A New Case of Phosphoglycerate Kinase Deficiency: PGK Creteil Associated With Rhabdomyolysis and Lacking Hemolytic Anemia

By Raymonde Rosa, Claude George, Michel Fardeau, Marie-Claude Calvin, Maurice Rapin, and Jean Rosa

A new case of phosphoglycerate kinase (PGK) deficiency is described. The propositus displayed episodes of rhabdomyolysis crises and acute renal failure but did not exhibit any sign of hemolysis. A severe deficiency in phosphoglycerate kinase was revealed in muscle and was also found in erythrocytes, white cells and platelets. A partial defect in the same enzyme was present in the mother's and the two daughters' erythrocytes, indicating a X-linked recessive genetic transmission of the enzyme defect. In the propositus, erythrocyte ATP concentration was normal, although 2,3-diphosphoglycerate and triose phosphate levels were moderately increased. Lactate production from glucose, in vitro, was close to normal in intact red cells. The partial PGK was characterized by an increased $K_m$ for ADP and more especially for ATP, reduced thermostability, and diminished electrophoretic mobility. Lack of this enzyme, which is a key step in the glycolytic process (generation of one molecule of ATP), is thought to be responsible for rhabdomyolysis, a fact that has not been reported previously.

EXCERPTIONAL Rhabdomyolysis has been described in the hereditary lack of muscle phosphorylase or phosphofructokinase (PFK) and may be complicated by acute renal failure. Other exertional and familial myoglobinurias have been reported in which the enzymatic defects have not been characterized. In addition, hereditary rhabdomyolysis without a direct relationship to muscular exercise has been found in the absence of carnitine palmitoyltransferase or in the malignant postanesthesia hyperpyrexia syndrome.

In our patient with recurrent exertional rhabdomyolysis and renal failure, a deficiency in muscle phosphoglycerate kinase (PGK) was discovered (e.g. ATP: 3-phospho-D-glycerate 1-phosphotransferase). This enzyme, which is a key step in the glycolytic process, generates one molecule of adenosine triphosphate (ATP) by conversion of 1,3-diphosphoglycerate into 3-phosphoglycerate. Until now, the previous cases of PGK deficiency were associated most often with hemolytic anemia and mental retardation without muscular manifestation. In only one case has it been demonstrated in the muscle of a patient who exhibited muscular atrophy. More recently, a family has been described that exhibits partially deficient PGK; however, this deficiency was not associated with hemolytic anemia nor with neuromuscular and mental manifestations. The kinetic properties and structural characteristics of this variant have been evaluated. Some studies of the characteristics of the residual PGK of red cells have been carried out in other PGK-deficient males: in one case, increases in the isoelectric point and thermal stability were found. Another case was described with reduced affinity for the substrate, lower heat stability, and slower electrophoretic mobility. More recently, PGK Uppsala was reported with faster electrophoretic mobility, lower affinity for 3-phosphoglycerate, and lower heat stability.

In this article, we report a new case of PGK deficiency that is associated with rhabdomyolysis crises but not with hemolytic anemia. This new case is the first in which a severe PGK deficiency does not result in hemolysis, but is manifested in muscular abnormalities.

MATERIALS AND METHODS

All substrates and commercial enzymes were purchased from Boehringer Mannheim Biochemicals, France. Buffer salts were obtained from Merck, Chemical Division, Darmstadt (RFA). Cellogel strips were purchased from Chemetron Corp., Medical Products, Milano, Italy. a-Cellulose and Sigma cell type 50 were from Sigma Chemical Co., St. Louis, Mo. Carboxymethyl Sephadex (CM Sephadex) was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden.

Muscle Study

The muscular biopsy was performed under local anesthesia (with the patient's consent) several days after recovery of normal renal function. Skeletal muscle tissue obtained from the quadriceps muscle was immediately frozen in liquid nitrogen and stored at -80°C until use. Muscle specimens were obtained after informed consent, from subjects without demonstrable disease and who undergo an orthopaedic operation.

Microscopy Study

The muscular biopsy was studied by light microscopy of cryostat frozen sections, as well as by light and electron microscopy of section...
from specimens embedded by Spurr's method. The technical points have been reported previously.\textsuperscript{27}

**Enzymatic Study**

Extracts of skeletal muscle obtained from biopsy were prepared first by homogenization in a solution containing 5 mM Tris-HCl, pH 7.5, 0.7 mM $\beta$-mercaptoethanol, and 2.7 mM EDTA and centrifuging at 30,000 g for 30 min. The clear supernatant fluid was immediately used for enzymatic assays. Protein content of muscle extracts was assayed by ultraviolet absorption.\textsuperscript{28}

Enzymatic assays were performed according to Beutler et al.\textsuperscript{29} with some modifications. In the case of PGK activity, 5 mM sodium fluoride was added to the medium to prevent the effect of enolase which is much more active in muscle than in red cells.

**Blood Study**

Blood, collected in heparin or in an acid-citric-dextrose (ACD) solution, was passed through a mixture of $\alpha$-cellulose and Sigma cell type 50 in 0.15 M NaCl, and the washed red cells were hemolyzed according to Beutler et al.\textsuperscript{30} Leukocytes were prepared by sedimentation according to Evans and Kaplan,\textsuperscript{31} and platelets were separated by differential centrifugation.

**Enzymatic Assays**

Enzymatic assays were performed according to Beutler et al.\textsuperscript{32} For the backward PGK reaction, the assay medium contained 100 mM Tris-HCl, 0.5 mM EDTA (pH 8.0), 10 mM MgCl\textsubscript{2}, 0.2 mM NADH, 3 mM neutralized ATP, 40 U of glyceraldehyde 3-phosphate dehydrogenase, and 2 mM 3-phosphoglycerate.

For the forward reaction of PGK, 1,3-diphosphoglycerate was prepared according to Rose.\textsuperscript{33} The assay system contained 40 mM triethanolamine-HCl buffer (pH 7.5), 8 mM MgCl\textsubscript{2}, 40 mM KCl, 0.14 mM NADH, 2.6 mM ADP, 1.13 mM 1,3-diphosphoglycerate, 1.3 U/ml of phosphoglycerate mutase 1.3 U/ml of enolase 1.3 U/ml of pyruvate kinase, and 1.8 U/ml of lactate dehydrogenase. For both the backward and the forward reactions, the decrease of absorbance at 340 nm produced by the oxidation of NADH was followed spectrophotometrically.

**Glycolytic Intermediates and Other Red Cell Compounds**

Measurement of the concentration of glycolytic intermediates was performed according to Oelschlegel\textsuperscript{34} and that of reduced glutathione according to Kaplan and Dreyfus.\textsuperscript{35}

**Partial Purification of PGK**

In order to evaluate the $K_m$ of PGK for ADP and 1,3-diphosphoglycerate, it was necessary to study the forward reaction of this enzyme. To perform this forward reaction, we first had to obtain PGK free of glyceraldehyde 3-phosphate dehydrogenase and adenyate kinase. These two enzymes interfered in the assay of PGK when 1,3-diphosphoglycerate and ADP were used as substrates. This interference was particularly marked in deficient PGK. To perform this partial purification, a hemolysate was made from washed red cells to which one volume of water and one of toluene were added. After centrifugation at 15,000 g for 20 min, the supernatant toluene and the lipid layer were removed. The remaining hemolysate, still containing stroma, was applied to a CM-Sephadex column equilibrated in 10 mM sodium phosphate buffer at pH 6.4 and containing 1 mM EDTA and 1 mM $\beta$-mercaptoethanol. The stroma were eluted in the first fractions and hemoglobin remained on the column. Adenylate kinase was eluted by the equilibrating buffer containing 25 mM KCl and 1 mM AMP. Elution of PGK was then performed in the same buffer but with the addition of 4 mM of 3-phosphoglycerate in place of AMP. Glyceraldehyde 3-phosphate dehydrogenase remained on the column and could be eluted with the equilibrating buffer containing 100 mM KCl. The fractions displaying PGK activity were pooled and concentrated by ultrafiltration in a Diaflo system (Amicon). They did not contain any detectable adenylate kinase or glyceraldehyde phosphate dehydrogenase activity.

**Electrophoretic Study**

Electrophoresis of PGK was performed according to methods previously described for monophosphoglycerate mutase,\textsuperscript{36} i.e., on Cellogel strips in a 0.075 M Tris-EDTA citric acid buffer (pH 8.0) at 220 V for 3 hr. For normal controls, the samples applied to the strips were 2 $\mu$l of 1/10 hemolysate or 1/50 muscle extracts, and in the case of PGK-deficient subject, 5–10 $\mu$l of 1/3 hemolysate or 1/10 muscle extracts. After electrophoresis, PGK activity was revealed by pouring 4 ml of a mixture containing 1% agarose, in the same medium as that used for the assay (backward reaction), over the strip. PGK activity appeared as dark bands on a fluorescent background and was detected with ultraviolet light ($\lambda$ 340 nm) after 5–30 min incubation at 25°C.

**CASE REPORT**

A 31-yr-old white man of French origin was in generally good health and has a normal intellectual development, but since his childhood, several symptoms occurred during physical exercises that required its termination. These were painful cramps (especially in the legs) associated with abdominal pain, vomiting, and dizziness. However, moderate effort was possible for a long while without symptoms. Fasting was tolerated without trouble. No history of dark urine was noted until the age of 21 when, after a 100-yd run, myalgias appeared, followed by brown urine emission for 3 days. At that time, the urea nitrogen was 57 mg, the total lipids 450 mg, the cholesterol 150 mg/dl of serum, and the hemoglobin 14.3 g/dl of blood. A muscular biopsy was performed that disclosed normal phosphorylase activity. Electromyographic examination showed modest abnormalities of the myopathy type. No diagnosis was made at that time.

In January 1978, the patient took part in a family celebration where he danced and had a copious dinner that was followed by vomiting. Seven days later, the patient developed oliguria and oedema of the face but waited 6 days to seek medical advice. General physical and neurologic examinations gave normal results. The patient had normal muscle bulk and was not icteric. His spleen was not enlarged. The blood urea nitrogen was 165 mg, the serum creatinine 20 mg/dl, and the potassium was 6.4 meg/liter. Creatine phosphokinase (CPK) was 50 IU (normal: below 50 IU). The patient passed 4 liters of urine during the first 24 hr, did not require extra renal epuration, and renal function recovered completely.

Several assays performed over several months after complete recovery of a normal renal function continuously showed normal hemoglobin, haptoglobin, and bilirubin levels, as well as reticulocyte, leukocyte, and platelet counts. CPK assayed after moderate but sustained physical effort was 1025 IU. After overnight fasting, serum cholesterol was 220 mg and triglycerides 75 mg/dl. Polyacrylamide gel electrophoresis of the serum showed normal amounts of
very low density and low density lipoprotein. Ischemic exercise test was performed without pain or contracture and was followed by a rise in venous lactate less than that observed in the 6 normal subjects (Fig. 1) when maximum increases are considered.

No history of cramps during exercise was noted in the patient's family (his father had 4 sisters and 4 brothers with a total of 21 children; his mother had only 1 unmarried sister). His two daughters, 3 and 5 yr old, had normal physical activity relative to their ages and normal hemoglobin levels and reticulocyte counts.

RESULTS

Microscopy Study of Muscle

Very small changes were evidenced by light microscopy examination of muscle. Few angulous and atrophic fibers could be seen, dispersed with fibers of normal size and structure. A predominant number of type II fibers (normal within the quadriceps) was found, with a limited grouping of type I fibers without abnormality in the internal structure. No sudanophilic lipid storage was demonstrated. Phosphorylase activity was substantial. No glycogen storage was found with periodic acid Schiff coloration, but the two types of fibers demonstrated the same intensity of staining. However, a diffuse moderate peripheral and intermyofibrillar accumulation of glycogen granules was shown by electron microscopy (Fig. 2). The number of satellite cells was increased.

Enzymatic Studies

In muscle extracts, a marked PGK deficiency (about 25% of normal) was disclosed, contrasting with the high activities of three other enzymes of the glycolytic pathway (Table 1). When incubated without a sulfhydryl group (SH) protector, such as β-mercaptoethanol, the PGK activity decreased to about 5% of normal.

In red cells, the patient exhibited PGK activity amounting to about 3% of the normal mean (Table 2). All other glycolytic enzymes—adenylate kinase, adenine deaminase, glutathione reductase—displayed activities within the normal range. The PGK defect was also found in some other tissues of the patient (Table 2). The residual activity was very low in platelets, as well as in red cells. As in muscle, PGK activity of leukocytes was about 25% that of normal control. The patient's mother and his two daughters displayed partial RBC PGK deficiency (Table 2), which is in accordance with the X-linked genetic transmission of the defect.14

Glycolytic Intermediates and Other RBC Compounds

The levels of glycolytic intermediates were close to those in the normal control (Table 3), with the excep-

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**Fig. 1.** Plasma lactate levels during ischemic exercise of the forearm muscles. The response of the patient (full line) is compared to the response in 6 normal subjects (dotted lines). C1 and C2 indicate two assay before exercise.

**Fig. 2.** Muscle biopsy. Transverse section of a muscle fiber; electron micrograph × 32,000. Subsarcolemmal and intermyofibrillary accumulation of glycogen granules; lypofuscin body visible near a muscular pole.

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**Table 1.** Muscle Glycolytic Enzyme Activities

<table>
<thead>
<tr>
<th></th>
<th>U/mg of Protein</th>
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<tbody>
<tr>
<td>PGK</td>
<td>0.56</td>
</tr>
<tr>
<td>PGM</td>
<td>7.65</td>
</tr>
<tr>
<td>PK</td>
<td>3.31</td>
</tr>
<tr>
<td>PFK</td>
<td>0.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGK</td>
<td>0.56</td>
<td>2.11</td>
<td>1.52–2.70</td>
</tr>
<tr>
<td>PGM</td>
<td>7.65</td>
<td>4.24</td>
<td>2.08–5.13</td>
</tr>
<tr>
<td>PK</td>
<td>3.31</td>
<td>2.10</td>
<td>1.08–2.61</td>
</tr>
<tr>
<td>PFK</td>
<td>0.38</td>
<td>0.26</td>
<td>0.12–0.39</td>
</tr>
</tbody>
</table>

PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PK, pyruvate kinase; PFK, phosphofructokinase.

*Values obtained on five controls.
Table 2. The PGK Activities of the Red Cells and of Some of the Patient’s Tissues

<table>
<thead>
<tr>
<th></th>
<th>U/g Hb</th>
<th>U/mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>3.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Mother</td>
<td>39</td>
<td>-</td>
</tr>
<tr>
<td>Father</td>
<td>128</td>
<td>-</td>
</tr>
<tr>
<td>1st Daughter Ch.</td>
<td>84.7</td>
<td>-</td>
</tr>
<tr>
<td>2nd Daughter S.</td>
<td>79.8</td>
<td>-</td>
</tr>
<tr>
<td>Normal mean</td>
<td>142 ± 33*</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>(0.7-1.0)†</td>
<td>(0.55-0.99)†</td>
</tr>
</tbody>
</table>

*Normal mean ± SD from 50 determinations.
†Normal mean and range from 5 determinations.

Red Cell Incubation

Incubation of washed red cells with glucose showed glucose consumption and lactate production to be close to normal (Table 4).

PGK Activity and Red Cell Age

PGK activity was compared to two other enzymatic activities in old and young red cells. Table 5 shows that the residual activity of PGK in old cells relative to that in young cells is much lower in the patient (37%) than in the control (80%). By comparison, pyruvate kinase (PK) and glutamic-oxalo-acetic transaminase (GOT), well known for their age-dependent activities, were very close to normal.

Kinetic Properties of PGK

The kinetic properties of the partially purified PGK of the patient’s RBC (Table 6) included a normal pH optimum and normal \(K_m\) for 3-phosphoglycerate and 1,3-diphosphoglycerate. However, the \(K_m\) values for ADP and ATP were significantly elevated. The increase was higher for ATP (16 times the normal \(K_m\)) than for ADP (3 times normal). In addition, the ratio of the forward to the backward reaction was higher in the patient sample than in the control.

The PGK of the patient’s red cell exhibited thermal instability at 45°C (Fig. 3), with a loss of about 80% of its activity in 30 min. Similar thermostability was found in muscle extracts of the patient.

Electrophoretic Study

The electrophoretic pattern given by the red cell PGK at pH 8.0 (Fig. 4) exhibited one band that migrated behind that of hemoglobin. The patient’s PGK band was less anodic than that of the control. This pattern is identical in red cells, muscle, and leukocytes.
Table 6. Kinetic Properties of Red Cell PGK

<table>
<thead>
<tr>
<th>Properties</th>
<th>PGK Cretal</th>
<th>Normal PGK</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH Optimum</td>
<td>6–7.5</td>
<td>6–7.5</td>
</tr>
<tr>
<td>3-PGA (μM)</td>
<td>300</td>
<td>300–470</td>
</tr>
<tr>
<td>1,3-DPG (μM)</td>
<td>24</td>
<td>24–30</td>
</tr>
<tr>
<td>ATP (μM)</td>
<td>3,300</td>
<td>210–260</td>
</tr>
<tr>
<td>ADP (μM)</td>
<td>1,110</td>
<td>200–230</td>
</tr>
<tr>
<td>V_max (forward)</td>
<td>7.9</td>
<td>5.63–6.60</td>
</tr>
<tr>
<td>V_max (backward)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All measurements were performed as described in Materials and Methods. For the pH optimum, Tris-maleic acid buffers were used at pHs between 5 and 8.5. The apparent K_m’s were obtained graphically from standard double reciprocal Lineweaver-Burk plots. The control values in the table represent the range from five normal samples.

DISCUSSION

The medical history of this patient was one of acute exertional rhabdomyolysis with bouts of acute renal failure and was suggestive of muscular enzyme deficiency, but phosphorylase or PFK activity were not reduced, a fact that correlates with the absence of gross glycogen accumulation in muscle. Carnitine palmitoyltransferase activity was not assayed, but several features excluded its responsibility: no relation of rhabdomyolysis with fasting; no accumulation of lipids in muscle.

Considering that in cases of PFK deficiency, the enzyme activity is lacking in erythrocytes as well as in muscle, we examined the glycolytic pathway in red blood cells of the patient and found a marked decrease in PGK activity that was subsequently demonstrated in muscle, leukocytes, and platelets. This deficiency is presumably responsible for the muscular disease, a fact that had not been reported. Several patients with erythrocyte PGK deficiency have been described without mention of rhabdomyolysis but with hemolytic anemia and mental retardation. In only one case was a PGK muscular defect found that was associated with muscle atrophy and hemolytic anemia. This apparent discrepancy could probably be explained by the fact that other patients were very young or severely impeded by mental retardation and unable to perform an exhausting muscular exercise.

The fact that a deficiency in such a key enzyme of the glycolytic process induced exertional rhabdomyolysis is not surprising since muscle glycogen and blood-borne glucose contribute substantially to the energy needs induced by exercise. As in McArdle’s disease or deficiency in PFK, the cell lysis is thought to be due to a lack of ATP production. However, the modest decrease in PGK activity observed in the patient’s muscle would be unlikely to produce any limitation in the rate of ATP synthesis. Nevertheless, we must stress that the values of some other enzymatic activities were much higher in the patient’s muscle than in the controls, suggesting that the patient’s muscle cells contain newly synthetized enzymes, which...
may explain the relatively high activity of PGK found after a crisis of myoglobinuria. It is likely that the activity of this heat-unstable PGK would be further decreased if the biopsy was performed in a period of time far from an episode of myolysis. On the other hand, our patient did not exhibit hemolysis, though paradoxically, PGK deficiency appeared more severe in the red cells than in the muscle. Furthermore, we observed that the defect was less marked in cells with a nucleus, such as in muscle and leukocytes, compared to cells deprived of a nucleus, such as red cells and platelets, that cannot synthesize new PGK molecules. We must emphasize that our method of preparing the hemolysate is not different from that used by the other authors. Indeed the hemolysate contained EDTA and β-mercaptoethanol to protect PGK from oxidation. We can assume that the PGK value of our mutant does not result from an artificial lowering due to the hemolysate preparation.

PGK deficiency is X-linked, as previously demonstrated by Valentine et al.14 The genetic transmission of this defect in our patient's family is in accordance with this fact. Thus, the patient's mother and his two daughters display partial red cell PGK deficiency, whereas the patient's father shows normal red cell PGK activity. The differences between the values of the mother and those of the daughters could be compared to those found in individuals with heterozygous glucose-6-phosphate dehydrogenase deficiency and are consistent with the existence of two separate red cell populations (enzyme deficient and normal cells), as previously suggested in PGK deficiency14 and as predicted by the X-inactivation hypothesis.40,41 The heterozygotes of our family did not exhibit any clinical symptoms, thereby resembling other heterozygotes for PGK and glucose-6-phosphate dehydrogenase deficiencies.

By contrast, our patient has a marked defect. Such a low red cell PGK activity should have severe consequences for red cell glycolysis, since PGK is one of the enzymes that provide the ATP necessary for the maintenance of the cell. The reason why the red cells of our patient are not impaired by the enzymatic defect is difficult to understand. Similar absence of hemolysis in one case of red cell PGK deficiency has been previously reported by Krietsch et al.23 in a large kindred family, but their red cell PGK defect was less severe than that of our patient. In the family described by Krietsch, the deficient subjects displayed 20% of PGK activity in their red cells and no clinical manifestation was described. To the contrary, in three other cases of PGK deficiency,13,17,18 residual PGK activities of 23%, 30%, 42%, respectively, were found associated with hemolytic anemia. It must be observed that the red cells of our patient exhibited normal levels of ATP, little increase in 2,3-DPG, normal glucose consumption, and lactate production. These data demonstrate that the defect leads to a mild perturbation in red cell glycolysis. This fact is especially surprising because the very low erythrocyte PGK activity is further impaired by elevated nucleotide kinetic constants and thermal instability. However, we must emphasize that the affinity of the patient's PGK is more decreased for ATP than for ADP, which probably promotes the reversible reaction of PGK towards lactate formation and corroborates the finding that the ratio of the forward to the backward reaction is higher in the patient's than in the control hemolysate. We must emphasize that the ratio found by Krietsch et al.24 in normal controls is much lower than that found by us. This apparent discrepancy is probably due to the difference between the techniques used in evaluation of the forward reaction of PGK. By using their procedure, we have obtained control values of 2.5–2.9, which are close to that found by Krietsch et al. (2.87) for the ratio of the forward/backward reaction of PGK. In this case, our mutant displayed a ratio of 3.1, which is again higher than that of the control. Anyhow, the relatively "favorable" ratio led us to think that the red cell PGK defect of our patient is less severe than it appears. Indeed, the evaluation in hemolysate has been made by the backward reaction, which is 2 or 3 times lower (depending on whether our technique or Krietsch's is used) than the forward, which is physiologically operating. Under these conditions, the value of PGK activity of the patient's red cells would be at least 11 U/g Hb. We can postulate that this value is not rate-limiting and is probably higher than that of hexokinase (0.84 U/g Hb) in the patient's red cells, though we must recall that, in vivo, PGK operates below substrate saturation concentration.

In conclusion, this case of recurring rhabdomyolysis seems related to a genetically transmitted lack of PGK activity. The way in which the disease has been demonstrated (analysis of red blood cell enzymes, despite the lack of hemolysis) offers a way of finding the responsible enzyme in recurrent rhabdomyolysis from an unknown cause. The various modifications in the kinetic characteristics of the patient's PGK are consistent with the suggestion that this case of a PGK defect is a new one, differing distinctly from the known PGK variants. We believe that the origin of the defect may lie in a
mutation of the structural gene. The electrophoretic pattern affords additional evidence for the existence of this mutation. Further investigations, such as utilization of specific antibodies and structural analysis, are required for the further characterization of this mutation.

REFERENCES

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