Effect of Adrenaline on Blood ATP Level in Diabetic Patients

By

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Numerous studies have been made to clarify what is the fundamental metabolic disturbance in diabetes mellitus. Our knowledge of this problem to-day might be briefly summarized as follows. There are three principal disturbances of carbohydrate metabolism in diabetes mellitus. The first is the disturbance in the phosphorylation process of glucose into glucose-6-phosphate by hexokinase and adenosine triphosphate (ATP); the second is the disturbance in aerobic oxidation of pyruvic acid; and the third disturbance is the decreased production of high energy-bound phosphate. The first disturbance is aggravated by the third disturbance.

The depletion of ATP in diabetic coma rat liver and the decreased phosphorylation of thiamine in diabetic rat liver homogenate, which were found by Kaplan and his co-workers) and Foa and his colleagues) respectively, indicate the disturbance of ATP formation or of oxidative phosphorylation in diabetes mellitus. Most of these findings were obtained in experimental diabetic animals and it is doubtful whether the metabolic disturbances of human diabetes are the same as those of experimental ones. Our studies reconfirmed that the fasting blood ATP level of diabetic patients and the liver seven minutes hydrolyzed phosphorus level of alloxan diabetic rabbits without severe ketosis were the same as those of nondiabetic test subjects and normal rabbits. The present study was undertaken to confirm the effect of adrenaline on human blood ATP level, and to determine whether the fluctuation of blood ATP provoked by adrenaline administration is not different between normal adults and nonketosic diabetic patients.

Experimental

Method

Five diabetic patients and five healthy adults were chosen for this study. After an overnight fast, 0.5 mg. of adrenaline hydrochloride
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(Sankyo Company) was injected subcutaneously and blood specimens were obtained by antecubital venipuncture for the blood ATP estimation prior to, and every one hour after adrenaline injection for five hours. Other blood specimens were collected from the ear lobe for the blood sugar estimation. The test subjects were kept in bed during this study.

**Estimation of blood ATP.** The blood ATP was estimated as "pyrophosphate" fraction obtained by ten minutes' hydrolysis with boiling normal hydrochloric acid, after which the liberated inorganic phosphorus was measured by the Lohmann's procedure\(^4\)\(^5\). 2.0 cc. of venous blood was pipetted into a test tube containing 2.0 cc. of 3.8% sodium citrate solution; 4.0 cc. of 10% trichloroacetic acid was added to this test tube; the mixture was shaken vigorously and filtered. 3.0 c.c. of filtrate and 3.0 cc. of 2 N HCl were pipetted into a test tube and shaken. After the pipetting of 2.0 cc. of this mixture into 25 cc. volumetric flask for the determination of blood inorganic phosphorus, this test tube was covered and immersed in a boiling water bath for just ten minutes. 2.0 cc. of the boiled mixture was pipetted into 25 cc. volumetric flask for the determination of acid-labile phosphorus of ATP. 5.0 cc. of 2.5% ammonium molybdate 5 N H\(_2\)SO\(_4\) solution, 1.0 cc. of \(\alpha\)-aminonaphtholsulfonic acid solution and adequate amount of water were added to these volumetric flasks; kept at 37\(^\circ\)C for ten minutes and cooled by running water. Determination of phosphorus was made photoelectrically with a 640 filter.

**Results**

**Blood ATP.** A decrease in blood ATP was produced by the injection of adrenaline in both nondiabetics and diabetics. In the normal group, blood ATP was lowest at 2nd hour and returned to the initial level or over the initial value within five hours except in one case (Fig. 1). In the diabetic group, the blood ATP decreased remarkably and reached the lowest value at 2nd hour in three of the five cases and at 4th hour in the other two cases. In three of the five cases, this decrease in blood ATP continued steadily, and didn’t return to the initial level until 5th hour in all diabetic cases. The difference of the average decrease rate of blood ATP in percent between diabetic and nondiabetic group is statistically significant. Similar results on liver seven minutes hydrolyzed phosphorus was obtained in normal and alloxan diabetic rabbits.\(^6\)

**Blood inorganic phosphorus.** In the normal group, the administration of adrenaline caused first a decrease in blood inorganic phosphorous and this decrease phase rebounded to a definite increase phase from 3rd hour. In the diabetic group, on the contrary, the blood inorganic phosphorus increased slightly without a prior decrease phase and returned to the initial level at 5th hour (Fig. 2).
TABLE I

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<th>3</th>
<th>4</th>
<th>5 hrs.</th>
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Blood sugar. The elevation of blood sugar induced by adrenaline was almost equal in both groups, but the hyperglycemic phase protracted longer in the diabetic group than in the nondiabetic group as did those in Saito’s report\(^7\) in our clinic.

**DISCUSSION**

The basic information and the clinical understanding of blood ATP has remained poorly delineated to the present time and it was not definitely settled yet whether human erythrocytes contain both ADP and ATP, or only ATP until recent years.\(^8\)-\(^11\) Most investigators think that the blood labile phosphorus is roughly the labile phosphorus of ATP, inasmuch as blood contains very small amounts of ADP in comparison to ATP.

The ATP contained in erythrocytes is formed closely at the cell mem-
The blood ATP is produced as the result of anaerobic oxidation of glucose. This is supported by Dische’s experiments and the fact that phosphorus uptake by erythrocytes is inhibited by iodoacetic acid (which poisons the triose phosphate dehydrogenase) or by sodium fluoride (which poisons the enolase step by removing Mg, forming the complex MgFPO₄)¹⁴), or by withholding glucose¹⁵) but not by deprivation of oxygen¹⁶), and more clearly depicted by the studies with radioactive phosphate¹⁷),¹⁸). The blood ATP is crudely regarded as erythrocyte ATP, because the glycolytic activity of erythrocytes is far great as compared with those of leukocytes and platelets as a whole.¹⁹) No evidence of aerobic oxidation and no ability to utilize pyruvate were confirmed in mammalian mature erythrocytes in contrast to avian erythrocytes²¹).

The significance of blood ATP is thought to be as follows.

1) The blood ATP reflects the metabolic conditions in blood cells. The reticulocyte, which has a high glycolytic rate and an aerobic metabolism, has a high concentration of ATP compared with that of mature erythrocytes;²²) the glycolytic power of the blood of various vertebrate species varies in the same direction as the content of ATP²⁵). The glycolysis in erythrocytes, however, is influenced by the physicochemical changes of blood, as described below.

2) The ATP content of blood cells is regulated by the physicochemical
conditions of blood itself. These are: pH, ionic milieu, conditions of various coenzymes, etc. A slight decrease in blood ATP was observed in diabetic acidosis.\textsuperscript{26}

3) ATP contained in erythrocytes has a great role in the maintenance of the biconcave shape of erythrocytes, involving the function of certain contractile elements in this cell stroma similar to actomyosin in muscle cells.\textsuperscript{27}

4) Blood ATP serves as one of the carriers of phosphorus which may be utilized in tissue. According to our experimental results described elsewhere\textsuperscript{28}, however, this thought seems to be questionable. Our experiments confirmed that the administration of glucose caused both an increase in blood ATP and a decrease in blood inorganic phosphorus. If the blood ATP is a reserve of labile phosphorus, the decrease in blood inorganic phosphorus might be accompanied by a decrease in blood ATP.

5) The blood ATP is a reflection of the soft tissue ATP or an indicator of the state of labile phosphorus reserve of the body. The causes which provoke changes of tissue ATP, may produce similar changes of blood ATP. The next fact seems to support this thought. The decrease in blood ATP after adrenaline injection was observed by Imaizumi\textsuperscript{29} in normal rabbits, and the same decrease in rabbit liver seven minutes' hydrolyzed phosphorus was observed by Ujiie in our laboratory.\textsuperscript{6}

The present experimental results showed that blood ATP decreased following adrenaline injection and this decrease in blood ATP was more remarkable in diabetic patients than in normal adults. The glycolysis in diabetic blood has been reported to be equal to that in nondiabetic blood.\textsuperscript{30} The inhibitory effects of adrenaline on glucose uptake of blood cells and glycolysis in blood were observed by Dietrich\textsuperscript{31}, Himmerich and Tschernjak\textsuperscript{32}, and this inhibitory action of adrenaline may be one of the causes of depression of blood ATP after adrenaline injection. The remarkable decrease of blood ATP in the diabetic group in the present study seems to suggest that the uptake of glucose or glycolysis in blood cells are more strongly inhibited by adrenaline in diabetic patients than in healthy adults, probably due to the latent lack of hexokinase or phospho-hexokinase in the former group.

Adrenaline accelerates the breakdown of liver glycogen by accelerating the activation of phosphorylase in the liver\textsuperscript{33}-\textsuperscript{36} and, on the other hand, inhibits the peripheral utilization of glucose\textsuperscript{37,38}. This is the reason for adrenaline hyperglycemia. Uncoupling of oxidative phosphorylation by adrenaline was demonstrated by in vitro experiment\textsuperscript{39}, but the experimental results of acceleration of oxygen consumption by small doses of adrenaline in vivo\textsuperscript{40} and of the decrease in liver seven minutes hydrolyzed phosphorus content\textsuperscript{9}, seem to suggest that adrenaline produces an un-
coupling of oxidative phosphorylation in vivo, too. The respiration of erythrocytes is inhibited by larger doses of adrenaline (0.075–0.1 mg./1 cc. blood), but accelerated by smaller doses of adrenaline (0.005–0.0005 mg./1 cc. blood). We feel that the decrease in blood ATP is elicited by the same or similar metabolic process as that of other soft tissues. In other words, the decrease in blood ATP is a reflection of the depletion in soft tissue ATP, which may occur as the result of a decrease in utilization of glucose, increase in the requirement of ATP to synthesize glycogen from lactic acid released massively by the breakdown of muscle glycogen, and decrease in the ATP formation because of uncoupling of oxidative phosphorylation. A decrease of oxidative phosphorylation is observed in diabetes mellitus. We feel that the tendency of oxidative phosphorylation to decrease in diabetes mellitus was aggravated by adrenaline, and the decrease in blood ATP became more remarkable in the present experiment.

In the present experiment, the blood inorganic phosphorus changed biphasically. It decreased during the first two hours in the nondiabetic group, and in the diabetic group it decreased slightly or not at all. The decrease phase of inorganic phosphorus was followed by the increase phase, which was observed three or four hours after adrenaline administration. The decrease in blood phosphorus induced by adrenaline, which was observed by many workers, was explained as the result of the formation of hexose phosphate in muscle, and this effect was thought to be due to the action of insulin secreted from the pancreas reacting to the adrenaline hyperglycemia. This explanation, however, seems very doubtful, because there is no demonstration of insulin secretion in adrenaline hyperglycemia. The increase in blood inorganic phosphorus seems in some part to the result of a breakdown of glycogen into glucose and phosphate in both liver and peripheral tissue. The absence of a decrease phase in the diabetic group suggests that the disturbance of glucose uptake in diabetic tissue was aggravated by the inhibitory action of adrenaline.

To summarize, the blood ATP level became lowered by adrenaline injection, and this decrease in blood ATP is more prominent in diabetic patients than in normal adults, even though there is no significant difference in fasting blood ATP level between them. The present experiment suggests that ATP formation in blood cells is more easily affected by adrenaline in diabetic patients as compared with healthy adults, and may indicate that the ATP formation in blood cells of diabetic patients is latently damaged as was observed in other soft tissues of experimental diabetic animals.
Blood ATP Level in Diabetic Patients

Summary

The change in the blood ATP level of five diabetic patients and five normal adults was observed hourly for five hours following adrenaline injection (0.5 mg.). Adrenaline injection produced a definite decrease in blood ATP and this decrease was more prominent in the diabetic group than in the normal persons. The change of blood inorganic phosphorus elicited by adrenaline was biphasic in healthy adults, with a decrease phase and an increase phase. In the diabetic group, this initial decrease phase was absent or very slight. These results seem to suggest a latent disturbance of ATP formation in diabetic patients.

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