ADENOSINETRIPHOSPHATASE ACTIVITY IN THE LIVER DURING ASKEROL DEFICIENCY

BY

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INTRODUCTION

As early as 1907, Suzuki, Yoshimura, and Takaisha (27, 31) reported that rice bran contained enzymes which would split phosphoric acid from phytin. In 1908 McCollum and Hart (23) cited evidence that animal tissues contained an enzyme similar to the rice bran enzyme. Blood and liver seemed to contain these enzymes, but muscle and kidney did not. Grosser and Husler (15) found an enzyme in bone, kidney, spleen, and pancreas which would hydrolyze glycerophosphate. The glycerol phosphate obtained by synthesis and that obtained from hydrolysis of lecithin were both attacked equally well. Robison (29) noted that in the presence of soluble calcium salts the enzyme acts upon hexose monophosphate with the formation of a tricalcium phosphate precipitate.

In 1923 Robison (30) reported that an enzyme was present in the ossifying cartilage of young rats and rabbits which was capable of hydrolyzing hexose monophosphate, liberating free phosphoric acid. He also demonstrated (28) that calcium phosphate could be deposited in bones taken from rachitic rats when immersed in solutions of calcium hexose monophosphate or calcium glycerophosphate at 37° C. and a pH of 8.4 to 9.4. This suggested to him the query of whether, in physiological intact tissue, bone might not be laid down in some such manner.

Since this time, it has been satisfactorily proven that the phosphatases are important in the metabolism of carbohydrates, nucleotides, and phospholipids, as well as in bone formation.

Harden and Young were the first to discover that phosphorylation of hexoses occured in alcoholic fermentation. Considerable time elapsed before this was considered something more than just a means of modeling the hexose molecule to fit it for fermentative breakdown. It later became evident that the primary phosphate ester bond of hexose changes into a new type of energy-rich phosphate bond. During various metabolic processes, phosphate is introduced into compounds not merely to facilitate their breakdown, but also as a prospective carrier of energy (18).

The discovery of creatine phosphate led to a better understanding of energy metabolism. The compound was isolated by Fiske and Subbarow (13, 14) from a protein-free muscle filtrate. Eggleton and Eggleton (7, 8) and Fiske and Subbarow (14) observed that cr-ph*, when decomposed during a long series of muscle contractions, was reconstucted quite rapidly during recovery in oxygen. Likewise, anaerobically, cr-ph was resynthesized very effectively at

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*The following abbreviations will be used:
cr-ph -- creatine phosphate ADP -- adenosinediphosphate
ATP -- adenosinetriphosphate AA -- adenylic acid
ATP-ase -- adenosinetriphosphatase

the expense of glycogen. Interest in the compound became stronger after Meyerhof and Suranyi (25) found that large amounts of heat were released by the enzymatic decomposition of cr-ph. Meyerhof, Lundsgaard, and Blaschko (26) measured the amount of energy released by the breakdown of cr-ph and glycogen. They found that irrespective of its origin from either cr-ph or glycogen, equivalent amounts of energy obtained did the same amount of mechanical work.

Later Lundsgaard (20) established the mechanism of muscular contraction, when he showed that anaerobic contraction proceeded qualitatively after complete blocking of glycolysis by iodoacetic acid. A muscle poisoned with iodoacetic acid contracts as long as there is cr-ph present, but when this compound breaks down completely, contraction ceases. Here, unlike in normal muscle, there is no resynthesis of the phosphorus compound. To explain these facts, it was assumed that normally the energy for contraction is derived from the breakdown of phosphocreatine. At this stage, glycogen is converted to lactic acid. During recovery of the muscle (relaxation), when most of the lactic acid is resynthesized to glycogen, some of the energy produced (resulting from the oxidation of lactic acid or its equivalent) is utilized for the resynthesis of phosphocreatine.

During anaerobic resynthesis of creatine phosphate, Lundsgaard (20) found a remarkable efficiency of glycolysis. By breakdown of one half mole of glucose to lactic acid, approximately two moles of creatine phosphate were reformed, and the active heat energy of glycolysis is utilized for the conversion into phosphate bond energy.

The availability of the energy-rich phosphate bond suggested that the energy utilized in the muscle under all circumstances was derived from energy-rich phosphate bonds, supplied constantly by glycolytic or oxidative foodstuff disintegration (18).

In anaerobic metabolism glucose, hexosemonophosphate, adenylic acid, and adenosinediphosphate act as phosphate acceptors during alcoholic fermentation, while creatine plays that role during muscle glycolysis (17).

Inorganic phosphorus is liberated in the working muscle at a higher rate than glycogen is esterified. The inorganic phosphorus originated from ATP and not from cr-ph (21). ATP is a universal constituent of all cells, and contains three hydrolyzable phosphate groups. The first phosphate is joined in an energy-poor linkage to ribose, while the other two are energy-rich linkages.

The structural formula of adenosinetriphosphate will help to illustrate energy-poor and energy-rich linkages (figure I).

ADENOSINETRIPHOSPHATE

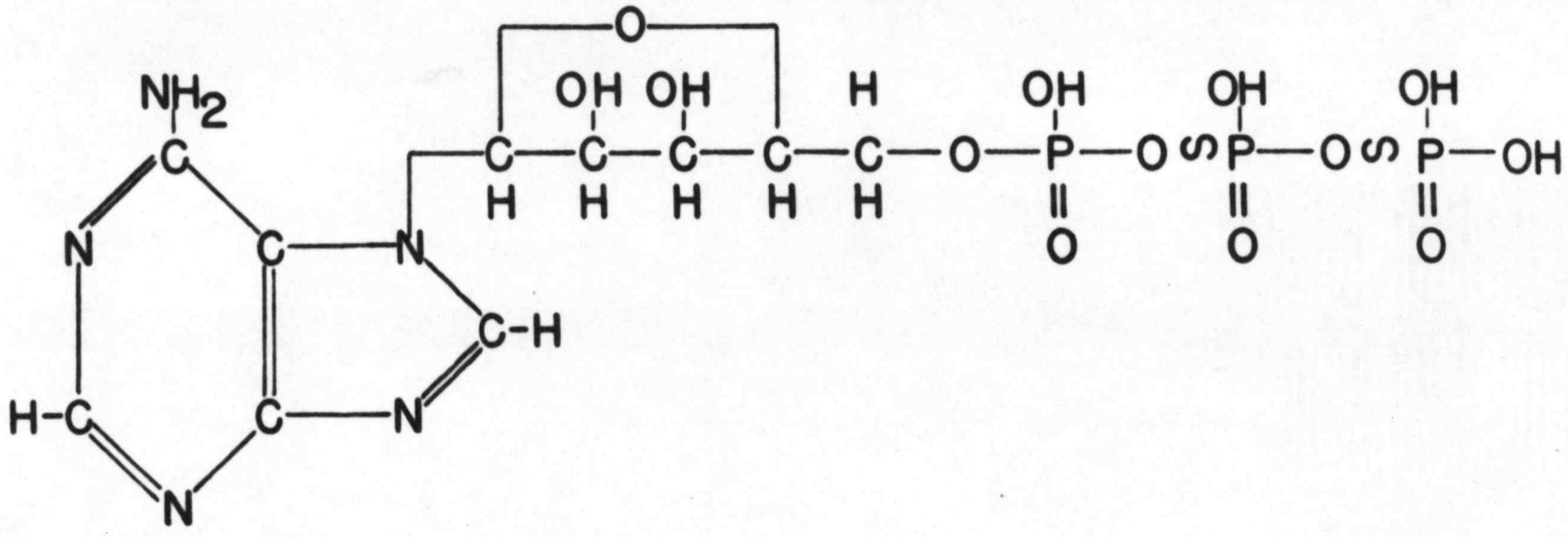


Figure I

The linkages indicated by ~ ph are energy-rich, and the first phosphate (CH_2-O-P) is energy-poor.

Energy-poor phosphate ester bonds are phosphates linked to an alcoholic hydroxyl group. The energy of the bond is between 2,000-4,000 calories per mole. Other examples of this type of linkage are phospho-glycerol, 3-phospho-glyceric acid, and 2-phospho-glyceric acid.

Energy-rich phosphate linkages are usually connected to an unsaturated radical. Examples of this type are found in the following compounds (figure II): 1,3-diphosphoglyceric acid, phospho-enol-pyruvic acid, and phosphocreatine. The energy of the bond of these compounds is between 9,000 and 11,000 calories per mole.

ATP is in enzymatic equilibrium with creatine according to the equation

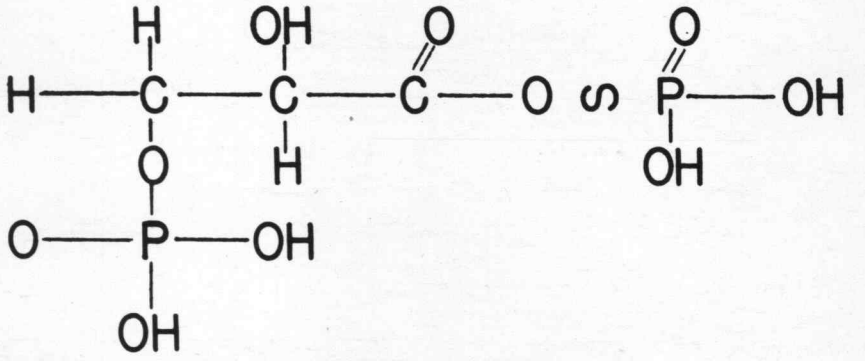
$$ATP + cr \rightleftharpoons ADP + cr\text{-}ph.$$

In the resting or recovering muscle ADP accepts ph from cr-ph, phosphopyruvate, or glycerophosphate to form ATP.

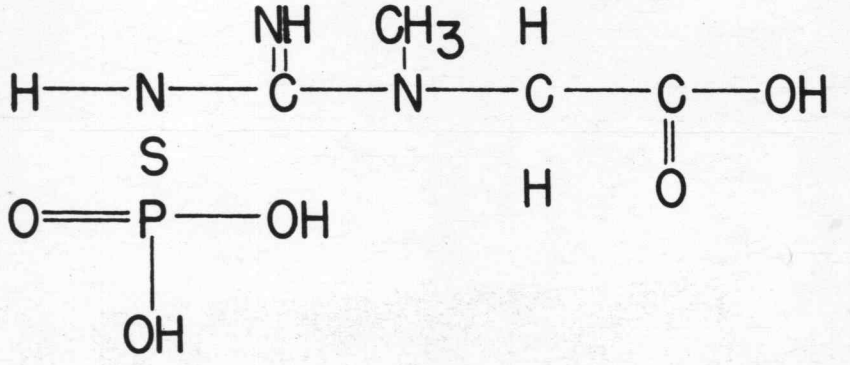
In dephosphorylation of ATP energy is liberated according to the following reaction:

$$ATP \rightleftharpoons ADP + H_3PO_4 + 11{,}000 \text{ calories.}$$

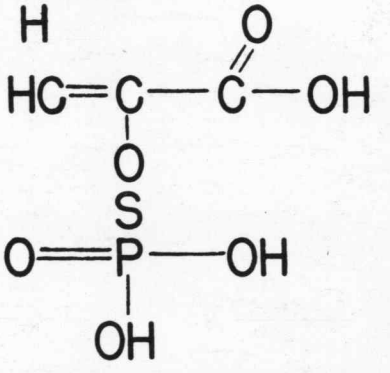
There is reason to believe that ATP is not dephosphorylated spontaneously, but rather by an enzymatic reaction, myosin acting as the phosphate transfer system. The major protein component of muscular tissue, myosin, has occupied



1, 3- DI-PHOSPHO-GLYCERIC ACID



PHOSPHO CREATINE



PHOSPHO-ENOL-PYRUVIC ACID

Figure II

the attention of many investigators because of its importance as the contractile element of living muscle. When isolated from the tissue it has many properties of the globulins, but its extreme molecular asymmetry gives rise to interesting properties which on the whole are similar to the corpuscular proteins.

In addition to its role as the contractile element of muscle, there now exists the possibility that myosin is also an enzyme. In 1939 Engelhardt and Ljubimova (9) announced that adenosinetriphosphatase activity was almost entirely associated with the myosin fraction of muscle. As will be described later, Kalckar (16) has labeled the active enzyme in myosin as adenylpyrophosphatase, and states that it is specific for ATP.

von Euler and Adler (10) and Lutwak-Mann and Mann (22) found an enzyme in yeast that would catalyze the transfer of phosphate from ATP to hexoses. von Euler called this enzyme "heterophosphatase". In earlier work Meyerhof (24) obtained an enzyme from autolyzed yeast which enabled old muscle juice to regain its ability to ferment glucose. He called this enzyme "hexokinase". Hexokinase is identical with heterophosphatase. The reaction is as follows:

ATP + 2 glucose ---→ AA + 2 glucose-6-phosphate

Colowick and Kalckar (3) found that the reaction is more complex than the one proposed by Meyerhof. With the yeast hexokinase, ATP could transfer only one phosphate to glucose. The reaction is as follows:

ATP + glucose ----→ ADP + glucose-6-phosphate

It was found that if a heat stable protein from rabbit muscle were added to the yeast hexokinase system, one phosphate from ADP could also be transferred to glucose. This is not a simple reaction, but a dismutation of adenosinediphosphate according to the following:

2 ADP ⇌ AA + ATP

The ATP which is formed may then phosphorylate hexoses by adenylpyrophosphatase action. Kalckar has called this heat stable protein which activates the yeast hexokinase system "myokinase". Myokinase is a protein which possesses an unusually high stability toward boiling with mineral acids as well as toward precipitation with trichloroacetic acid. Myokinase is found in large amounts in skeletal muscle of rabbit and frog. It is present in traces in heart and brain, but is absent in liver and kidney (4).

In a recent publication Kalckar (16) has labeled the enzyme in myosin as adenylpyrophosphatase, and cites evidence that it is specific for adenosinetriphosphate. The addition of myokinase causes dismutation of the diphosphate. Liver extract hydrolyzes the triphosphate to adenylic acid, and is for this reason thought by Kalckar

to contain both a di- and tri-phosphatase, or one enzyme which hydrolyzes both forms.

Bailey (2) has compared adenosinetriphosphatase activity of liver tissue with that of muscle tissue, using calcium as the activator. The dephosphorylation caused by adenosinetriphosphatase in liver was not as great as the muscular dephosphorylation. Bailey concluded from this that the liver enzyme and the muscle enzyme were not identical.

In 1936 Wulzen and Bahrs (36) discovered a deficiency disease in guinea pigs raised on a diet consisting of skim milk, adequate amounts of copper, iron, the necessary vitamins, straw, and iodized salt. The first sign of the deficiency was the development of stiffness at the wrist joint. During this deficiency there occured a general derangement of the phosphorus and calcium metabolism. The characteristic symptoms of the disease were calcium deposits parallel to muscle fiber, and in the aorta, cartilage, and intestine. Animals raised on a diet consisting of rolled barley, greens, salt, and water did not exhibit this characteristic stiffness. van Wagtendonk and Wulzen (33) reported that the syndrome could be alleviated within five days upon administration of one gram of raw cream to each animal per day. With raw cream as original material they were able to concentrate a factor which, in a daily dosage of 0.1 microgram, was able to

cure the stiffness induced by the milk diet within five days.

van Wagtendonk (34) reported the changes occuring in the phosphorus metabolism during the deficiency. The inorganic phosphorus shows a constant increase in the liver and kidney, during the deficiency, while the concentration of the easily hydrolyzable phosphorus, representing about 67% of the adenosinetriphosphate and 50% of the adenosinediphosphate, decreased considerably.

ATP is important in cellular economy because, as already pointed out, it possesses the ability to store within its structure the energy which is made available by the processes of anaerobic and aerobic metabolism. Adenylpyrophosphatase is the enzyme controlling the dephosphorylation of ATP to inorganic phosphorus and adenylic acid.

Inorganic phosphorus increased during the deficiency in the liver (34) and the muscle (35) at the expense of adenosinetriphosphate and -diphosphate. This indicates that the ATP-ase activity may have increased. Other factors, however, may have a counter influence on this enzyme. With these ideas in mind it was decided to carry on an investigation of the adenosinetriphosphatase activity of the liver during the deficiency.

EXPERIMENTAL

Approximately fifty animals were used in this series of determinations. These animals were divided into three groups. The first group consisted of five normal animals receiving a diet of rolled barley, greens, straw, salt, and water. The second group of twenty-one animals was placed on the deficient diet as described by van Wagtendonk (34). This diet had the following composition:

(1)	skim milk powder	16 g.
(2)	copper sulfate	0.25 mg.
(3)	ferric chloride	0.25 mg.
(4)	water	84 g.

Two feedings per day were required, one in the morning and one in the evening. To the morning diet a solution of water-soluble vitamins was added in such a concentration that the average daily vitamin intake was as follows:

(1)	thiamine hydrochloride	0.2 mg.
(2)	riboflavin	0.5 mg.
(3)	pyridoxine hydrochloride	0.1 mg.
(4)	nicotinic acid	1.0 mg.
(5)	ca-pantothenate	0.1 mg.
(6)	inositol	10 mg.
(7)	p-amino-benzoic acid	2.0 mg.
(8)	choline	50 mg.
(9)	biotin (S.M.A. Concentrate S-200)	0.01 mg.

A solution of the fat-soluble vitamins in cottonseed oil

was added to the evening diet. The average daily intake was as follows:

(1) β -carotene	150 I.U.
(2) viosterol	40 I.U.
(3) α -tocopherol	0.1 mg.
(4) 2-methyl-1,4-naphthoquinone	0.1 mg.

Once a week 50 mg. of crystalline l-ascorbic acid, dissolved in water immediately before use, were administered by mouth. Water and iodized salt were provided ad libitum.

The third group of animals was also placed on the deficient diet, but received during the last period of the experiment a ten gamma dosage of the anti-stiffness factor.

All of the animals were housed in wire cages upon autoclaved straw, the straw providing a source of roughage.

The experimental animals were killed by a blow on the head. The liver was immediately removed and two grams of the tissue weighed on an ordinary analytical balance. A tissue homogenate in water was prepared by using an apparatus consisting of a test tube and a close-fitting pestle (32). The homogenizer was powered by a cone driven stirring motor of the Cenco type, operated at a speed of about 1000 r.p.m.

An assay was made on the ATP used, in which total phosphorus, inorganic phosphorus, and easily hydrolyzable

phosphorus were determined.* The method of Fiske and Subbarow (12) was used to determine the total phosphorus and inorganic phosphorus, while the easily hydrolyzable phosphorus was determined according to Lohmann (19).

The empirical formula of ATP shows that the ratio of easily hydrolyzable phosphorus to total phosphorus should be around 2/3. The calculated ratio was 0.66 and the ATP upon analysis gave a ratio of 0.61.

The sodium salt of ATP was prepared by the method of Bailey (2). Seventy milligrams of the barium salt were dissolved in a small amount of cold N HCl and the solution then diluted with a small amount of distilled water. A saturated solution of sodium sulfate was added dropwise until all of the barium sulfate precipitated. After centrifuging the barium precipitate off, the solution was adjusted to a pH of 7 with a Beckmann pH meter. The solution of the sodium ATP salt was then diluted to 10 ml., which made the concentration 0.009 molar.

In formulating a quantitative method for the estimation of ATP-ase two factors are desirable: (a) the rate of reaction should be linear during incubation time, and (b) the rate must be directly proportional to the amount

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*We would like to thank Dr. V.L. Koenig of the Armour Research Laboratories for supplying us with the ATP used in this project.

of enzyme present. The tissue homogenate method of DuBois and Potter (6) meets these requirements, and the investigation was undertaken using their scheme of analysis. This method is based on tissue dilution of extracts, and the measurement of their activity in the presence of an excess of substrate and cofactors.

A 10% tissue homogenate (32) in water was prepared. Concentrations of one, two, three, four, and seven mg. of tissue per 0.2 ml. of homogenate were needed for the determinations, so they were made up as follows:

(1) 0.5 ml. of the original 10% concentration was diluted to 10 ml. This gave a final concentration of 1 mg. per 0.2 ml.

(2) 1.0 ml. of original was diluted to 10 ml. This gave 2 mg. of tissue per 0.2 ml.

(3) 1.5 ml. original diluted to 10 ml, giving 3 mg. of tissue per 0.2 ml.

(4) 2 ml. original diluted to 10 ml., giving 4 mg. of tissue per 0.2 ml.

(5) 3.5 ml. original diluted to 10 ml., giving 7 mg. of tissue per 0.2 ml.

0.2 ml. of each of the above concentrations were pipetted into small test tubes. A duplicate was run for each tissue concentration. To the small test tubes were added 0.2 ml. of 0.1 M sodium succinate buffer (pH 6.5)

and 0.2 ml. of 0.009 molar ATP. The tubes were mounted in a wire rack, and placed in a constant temperature bath for an incubation period of ten minutes at 30°C. At the end of this period 0.1 ml. of 50% trichloroacetic acid was added to each tube in order to terminate the reaction. The mixture was centrifuged in an angle centrifuge, and 0.4 ml. of the supernatant liquid was removed for analysis. The inorganic phosphorus liberated from ATP was measured in a Klett-Summerson photoelectric colorimeter by the method of Fiske and Subbarow (12), a final volume of 10 ml. being used.

The inorganic phosphorus in the tissue homogenates was also determined. These values were subtracted from the inorganic phosphorus value obtained for the incubation mixture. The inorganic phosphorus found in the ATP was also subtracted from the incubation mixture value. For the determination of inorganic phosphorus present in the tissue, the ATP was omitted from the tubes, and 0.2 ml. of distilled water added instead. The inorganic phosphorus was then determined after ten minutes and at 30°C. by the same method. The values obtained for 7 mg. tissue concentration were not used because after 4 mg. an exact linear relationship was not obtained.

The quantity of adenosinetriphosphatase which will liberate one gamma of inorganic phosphorus from ATP in 10

minutes has been defined as one adenosinetriphosphatase unit.

In order to determine the activation of adenosinetriphosphatase by calcium ions, a series of determinations were made on normal and deficient animals in which 0.1 ml. of 0.04 M $CaCl_2$ was added to each of the tubes. When calcium activation was being determined, a parallel run was made on the same tissue homogenate in which the calcium was omitted.

The calculations of the adenosinetriphosphatase units are as follows:

(1) Total inorganic ph = $\frac{\text{conc. of stand. in gammas}}{\text{reading of standard}} \times$ reading of unknown $\times$ 7/4.

(2) Total inorganic ph - inorganic ph in ATP - inorganic ph in tissue = gammas of inorganic ph liberated.

(3) $\frac{\text{Gammas inorganic ph liberated}}{\text{mg. tissue concentration}}$ = ATP-ase units.

The standard referred to in calculation (1) was made up according to Fiske and Subbarow (12).

It was found that adenosinetriphosphatase was being determined instead of adenosinepyrophosphatase. ATP-ase splits off one labile phosphorus from ATP, while adenosinepyrophosphatase would cleave both phosphorus groups from ATP.

According to Figure III, 4 mg. of tissue homogenate liberates 8.00 gammas of inorganic phosphorus from 0.003 molar ATP. Theoretically, 65.1 gammas of inorganic phosphorus should be released if one labile phosphorus is split off. It was assumed that if enough enzyme were present 65.1 gammas of inorganic phosphorus could be liberated. It is not likely that adenylpyrophosphatase is being determined for in this case 130.2 gammas of inorganic phosphorus would have to be split off.

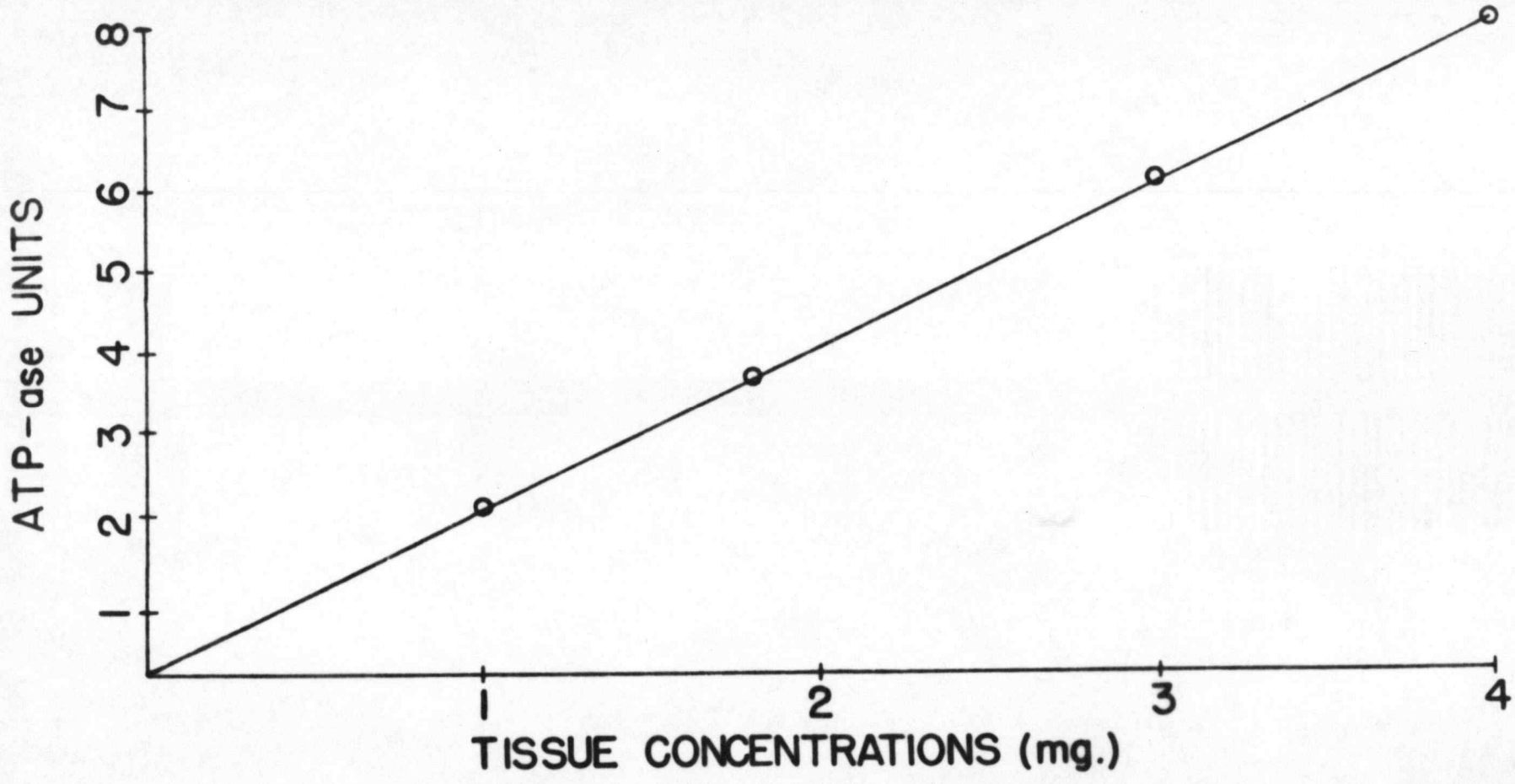
HYDROLYSIS OF ATP WITH DIFFERENT TISSUE CONCENTRATIONS
Normal Animals
ATP-ase UNITS
1
2
3
4
5
6
7
8
1
2
3
4
TISSUE CONCENTRATIONS (mg.)

Figure III

RESULTS

The data were tabulated and analyzed statistically according to the method of Fisher (11). ATP-ase units are calculated as the amount of inorganic phosphorus liberated in ten minutes at 30° C. per milligram of tissue.

Figure III gives the linear relationship obtained for normal animals when milligrams of tissue homogenate are plotted against ATP-ase units.

The adenosinetriphosphatase activity in the liver of normal and deficient animals is given in Table I. The ATP-ase activity is lowered almost immediately after the animals were given the deficient diet, and remained on this low level until after eight or nine weeks. No significant differences were noted between normal and deficient values during the later stages of the deficiency.

The ATP-ase activity of liver tissue increases significantly in deficient animals after 0.04 M $CaCl_2$ has been added (Table II). With normal animals this increase is not significant.

A marked increase of ATP-ase activity occured when daily dosages of 10 gammas of the anti-stiffness factor were administered to fifteen week deficient animals over a period lasting from one to six days (Table III). The activation caused by addition of the factor to the fifteen week deficient animals is significantly greater than the increase obtained when calcium ions are added.

TABLE I

ADENOSINETRIPHOSPHATASE ACTIVITY IN THE LIVER OF NORMAL AND DEFICIENT ANIMALS

AGE IN WEEKS	NO. OF DETNS.	TIME ON DIET IN WEEKS	ATP-ase UNITS ± STANDARD ERRORS	SIGNIFICANT* DIFFERENCES
30	15	Normal	2.05 ± 0.028	
15	13	2	1.60 ± 0.124	3.5
35	9	5	1.38 ± 0.065	9.6
21	2	8	1.85 ± 0.017	6.2
28	7	15	2.01 ± 0.085	.45
39	11	26	1.65 ± 0.085	4.5
60	7	45	1.97 ± 0.129	0.6

* The significant differences were determined against the normal value of 2.05 ± 0.028.

TABLE II

ADENOSINETRIPHOSPHATASE ACTIVITY IN THE LIVER OF NORMAL AND DEFICIENT ANIMALS WITH CALCIUM ADDED

AGE IN WEEKS	NO. OF DETNS.	TIME ON DIET IN WEEKS	ATP-ase UNITS ± STANDARD ERRORS	SIGNIFICANT* DIFFERENCES
25	8	Normal	2.33± 0.20	1.6
35	8	5	1.85 ± 0.11	3.6
30	8	15	3.00 ± 0.30	3.2
60	8	45	2.85 ± 0.25	3.1

* The significant differences were determined against the values for the same deficency without calcium.

TABLE III

EFFECT OF ANTI-STIFFNESS FACTOR ON ADENOSINETRIPHOSPHATASE ACTIVITY IN THE LIVER OF ANIMALS DEFICIENT 15 WEEKS

AGE IN WEEKS	NO. OF DETNS.	CONCENTRATION OF DOSAGE	ATP-ase UNITS ± STANDARD ERRORS	SIGNIFICANT* DIFFERENCES
28	7	0	2.01 ± 0.09	
28	8	10	3.72 ± 0.44	3.9
28	8	20	4.98 ± 0.80	3.6
28	8	30	4.53 ± 0.61	4.1
28	8	40	3.49 ± 0.32	4.5
28	14	50	3.99 ± 0.51	3.8
28	8	60	3.67 ± 0.52	3.1

* The significant differences were determined against the values for 15 week deficient animals.

It is evident from the results reported in this thesis that a deficiency of the anti-stiffness factor causes a derangement in the activity of adenosinetriphosphatase in liver tissue. The explanation of this derangement, in light of previuos work, is as yet difficult.

DuBois and Potter (6), and Bailey (2) have demonstrated that calcium is the specific activator for the adenosinetriphosphatase of skeletal muscle. DuBois and Potter (6) have also shown that calcium ions activate ATP-ase in the liver. The presence of a calcium activated adenosinetriphosphatase in liver tissue raises the question as to the form and functions of the enzyme. The ATP-ase determinations in the liver, with and without calcium, suggest that calcium may exist in combination with adenosinetriphosphatase. The quantity of calcium combined with the enzyme at any one time would determine the activity of the system.

As stated previously, a disturbance of the phosphorus metabolism occurs during the deficiency disease and upon autopsy calcium triphosphate deposits may be found in connection with almost any body tissue. As a result of the deficiency of the anti-stiffness factor, the concentrations of the inorganic phosphorus and total non-diffusible calcium in the blood are higher than in normal animals or in animals on a deficient diet supplemented with this factor

(35). Colloidal calcium phosphate may then be formed and deposited in the tissues. The increase of deposited inorganic phosphorus would seem to indicate a raise in adenosinetriphosphatase activity. However, if calcium ions are the primary activating agent of ATP-ase, and if calcium is being removed, this would result in a lowering in the activity of the enzyme.

The increase in activity, effected by the addition of calcium to the ATP-ase system, is greater in deficient animals than in animals receiving a normal diet. This seems to indicate that calcium ions are present in stock animals in an amount sufficient to insure a stable enzymatic system. Therefore the addition of calcium to the deficient animals, where there is a decrease in the activating ions due to precipitation as $Ca_3(PO_4)_2$, would cause a greater increase in the activity of adenosinetriphosphatase.

The high values obtained when the deficient animals were given a dosage of the anti-stiffness factor suggests the possibility of a stimulating action. Heilbrunn (5) has advanced a theory, without reference to any particular enzyme system, that the stimulation of cells in general results in the release of calcium which then produces a tissue response. Evidence that acetylcholine increases ATP-ase activity has been reported by DuBois and Potter (5), and the evidence suggested that the effect might be mediated by calcium.

The assumption that calcium will be made available by the anti-stiffness factor when it is administered during the deficiency is not the only action indicated in the results obtained. When calcium ions are added to the ATP-ase system, an increase in the activity occurs. However, upon the addition of the anti-stiffness factor a much larger increase in ATP-ase activity is noted. This increase proves that the factor has a direct activating action on ATP-ase and is not merely a means of releasing calcium. The Heilbrunn theory may also apply here, but, as already implied, the reaction would only be secondary.

The linkage of physiological stimuli with the regulation of the rate of glycolysis and the performance of work may be controlled by the activation of the adenosine-triphosphatase system, since the rate of glycolysis and respiration is influenced by the availability of inorganic phosphate and adenylic acid.

1. ATP-ase activity in the liver of guinea pigs has been determined in normal animals and in animals developing a dietary stiffness disease. This activity was also determined in deficient animals that were receiving a daily dosage of the anti-stiffness factor.
2. It has been found that in deficient animals ATP-ase activity declines steadily during the first eight or nine weeks of the deficiency, after which time there is an increase to slightly below the normal value.
3. The activation of ATP-ase in the liver increases significantly in deficient animals after calcium ions are added. The activation increase is not significant when calcium ions are added to normal animal tissues.
4. A significant increase occurs in phosphatase values when a dosage of the anti-stiffness factor is administered to fifteen week deficient animals. Calcium ions did not cause as large an increase in ATP-ase activation as did the anti-stiffness factor.

1. Bahrs, A. M., and Wulzen, R., Proc. Soc. Exp. Biol. Med., 33:528 (1936).

2. Bailey, K., Biochem. Journal, 36:121 (1942).

3. Colowick, S. P., and Kalckar, H. M., J. Biol. Chem., 137:789 (1941).

4. Colowick, S. P., and Kalckar, H. M., J. Biol. Chem., 148:117 (1943).

5. DuBois, K. P., and Potter, V. R., J. Biol. Chem., 148: 451 (1943).

6. DuBois, K. P., and Potter, V. R., J. Biol. Chem., 150: 185 (1943).

7. Eggleton, P., and Eggleton, C. P., J. Physiol., 63:155 (1927).

8. Eggleton, P., and Eggleton, C. P., J. Physicl., 65:15 (1928).

9. Engelhardt, W. A., and Ljubimova, M. N., Nature, Lond., 144:668 (1939).

10. Euler, H. von, and Adler, E., Z. Physiol. Chem., 235: 122 (1935).

11. Fisher, R. A., Statistical methods for research workers, 8th ed., Edinburgh, Oliver and Boyde, 1941.

12. Fiske, C. H., and Subbarow, Y., J. Biol. Chem., 66: 375 (1925).

13. Fiske, C. H., and Subbarow, Y., Science, 67:169 (1928).

14. Fiske, C. H., and Subbarow, Y., J. Biol. Chem., 81: 829 (1929).

15. Grosser, P., and Husler, J., Biochem. Z., 39:1 (1912).

16. Kalckar, H. M., J. Biol. Chem., 153:355 (1944).

17. Lehmann, H., Biochem. Z., 288:338 (1936).

18. Lipmann, F., Advances in Enzymology, 1:99 (1941).

19. Lohmann, K., Biochem. Z., 254:381 (1932).

20. Lundsgaard, E., Biochem. Z., 217:162 (1930).

21. Lundsgaard, E., Annual Review of Biochem., 7:377 (1938).

22. Lutwak Mann, C., and Mann, T., Biochem. Z., 281:140 (1935).

23. McCollum, E. V., and Hart, E. B., J. Biol. Chem., 4: 497 (1908).

24. Meyerhof, O., Biochem. Z., 183:176 (1927).

25. Meyerhof, O., and Suranyi, J., Biochem. Z., 191:106 (1927).

26. Meyerhof, O., Lundsgaard, E., and Blaschko, H., Naturuwissenschaften, 18:787 (1930).

27. Plimmer, R. H. A., Biochem. J., 7:43 (1913).

28. Robison, R., Biochem. J., 16:809 (1922).

29. Robison, R., Biochem. J., 17:286 (1923).

30. Robison, R., and Soames, K. M., Biochem. J., 18:740 (1924).

31. Suzuki, Yoshimura, and Takaiski, Tokyo Imp. Univ. Coll. Agr. Bull., 7:503 (1907).

32. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Related Methods for the Study of Tissue Metabolism, Minneapolis, Burgess Publishing Co., 1945.

33. van Wagtendonk, W. J., and Wulzen, R., Arch. Biochem., 1:373 (1943).

34. van Wagtendonk, W. J., J. Biol. Chem., 155:337 (1944).

35. van Wagtendonk, W. J., and Lamfrom, H., J. Biol. Chem., 158:421 (1945).

36. Wulzen, R., and Bahrs, A. M., Physiol. Zool., 9:508 (1936).