THE EFFECTS OF SUCROSE AND VARIOUS SALTS ON THE GROWTH ANDLYSOSOMAL ENZYME ACTIVITY OF L5178Y CELLS

R. J. BERNACKI AND H. B. BOSMANN
Department of Pharmacology and Toxicology, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y. 14620, U.S.A.

SUMMARY

L5178Y murine leukemic cells do not seem to be affected by sucrose in the same manner as other mammalian cells. Although there is active uptake of sucrose with no concomitant metabolism, there is no increase in vacuolation or cell size and there is only a slight increase in acid phosphatase, acid protease and β-glucuronidase activities. However, the glycosidases are significantly elevated in the presence of 0.08 M sucrose. This may be due to a specific induction of these enzymes by the sucrose not related to a general increase in lysosomes.

As the sucrose concentration is raised, a depression of the growth rate and an elevation of glycosidase activities occurs, reaching a maximum at 0.08 M sucrose. This depression of growth with a concomitant increase in generation time is primarily a result of the increased osmolarity of the medium; the same effect is observed with increased concentrations of NaCl. Although the growth effects are similar with either sucrose or NaCl, the elevation of glycosidase activity occurs only with increased concentrations of sucrose.

Sucrose-induced vacuolation with an increase in cell size is not evident in L5178Y cells; therefore this effect does not seem to be common to all mammalian cells, even though it has been reported for LS cells, Chinese hamster fibroblasts, and chick bone rudiments and in vivo for liver cells.

INTRODUCTION

The review of De Duve & Wattiaux (1966) has demonstrated and defined the biochemistry of sucrose-induced vacuolation of cells. Sucrose, when added to suspension cultures of LS cells, caused an increase in vacuolation and an increase in acid phosphatase activity (Benassi, 1968). Similarly, Chinese hamster fibroblasts (Munro, 1968) and rat liver cells (Brewer & Heath, 1964; Wattiaux, Wattiaux-De Coninck, Rutgerts & Tulkens, 1964), in the presence of sucrose, have been shown to have increased vacuoles. Furthermore, Dingle, Fell & Glauert (1969) reported that in organ cultures of bone and cartilage, vacuolation was induced by sucrose and that this increased vacuolation was associated with an increased synthesis of lysosomal enzymes.

The objects of the present investigation were to find out the effects of sucrose and other salts on the growth and lysosomal enzyme activities (De Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955) of acid phosphatase, acid protease, β-glucuronidase and the glycosidases in L5178Y cells and to determine whether L5178Y cells, a murine leukemic cell line, respond as other mammalian cells respond in the presence of sucrose.
MATERIALS AND METHODS

Cell culture

L5178Y cells (mouse lymphoma cell line) were grown in suspension culture in sealed containers in Fischer's medium with 10% horse serum and were utilized in the exponential growth phase. The starting cultures contained approximately $6 \times 10^4$ cells/ml and remained in log phase to $10^6$ cells/ml. All experiments were performed with cells in exponential growth phase. Sucrose (reagent grade) supplied by Mann, cyclamic acid (sodium salt) and saccharin (o-sulphobenzimide, sodium salt) obtained from Sigma, and sodium chloride (reagent grade) obtained from Baker, were added in various concentrations to the culture medium. Fischer's medium in powder form and horse serum were supplied by Grand Island Biological Co., Buffalo, New York; to the supplied medium, penicillin (500 units/ml) and streptomycin (0.05 mg/ml) were added.

Cell counting and sizing

Cell numbers were determined with a Coulter counter, Research Model B. Cell volumes were calculated with reference to human red blood cells and ragweed pollen (19-20 μm diameter).

Sucrose uptake

Approximately $10^7$ cells were incubated in 100 ml of complete Fischer's medium plus 250 μCi of D-[14C]sucrose (uniformly labelled, specific activity 0.34 Ci/mmol supplied by New England Nuclear Corp.) for 6 h at 37 °C. At 30, 60, 120, and 360 min, 25-ml aliquots were removed, the cells were sedimented at 1000 g for 5 min, broken in 1 ml of 0.1% Triton X-100 with 30 strokes in a Ten Broeck homogenizer, and brought up to 5 ml with triple-distilled water. This extract was then centrifuged at 700 g for 10 min to remove cell debris. An 0.2-ml aliquot was plated on a glass fibre filter disk for counting in a PPO-POPOP-toluene counting fluid in a Nuclear Chicago liquid scintillation counter. The remaining extract was then lyophilized and brought up to 50 ml in ethanol for chromatography. Authentic standards ([14C]sucrose, glucose, fructose, and mannose) were simultaneously chromatographed with the extract on Whatman 3 MM paper in n-butanol:pyridine:0.1 N HCl (5:3:2, by volume) for 42 h by descending chromatography. The standards were stained by the alkaline silver nitrate procedure and the resulting radioactive strips were cut and the radioactivity determined by liquid scintillation counting. This procedure was used to determine if the incorporated [14C]sucrose was metabolized by the L5178Y cells to glucose and fructose.

Enzyme activity

The amount of glycosidase, acid phosphatase and β-glucuronidase activity was determined using p-nitrophenyl derivatives as substrates (Bosmann & Bernacki, 1970; Bosmann & Meritt, 1969; Bosmann, 1969), and acid protease activity was assayed using haemoglobin as a substrate (Anson, 1939; Barrett, 1967). At time zero (immediately after addition of sucrose to a final concentration of 0.08 M), a 200-ml aliquot of cells (approximately $10^7$ cells) was removed from both the culture containing the added sucrose and the original culture, which was previously brought up to the same volume in order to assure that the number of cells per ml would be the same in each culture. The cells were then sedimented by centrifugation at 1000 g for 5 min, washed twice with 0.9% NaCl, and the washed cellular pellet was resuspended in 3 ml of 0.1% Triton X-100. It was then homogenized with 30 strokes in a Ten Broeck homogenizer at 4 °C. The resulting extract was used as the enzyme source for the glycosidase, acid protease, acid phosphatase and β-glucuronidase determinations (Bosmann & Bernacki, 1970).

In each case 50 μl of the cell extracts were incubated with 6.0 μmol of the p-nitrophenyl derivative (the final volume was 1.050 ml, 0.05 M in citrate buffer, adjusted to pH 4.3) for 1 h at 37 °C. The reaction was terminated by the addition of 2 ml of 0.4 M glycine-NaOH buffer, pH 10.5. The reaction mixtures were centrifuged at 5000 g for 5 min and the optical density of the released p-nitrophenol in the supernatant was measured at 420 nm. From these data and a
Sucrose effects on L5178Y cells

**RESULTS**

**Effect of sucrose and various salts on growth of L5178Y cells**

The effect of various sucrose concentrations on the growth of L5178Y cells (Fig. 1) is found to be related to the final osmolarity of the medium. As the sucrose concentration is increased from 0.05 to 0.1 M, a longer lag phase is observed with a resulting increase in generation time. In log phase the control culture had a doubling time of 11.5 h while the 0.05 M sucrose culture had a doubling time of 14 h, the 0.08 M sucrose culture of 19 h, and the 0.1 M sucrose culture 22 h. A concentration of 0.2 M sucrose is toxic to the L5178Y cells and cell lysis occurs almost immediately. For comparison, cell cultures were grown in 0.025 and 0.05 M NaCl (Fig. 2). The results are very similar to those seen with sucrose. As the concentration of salt is increased, a decrease in growth is observed with an 18-h doubling time for 0.025 M NaCl and a zero growth rate for 0.05 M NaCl. The effects of sodium saccharin and sodium cyclamate were also observed; each salt was toxic at 0.025 M even though the pH of the medium was
adjusted to normal. With 0.025 M sodium saccharin or sodium cyclamate growth of cultures was completely inhibited whereas the same concentration of NaCl only depressed growth (Fig. 2).

Sucrose uptake and metabolism

Sucrose uptake (Fig. 3) was followed with uniformly labelled $[^{14}C]$sucrose for 6 h. The results show rapid incorporation during the first hour with a gradual decrease in uptake occurring after 2 h and a levelling off at 6 h, with any further increases in uptake presumably due to increase in cell number. Invertase activity (Fig. 4) was found to be absent when determined by chromatography of the cellular extracts obtained from the sucrose uptake study. Each extract (30, 60, 120, and 360 min incubation) chromatographed had a single peak of radioactivity, whether determined with a strip scanner or a liquid scintillation counter. This peak had an $R_F$ value of
Sucrose effects on L5178Y cells

approximately 0.74, which corresponded exactly to that of the standard uniformly labelled [\( ^{14}\text{C} \)] sucrose (Table 1). No labelled material was found in the area of glucose or fructose (Fig. 4).

Fig. 3. Sucrose uptake. Approximately \( 10^7 \) cells were incubated at 37 °C with 250 \( \mu \text{Ci} \) of \([U-^{14}\text{C}] \) sucrose; aliquots were removed for scintillation counting at 30, 60, 120 and 360 min, and the remaining samples were used for chromatography (see Fig. 4).

Fig. 4. Chromatography of the uptake of \([^{14}\text{C}] \) sucrose. \( L_5178Y \) cells were incubated with 250 \( \mu \text{Ci} \) of \([U-^{14}\text{C}] \) sucrose for 6 h. Aliquots were removed at 30, 60, 120 and 360 min for scintillation counting (see Fig. 3) and the remaining extract was used for chromatography. Authentic standards were run simultaneously and their \( R_f \) values (Table 1) are plotted against the \( R_f \) values obtained from \([^{14}\text{C}] \) sucrose by counting the radioactive strips either on a strip counter or a scintillation counter. None of the \([^{14}\text{C}] \) sucrose taken up by the \( L_5178Y \) cells was recovered as glucose or fructose.

Table 1. \( R_f \) values for chromatography on Whatman 3MM paper

<table>
<thead>
<tr>
<th>Sugar</th>
<th>( R_f ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose</td>
<td>1.000</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.830</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.940</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.743</td>
</tr>
</tbody>
</table>

* \( R_f \) values are based on mannose arbitrarily set to 1.000.

Effects of sucrose on lysosomal enzyme levels

Glycosidase, acid phosphatase, acid protease and \( \beta \)-glucuronidase activities were determined on logarithmic \( L_5178Y \) cells and are the same as the control activities or zero-time activities shown in Table 2. Activities were monitored over a period of 48 h.
on all the various sucrose concentrations and NaCl concentrations studied. As the sucrose concentrations were increased to 0.08 M the glycosidases showed the greatest significant increase; above 0.08 M, toxicity was evident and glycosidase activity levels became erratic. None of the enzyme activities changed significantly with low doses of NaCl (0.025, 0.03 M) as compared with the controls; cultures grown in 0.025 M sodium saccharin, 0.025 M sodium cyclamate, and 0.05 M NaCl were not assayed since no viable cells remained. Assays were performed at 24 and 48 h after the addition of the sucrose, but no significant changes occurred until 48 h after the addition of sucrose. The largest effects were the significant increases in the glycosidase activities after 48 h in 0.08 M sucrose (Table 2). Although acid phosphatase, acid protease and β-glucuronidase activities did increase, the increases were not as great as those of the glycosidases and were not significant at the \( P = 0.05 \) level. After 48 h the cells in 0.08 M sucrose were returned to normal medium and the glycosidase activities returned to normal range after 24 h.

### Table 2. The effect of 0.08 M sucrose after 48 h of incubation on glycosidase, acid phosphatase, β-glucuronidase and acid protease activities of L5178Y cells

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity in 0.08 M sucrose (48 h)</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control activity (zero time)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-acetyl-β-D-galactosaminidase†</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>β-galactosidase†</td>
<td>110.5</td>
<td></td>
</tr>
<tr>
<td>N-acetyl-β-D-glucosaminidase†</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>β-glucosidase†</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>α-mannosidase†</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td>β-glucuronidase†</td>
<td>315.5</td>
<td></td>
</tr>
<tr>
<td>acid phosphatase†</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>acid protease§</td>
<td>96.6</td>
<td></td>
</tr>
</tbody>
</table>

* * for the difference between the means was calculated using the Student \( t \) test and \( P \) determined from tables. Each assay was run in duplicate or triplicate, at least 8 times.
† Specific activity is expressed as nmol p-nitrophenol released/h/mg protein.
§ Specific activity is expressed as mg pronase equivalents/h/mg protein.

**Cell-sizing determinations**

Cells grown in 0.08 M sucrose, 0.03 M NaCl and control cells were sized using a model B Coulter counter in accordance with the procedures outlined in the Coulter counter manual. Since cell volumes vary in any population of cells, the mean cell volume obtained in this manner on 2 x 10^4 cells is more relevant than other procedures depicting increases in cell sizes of a few selected cells. Our results (Table 3) indicate that after 48 h of incubation in 0.08 M sucrose there was a slight decrease in cell size of magnitude similar to that found with the hypertonic 0.03 M NaCl medium.
Table 3. Comparison of volumes of cells grown in 0.08 M sucrose, 0.03 M sodium chloride and control medium, using a Coulter counter

<table>
<thead>
<tr>
<th>Culture</th>
<th>Mean cell volume ($\mu$m$^3$)</th>
<th>Diameter ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>945.2</td>
<td>12.20</td>
</tr>
<tr>
<td>0.08 M sucrose</td>
<td>850.4</td>
<td>11.76</td>
</tr>
<tr>
<td>0.03 M NaCl</td>
<td>880.9</td>
<td>11.90</td>
</tr>
</tbody>
</table>

DISCUSSION

The increase in glycosidase activities found after incubation in medium containing 0.08 M sucrose may be attributed to the stimulation or formation of lysosomes, as observed in other cells grown in the presence of sucrose. The results presented herein do not substantiate this hypothesis. No significant increases were found in the other lysosomal enzymes (acid phosphatase, acid protease or $\beta$-glucuronidase) and the increases in the glycosidase activities were not of the magnitude found for acid phosphatase in L5 cells (Benassi, 1968) or for acid protease and acid phosphatase found in rudiments (Dingle et al. 1969). Vacuolation was not evident with phase microscopy but because of the small size of the cells used (diameter 12.2 $\mu$m) the vacuoles or lysosomes may not have been apparent. No increase in mean cell volume was observed for cells grown in 0.08 M sucrose when compared to the mean cell volume of control cells.

The increases in activity found for the glycosidases varied from 22.7% for N-acetyl-$\beta$-D-glucosaminidase to 37.0% for $\alpha$-glucosidase and are significant. These increases in activity may be attributed to a more specific induction of hydrolytic enzymes geared toward the degradation of oligosaccharides, polysaccharides, disaccharides, or monosaccharide residues of glycoproteins which are induced by a non-metabolizable substrate such as sucrose. Whatever the mechanism may be (an increase in synthesis or a decrease in degradation of the glycosidases) there is an elevation in glycosidase activity which remains relatively constant after 48 h (chronic studies were performed for a period of 8 days showing no further increases in activities) and is reversible—the elevated activities return to normal after the cells are returned to a normal medium.

It is impossible to state at this time whether or not the vacuolation and elevation of lysosomal enzyme activities, which have been reported in bone cells, fibroblasts and liver cells, will be found to occur in other normal cell lines. Perhaps this phenomenon will be found to differ from cell to cell and from normal to malignant cell.

We would like to thank Mrs Gerilyn Pike, Mr Kenneth Case, and Miss Melinda Shea for valuable technical assistance.

This work was supported in part by Grant No. P529 from the American Cancer Society, Grant No. 1-P11-GM-15190 from the National Institutes of Health and Training Grant No. 5-To1-GM-00032 from the National Institutes of Health.

HBB is a Research Career Development Awardee of The National Institute of General Medical Sciences.
REFERENCES


(Received 24 August 1970)