Trehalose Can Be Used as a Parenteral Saccharide Source in Rabbits

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ABSTRACT  Trehalose is a saccharide that possesses no reducing group and so has possible use in parenteral nutrition, especially because it can be stored with amino acids without undergoing the Maillard reaction. To evaluate this possibility, a series of experiments were conducted. The activity of trehalase, an enzyme that metabolizes trehalose to glucose, was measured in rabbit serum and kidney. Conversion of trehalose to glucose and excretion of trehalose in the urine were measured in rabbits administered 10% trehalose intravenously. The effects on nutritional indices as indicators of its use as an energy source were also measured in rabbits infused with 8.23 g·kg⁻¹·d⁻¹ (4.12 g·kg⁻¹ on d 1) of trehalose for 5 d. Trehalase activity resembled maltase activity, both being high in the renal cortex (2.04 ± 0.71 and 2.93 ± 0.26 μmol·g⁻¹·min⁻¹, respectively), weak in the medulla, and undetectable in the serum. Serum glucose and insulin concentrations were increased significantly by trehalose infusion. Significant elevations were observed in serum glucose but not insulin levels by maltose infusion. On the other hand, urinary excretion of trehalose (1.1 ± 2.1% of dose) was significantly lower than that of maltose (10.1 ± 4.9% of dose). Similar effects of trehalose and maltose infusions as seen in normal rabbits occurred in rabbits with alloxan diabetes (urinary excretion rate, 3.8 ± 3.0% of the infused trehalose dose and 35.6 ± 9.7% of the infused maltose dose). Nitrogen balance was positive in the trehalose- and glucose-infused normal rabbits with significant difference from the control group infused with saline, suggesting that trehalose was used as an energy source. These results suggest that trehalose has the potential for use as a saccharide source for parenteral nutrition.


KEY WORDS:  ● rabbits     ● trehalase     ● trehalose     ● serum glucose     ● nitrogen-balance

Mono- or disaccharides such as glucose, fructose, xylitol, sorbitol and maltose are used as parenteral carbohydrate sources during periods when oral feeding is not possible due to an operation or for other reasons (Tahara et al. 1990). In parenteral nutritional supplementation, saccharides are usually infused with amino acids, electrolytes and vitamins. Because these saccharides possess reducing groups, they cause a brown discoloration as a result of Maillard reaction when mixed with amino acids for a long time (Fry and Stegink 1982). To prevent this problem, some methods such as storing the saccharides and amino acids in different containers, acetylating the most reactive amino acids (Mukai et al. 1980) and lowering the pH of the solvent (Mori et al. 1993) were developed. However, these procedures create other problems such as adding unnecessary work and inducing angialgia due to the nonphysiological properties of the solutions (Fujiyawa et al. 1996, Hasegawa et al. 1995, Ozaki et al. 1995). These problems have been considerably alleviated by methods in which the amino acids and saccharides are mixed together in a dual-chamber bag system (Mori et al. 1991). However, it would be of tremendous value if a new saccharide could be dissolved with amino acids in the same solution without resorting to any special treatment.

Trehalose is a nonreducing disaccharide composed of two D-glucose molecules, which we consume daily, mainly from mushrooms, algae, crustaceans and yeast (Sugimoto 1995). A method of inexpensively producing large amounts of trehalose was recently established (Sugimoto 1995), which is now possible to use on a commercial level. Due to its ability to maintain proteins and nucleic acids stable in cold or dry environments, trehalose recently was used as a stabilizer in foods (Rosser 1991), drugs and cosmetics (Kawano 1995). In addition, trehalose can be mixed in a single preparation with other nutrients such as amino acids and electrolytes, and does not participate in Maillard reactions when mixed with amino acids since the molecules do not contain reducing groups. If trehalose were adequately used in the body, it would be of great value as a new saccharide source for parenteral nutrition.

However, little is known about the use of trehalose as a saccharide energy source. Some reports exist concerning the presence of trehalose in organs such as the small intestine and kidney of animals and humans (Dahlqvist 1960, Ruf et al. 1990, Sasai-Takedatsu et al. 1996), and about racial differences in absorption of trehalose caused by deficiency in trehalase when ingested orally (Ushi-jima et al. 1995a, 1995b). Following intravenous injection of trehalose to rabbits, no trehalose was detected in urine until the concentration of trehalose in blood exceeded 1.59 mmol/L (Riby et al. 1990).
No information is available concerning utilization for energy of intravenously infused trehalose in spite of its properties as a possible element of parenteral solutions. We therefore conducted a study in rabbits to determine whether intravenously infused trehalose is hydrolyzed to glucose and utilized as a saccharide energy source. We first measured the activity of trehalase, the enzyme that catalyzes the hydrolysis of trehalose, in serum and kidney, the latter of which is considered to be the major organ for the metabolism of maltose, another disaccharide (Fujii et al. 1972), to evaluate possible use of trehalose. We then investigated the extent to which trehalose is converted to glucose when intravenously infused and the amount excreted in urine in comparison with maltose. We also investigated trehalose in rabbits with alloxan diabetes in which glucose tolerance is reduced. Having confirmed sufficient hydrolysis of intravenously infused trehalose to glucose, we investigated the effects of trehalose on nutritional indices by continuous infusion with amino acids, electrolytes and vitamins to food-deprived rabbits for 5 d compared with glucose.

MATERIALS AND METHODS

The protocol complied with the guidelines for the care and use of laboratory animals at Otsuka Pharmaceutical Factory, Inc. (Nanoto, Japan).

Experiment 1. Measurement of trehalase (EC 3.2.1.28) and maltase (EC 3.2.1.20) activities in the serum and the kidney. Trehalase and maltase activities were measured by modifying the methods described by Ohneda et al. (1973). Laparotomy was performed on male Japanese White rabbits (Kitayama Labs, Osaka, Japan) under pentobarbital anesthesia (Dainippon Pharmaceutic Co., Ltd., Osaka, Japan). After collecting blood from the abdominal aorta, the kidneys were removed. The blood obtained was centrifuged at 2000 × g for 20 min, and measurements were made in serum. The kidneys were dissected on ice into cortex and medulla after being washed with physiological saline (Otsuka Pharmaceutical Factory, Inc.). A 50-fold homogenate of cortex and a 10-fold homogenate of medulla were prepared with Krebs-Ringer-bicarbonate buffer (the enzyme solutions). Trehalose (α-D-glucopyranosyl α-D-glucopyranoside; Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) and maltose (Maltose Monohydrate; Wako Pure Chemical Industries, Ltd., Osaka, Japan) were dissolved in Krebs-Ringer-bicarbonate buffer to make 0.2 mol/L solutions (the substrate solutions). After preincubating 0.5 ml of each of the enzyme solution and the substrate solution at 37°C for 10 min, they were mixed and incubated at 37°C for an additional 60 min. Following incubation, the mixtures were chilled on ice and centrifuged at 2000 × g for 10 min, and the glucose in the supernatant was measured with DRI-CHEM5500 (Fuji Film, Tokyo, Japan). The amounts of glucose produced per liter serum or 1 g tissue were calculated as an index of the enzyme activity.

Experiment 2. Assessment of utilization of trehalose during 90-min continuous infusion in rabbits with alloxan diabetes. Alloxan diabetes was induced by injecting 10- to 13-wk-old male Japanese White rabbits (Kitayama Labs) with 2 ml/kg of 100 g/L alloxan (Sigma Chemical Co., St. Louis, MO) via an auricular vein. From the following evening, the rabbits were individually housed in metabolic cages, and deprived of food until the end of experiment. Two days after alloxan injection, a blood sample was collected from an auricular vein, and serum glucose was determined. The rabbits were then divided into three groups so that the serum glucose concentrations in each group would be the same. The groups were then continuously infused with either 100 g/L trehalose solution, 100 g/L maltose solution or saline (control) for 90 min via an auricular vein at a rate of 6.7 mL · kg⁻¹ · h⁻¹ with an infusion pump. Before the start of the infusion and 90 and 180 min after the start of the infusion, a blood sample was collected and treated as in Experiment 1. Urine was also collected and treated as in Experiment 2.

Experiment 3. Assessment of utilization of trehalose during 90-min continuous infusion in rabbits with alloxan diabetes. Alloxan diabetes was induced by injecting 10- to 13-wk-old male Japanese White rabbits (Kitayama Labs) with 2 ml/kg of 100 g/L alloxan (Sigma Chemical Co., St. Louis, MO) via an auricular vein. From the following evening, the rabbits were individually housed in metabolic cages, and deprived of food until the end of experiment. Two days after alloxan injection, a blood sample was collected from an auricular vein, and serum glucose was determined. The rabbits were then divided into three groups so that the serum glucose concentrations in each group would be the same. The groups were then continuously infused with either 100 g/L trehalose solution, 100 g/L maltose solution or saline (control) for 90 min via an auricular vein at a rate of 6.7 mL · kg⁻¹ · h⁻¹ with an infusion pump. Before the start of the infusion and 90 and 180 min after the start of the infusion, a blood sample was collected and treated as in Experiment 1. Urine was also collected and treated as in Experiment 2.

Experiment 4. Effect of trehalose on nutritional indices during 5-d continuous infusion in normal rabbits. Male Japanese White rabbits (10–13 wk of age; Kitayama Labs) were individually housed in metabolic cages. After overnight food deprivation, the rabbits were continuously infused with either 100 g/L trehalose solution, 100 g/L maltose solution, 50 g/L glucose solution (prepared from 700 g/L glucose solution, Otsuka Pharmaceutical Factory, Inc.), or saline (control) via an auricular vein with an infusion pump for 90 min at a rate of 6.7 mL · kg⁻¹ · h⁻¹ (10 mL · kg⁻¹ · 90 min⁻¹) without other nutrients. Before the infusion, and 30, 60, 90, 120 and 180 min after the start of the infusion, 3 mL of blood samples were collected from the auricular artery on the opposite side, and the serum was isolated and frozen at −80°C until measurement. Urine was collected during the 24 h immediately after start of the infusion, during which the rabbits were food-deprived, and after measuring its volume, a sample was stored at −80°C. The concentrations of glucose (enzyme method: Banauch et al. 1975), trehalose, maltose (high performance liquid chromatography method: modified from Ribi et al. 1990), and insulin (RIA2 antibody method: Nomura et al. 1984) were measured. The urine sample was used to measure the concentration of glucose, trehalose and maltose. Urinary excretion of trehalose and maltose was calculated as percentages of the amounts administered.

<table>
<thead>
<tr>
<th>Amount infused, unit · kg⁻¹ · d⁻¹</th>
<th>Control</th>
<th>Glucose</th>
<th>Trehalose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trehalose, g</td>
<td>0</td>
<td>0</td>
<td>8.23</td>
</tr>
<tr>
<td>Glucose, g</td>
<td>0</td>
<td>8.23</td>
<td>0</td>
</tr>
<tr>
<td>Amino acids1, g</td>
<td>3.29</td>
<td>3.29</td>
<td>3.29</td>
</tr>
<tr>
<td>Electrolytes</td>
<td>3.84</td>
<td>3.84</td>
<td>3.84</td>
</tr>
<tr>
<td>Na, mmol</td>
<td>2.74</td>
<td>2.74</td>
<td>2.74</td>
</tr>
<tr>
<td>K, mmol</td>
<td>3.84</td>
<td>3.84</td>
<td>3.84</td>
</tr>
<tr>
<td>Cl, mmol</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Ca, mmol</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Mg, mmol</td>
<td>0.46</td>
<td>0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>Pp, mmol</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Multivitamins2, mL</td>
<td>98.7</td>
<td>98.7</td>
<td>98.7</td>
</tr>
<tr>
<td>Total volume, mL</td>
<td>55</td>
<td>193</td>
<td>193</td>
</tr>
<tr>
<td>Total energy, kJ</td>
<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>Total nitrogen, g</td>
<td>63.3</td>
<td>63.3</td>
<td>63.3</td>
</tr>
</tbody>
</table>

1 The composition was as follows (gL): L-leucine, 14; L-iso-leucine, 8; L-valine, 10.5; L-threonine, 5.7; L-tryptophan, 2; L-methionine, 3.9; L-phenylalanine, 7; L-cystine, 1; L-tyrosine, 0.5; L-arginine, 10.5; L-histidine, 5; L-alanine, 8; L-proline, 5; L-serine, 3; L-glycine, 5.9; L-aspartic acid, 1; L-glutamic acid, 1.

2 The composition was as follows (mg/L): thiamine HCl, 0.975; riboflavin, 1.15; pyridoxine HCl, 1.225; vitamin B-12, 0.00125; ascorbic acid, 25; D-biotin, 0.015; folic acid, 0.1; panthenol, 3.5; nicotinic acid amid, 10; vitamin A, 825 (RE); cholecalciferol, 0.00125; tocopheryl acetate, 2.5; vitamin K, 0.5.

3 NPE/N: nonprotein energy/g nitrogen.
in the trehalose- and glucose-infused groups but no saccharide was administered served as the control. After the start of administration, urine volume was measured every 24 h except for the first 2 d, where all urine was collected together, and a sample was stored at −80°C for quantitative determinations. On d 3 after start of the infusion, pentobarbital (40 mg/kg) was administered by catheter after which the animals were weighed, blood was collected from the aorta, and organs (liver, kidneys and gastrocnemius muscle) were removed and weighed. Whole blood was used to measure lactic acid (lactate oxidase method: Richmond 1973), high density lipoprotein-cholesterol (heparin antithrombin method: Ozawa 1985). Glucose, trehalose, maltose, urea N, 3-methylhistidine (high performance liquid chromatography method: Fujiiwara et al. 1987). Na, K, Cl, and P were measured in urine.

**Statistical analysis.** The Fisher statistical software (Nakayama Shoten Co., Ltd., Tokyo, Japan) was used for analyzing the data. The data are expressed as means ± sd. Data with unequal variance were log-transformed before analysis. The differences between two groups were tested for significant difference by Student’s paired t-test (Expt. 1) or unpaired t-test (Expts. 2 and 3). The differences against pre-values or among groups were tested for significance by Dunnett’s t-test (Expts. 2 and 3) or Tukey-Kramer test, respectively, following one-way analysis of variance (Expts. 2 and 3). Differences were considered significant at P < 0.05.

**RESULTS**

**Experiment 1. Measurement of trehalase and maltase activities in serum and kidney.** Neither trehalase nor maltase activity was detected in the serum (Table 2). Trehalase and maltase activities were detected in the kidney, and the activities of both were more than 10-fold greater in the cortex than the medulla.

**Experiment 2. Assessment of utilization during 90-min continuous parenteral infusion in normal rabbits.** After beginning parenteral infusion of trehalose, the serum glucose concentration rose significantly and peaked at 90 min (Fig. 1A). After completion of the infusion, the serum glucose level decreased, and after 90 min had returned to its preinfusion value. Similar changes were observed in response to maltose infusion, but the proportional increase in serum glucose was less than in rabbits infused with trehalose. The dose of glucose infused was half that of trehalose and maltose. In these rabbits, the serum glucose concentration was significantly greater than baseline only at 60 min after start of the infusion and decreased to the preinfusion value within 30 min after completion of the infusion. Patterns of changes in serum insulin levels due to saccharide infusion were similar to those of serum glucose, but not significant except between the control and

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**TABLE 2**

Trehalase and maltase activity in serum and kidney from rabbits (Expt. 1)^

<table>
<thead>
<tr>
<th></th>
<th>Trehalase</th>
<th>Maltase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, mmol·L⁻¹·min⁻¹</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kidney, μmol·g⁻¹·min⁻¹</td>
<td>0.15 ± 0.13b</td>
<td>0.22 ± 0.10b</td>
</tr>
<tr>
<td>Medulla</td>
<td>2.04 ± 0.71a</td>
<td>2.93 ± 0.26a</td>
</tr>
<tr>
<td>Cortex</td>
<td>3.06 ± 0.79a</td>
<td>3.93 ± 0.28a</td>
</tr>
</tbody>
</table>

^1 Values are means ± sd, n = 3. Means with no letters in common differ significantly (P < 0.05, paired t-test).

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**FIGURE 1** Serum glucose (A) and insulin (B) concentrations in food-deprived normal rabbits infused with either saline (control), 100 g/L trehalose, 100 g/L maltose or 50 g/L glucose for 90 min (Expt. 2). Values are means ± sd, n = 4. *Significantly different from prevalue (P < 0.05, Dunnett’s test). Means with no letters in common at the same time points differ significantly (P < 0.05, Tukey-Kramer test).
Means with no letters in common differ significantly (P < 0.05). The weights of the gas-
trehalose was less than 1% of the total dose infused (data not shown). Urinary excretion of
controls except on d 2. Urinary glucose excretion in the trehalose-
rabbits did not urinate on d 1 after the infusion, measurements
Continuous Infusion in Normal Rabbits.
lose and maltose differed significantly (P < 0.05). In rabbits
There was no difference in glucose excretion between treha-
llose or maltose was infused (Fig. 1B). The serum trehalose con-
centration was lower than maltose concentration when trehalose
was infused than when saline or glucose was infused (Fig. 2).
There was no difference in glucose excretion between treha-
llose- and maltose-infused rabbits and among rabbits infused
with saline, glucose and trehalose. Urinary excretion of treha-
llose and maltose differed significantly (P < 0.05). In rabbits
infused with saline, 10.1 ± 4.9% of the dose was excreted in
the urine, compared with 1.1 ± 2.1% in those infused with
trehalose.

Experiment 3. Assessment of utilization of trehalose during 90-min continuous infusion in rabbits with alloxan dia-
betes. Despite overnight food-deprivation, serum glucose concentrations remained high 2 d after the alloxan injection
(Fig. 3A). Serum glucose levels rose further as a result of continuous infusion of trehalose or maltose, but the glucose
levels were not significantly different between the trehalose-
and maltose-infused groups. The elevated serum glucose con-
centrations in both groups persisted after completion of ad-
ministration for at least 90 min. There were no significant
differences in serum insulin levels among groups (Fig. 3B).

Urinary excretion of glucose was not significantly different
among groups. Urinary excretion of trehalose and maltose was
3.8 ± 3.0% and 35.6 ± 9.7%, respectively, of their respective
doses, and the difference was significant (P < 0.05).

Experiment 4. Effect on Nutritional Indices During 5-d Continuous Infusion in Normal Rabbits. Since many of the
rabbits did not urinate on d 1 after the infusion, measurements
were made on d 2. Urinary glucose excretion in the trehalose-
infused group did not differ from that in the glucose-infused
group, with neither being significantly different from the con-
trol group except on d 2 in the glucose group when it was
significantly greater (data not shown). Urinary excretion of
trehalose was less than 1% of the total dose infused (data not
shown).

Body weight loss did not significantly differ among the
three groups nor were there any differences in liver or
kidney weights (data not shown). The weights of the gas-
trocnenius muscle were significantly greater in the treha-
llose- and glucose-infused groups than in the control group
(9.62 ± 0.68, 9.70 ± 0.63 and 8.48 ± 0.64 g, respectively).
Cumulative nitrogen-balance (N-balance) was positive in
the trehalose- and glucose-infused rabbits with significant
difference from the control group infused with saline (Figs.
4A and B).

No significant differences were detected in insulin concen-
trations, total protein, albumin or albumin/globulin ratio (data
not shown). Blood urea nitrogen (BUN) was significantly
lower in the trehalose group than in the control group (Table
3). Although there were no significant differences among the
groups in serum triglycerides and phospholipids (data not
shown), nonesterified fatty acid concentration was signifi-
cantly lower in the trehalose-infused group than the control
group (Table 3). The levels of acetoacetic acid and β-hydroxy-
butyrate were lower in both the trehalose- and glucose-infused
groups than in the control group (Table 3). Serum Na and Cl
concentrations were higher in the trehalose-infused group
than in the glucose-infused and control groups (Table 3).
No significant differences among the groups in urinary electrolytes
and excretion of 3-methylhistidine were observed (data not
shown).

DISCUSSION
Trehalose, a disaccharide present in a variety of foods, has
no reducing group and consequently, does not cause the Maill-
lard reaction, even when mixed and stored with amino acids.
 Accordingly, it can be formulated with amino acids in a single
preparation for intravenous infusion, thus having possible use
as a new saccharide source for parenteral solutions. However,
for trehalose to be used as a saccharide source for parenteral
nutrition, it must be hydrolyzable to glucose after introduction
into the body.

To determine whether trehalose is converted to glucose in
the body, we measured the activity of the trehalose-degrading
enzyme (trehalase) in rabbits, as they are often used in disac-
charide research. No trehalase activity was detected in the
serum of rabbits, and simultaneous attempts to measure mal-
tase activity in the serum were also negative. Maltese
activity was detected in the blood of rats (Fujii et al. 1972), but
it was not to be found in the blood of rabbits and humans
(Ohneda et al. 1973). The results of our study corroborate
these findings. In contrast, trehalase activity was found in the
kidney, with activity being higher in the cortex than in the
medulla, similar to maltase (Ohneda et al. 1973). Based on
this enzyme activity in vitro, it was hypothesized that trehalose
could be used as a saccharide source for intravenous admin-
istration. To determine this, we next assessed its utilization in
vivo.

After trehalose was infused into the auricular vein of nor-
mal rabbits, the serum glucose levels rose more than when
maltose was infused. In contrast, the increase of trehalose
concentration in serum after trehalose infusion tended to be
less than when maltose was infused. Moreover, the urinary
excretion of trehalose was about 1% of dose, considerably
lower than the 10% that was excreted when maltose was
administered. For this reason, trehalose is thought to be con-
verted into glucose in the body to a greater extent than
maltose.

Maltase and trehalase activities are lower than normal in

3 Abbreviations used: BUN, blood urea nitrogen; N-balance, nitrogen-bal-
ance.
kidney brush border membranes from diabetic rabbits (Itoh et al. 1989). We studied utilization of trehalose in diabetic rabbits induced with alloxan. Although changes in serum glucose and insulin levels in diabetic rabbits receiving trehalose or maltose showed different patterns than those observed in normal rabbits, there apparently was greater conversion of trehalose compared with maltose to glucose in the diabetic rabbits. More trehalose was excreted in urine in diabetic rabbits than in normal rabbits, but whereas more than 30% of the dose of maltose was excreted in urine, less than 4% of trehalose was excreted, indicating that trehalose was converted to glucose in sufficient amounts even when glucose tolerance was reduced. However, since urinary excretion of glucose also increased to a considerable degree in diabetic rabbits compared with normal rabbits even in the trehalose group, it is necessary to consider the rate of infusion to deter-

FIGURE 3  Serum glucose (A) and insulin (B) levels in food-deprived rabbits with alloxan diabetes infused with either saline (control), 100 g/L trehalose or 100 g/L maltose for 90 min (Expt. 3). Alloxan (2 mL/kg of 100 g/L) was injected to rabbits via an auricular vein to induce diabetes. Values are means ± sd, n = 5. *Significantly different from prevalue (P < 0.05, Dunnett’s test). Means with no letters in common at the same time points differ significantly (P < 0.05, Tukey-Kramer test).

FIGURE 4  Daily (A) and cumulative (B) Nitrogen-balance (N-balance) in rabbits infused with either saline (control), trehalose or glucose for 5 d (Expt. 4). Oral intake of food and water was not allowed during the study period. Rabbits were infused with trehalose or glucose at 8.23 g · kg⁻¹ · d⁻¹ in combination with amino acids, electrolytes and vitamins. Controls were infused with saline instead of saccharide, with all other components being the same. Urine was collected every 24 h except for the first 2 d, where all urine was collected together. Nitrogen-balance was calculated by subtracting total excreted nitrogen from total infused nitrogen. Values are means ± sd, n = 5. Means with no letters in common at the same time points or in the 5-d accumulation differ significantly (P < 0.05, Tukey-Kramer test).


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**LITERATURE CITED**


