University), **32**, 328 (1954). Oesper, P., in McElroy and Glass, "Phosphorus Metabolism", **1**, 521 (1951). Burton, K., and Krebs, H. A., *Biochem. J.*, **54**, 94 (1953). Levintow, L., and Meister, A., *J. Biol. Chem.*, **209**, 265 (1954). Burton, K., *Biochem. J.*, **59**, 44 (1955). Morales, M. F., Botts, J., and Hill, T. L., *Physiol. Rev.*, **35**, 475 (1955).
[2] Guinodman, L. M., *Biokhimya*, **19**, 666 (1954).
[3] Podolsky, R. Y., and Morales, M. F., *J. Biol. Chem.*, **218**, 945 (1956).

Blood Anticoagulant Activity of Sulphated Ovular Mucins of Amphibians

The mucin constituting the gelatinous layer which envelops the eggs of *Bufo bufo*, if digested with papain and then sulphated, shows a marked anticoagulant activity on the fibrinogen and the whole blood plasma[1]. A similar anticoagulant activity has now been shown by the ovular mucins of other species of amphibians, sulphated without any previous proteolysis.

The ovular mucins of *Discoglossus pictus*, axolotl and *Bufo bufo*, removed from the eggs either by hand or by treatment with sodium thioglycolate[2], were lyophilized, desiccated *in vacuo* over phosphorus pentoxide, and sulphated with chlorosulphonic acid (0·7 ml. per gm. of dry substance) for 12 hr. at − 16° C. The sulphated derivatives were neutralized, dialysed and filtered; no further purification was attempted.

The anticoagulant activity has been determined by the prolongation of the coagulation times of fibrinogen added to thrombin, and of oxalated bovine plasma after recalcification. The determinations were carried out as follows.

(1) *Fibrinogen*. 0·1 ml. of sulphated mucin solution was mixed with 0·5 ml. of a 0·5 per cent solution of fibrinogen (Bovine Plasma Fraction I, Armour Co.) in saline, and 0·1 ml. of a 0·1 per cent solution of Trombasi Roussel (a powder extracted from the blood plasma, and containing thrombin) was added. The coagulation times at 37° C. were determined, as well as those of the controls (0·1 ml. of distilled water instead of the mucin) and those given in the presence of a 0·0001 per cent solution of standard heparin (100 units per mgm.).

Table 1

	Concentration (μgm./ml.)	Coagulation times	
		Fibrinogen	Plasma
Controls		4 min. 34 sec.	7 min. 10 sec.
Heparin	1	6 min. 18 sec.	10 min. 35 sec.
Discoglossus	2·5	6 min. 20 sec.	—
	60	—	10 min. 28 sec.
Axolotl	9	6 min. 10 sec.	—
	100	—	10 min. 30 sec.
Bufo bufo	2·5	6 min. 20 sec.	—
	25	—	10 min. 22 sec.

(2) *Whole plasma*. 0·5 ml. of oxalated bovine plasma (9 parts of blood and 1 part of 0·1 *M* neutral potassium oxalate) was recalcified with 0·1 ml. of 2 per cent calcium chloride, and mixed with 0·1 ml. of sulphated mucin solution, or distilled water, or 0·0001 per cent standard heparin solution. The coagulation times were determined at room temperature (about 18° C.).

The results are summarized in Table 1, which only gives the results for concentrations of mucins showing approximately the same anticoagulant activity as 0·0001 per cent heparin, that is, prolonging the coagulation time of fibrinogen by about 40 per cent, and that of plasma by about 50 per cent. The results in Table 1 are the averages of sixteen determinations for fibrinogen, and of nine for the plasma.

From these results, the anticoagulant activities of the sulphated mucins could be expressed in heparin units as in Table 2.

Table 2. Anticoagulant Activity in Heparin Units

Mucin	Fibrinogen	Plasma
Discoglossus	40	1·5
Axolotl	10	1
Bufo bufo	40	4

The anticoagulant activity induced by sulphation is probably due to the sulphamic groups arising in the esterification with sulphuric acid of the hexosamines present in the mucin substance[1]. The much lower activity for the whole plasma than that for fibrinogen, in respect to the heparin, may depend on the inability of the sulphated mucins to combine with the plasma fractions which activate heparin.

A. Minganti
G. Bellia

Zoological Institute,
University of Palermo.

[1] Minganti, A., and Azzolina, G., *Ric. Scient.*, **26**, 3674 (1956).
[2] Minganti, A., *Exp. Cell Research*, Supp. 3, 248 (1955).

Antigenicity of Antibodies of the Same Species

In previous papers[1] rare human sera were described which possess the ability to agglutinate human red cells sensitized with incomplete isoantibodies. The possible application of such sera in blood group laboratories was stressed. Based on the absorption experiments, the opinion was expressed that the agglutinating factor contained in these sera is an 'anti-antibody', which reacts with human immune globulins denatured in serological reaction.