Methylglyoxal Formation in Rat Liver Cells*

Junko Sato,† Yeu-ming Wang, and Jan van Eys

From the Department of Pediatrics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

The formation of methylglyoxal from triose phosphates was studied in dialyzed whole cell homogenates of rat liver. In the cell-free system, methylglyoxal is largely derived from dihydroxyacetone phosphate (DHAP). The addition of the cofactor of glyoxalase, reduced glutathione (GSH), to the system inhibits the accumulation of methylglyoxal from DHAP, whereas the absence of GSH promotes the accumulation of methylglyoxal regardless of the presence or absence of NAD.

To determine whether methylglyoxal synthase activity (EC 4.2.99.11) is present in the intact cell system, methylglyoxal formation from xylitol and glucose was also studied in a single cell suspension of liver of rats fasted for 48 h. Rat liver cells utilize glyceraldehyde 3-phosphate as a precursor of methylglyoxal, whereas DHAP is converted to a lesser degree, probably as a result of lack of permeability through the cell membrane. Methylglyoxal is converted to pyruvate and l-lactic acid by α-ketoaldehyde dehydrogenase activity, and to d-lactic acid formation through the glyoxalase system. The conversion and dilution of [U-14C]xylitol and [U-14C]glucose to [14C]methylglyoxal and [14C]pyruvate was quantitated in the presence and absence of NaF and diamide through the formation of the 2,4-dinitrophenylhydrazones of methylglyoxal and pyruvate. In the presence of inhibitors, the conversion of the substrates into [14C]methylglyoxal is less than 0.4% from glucose and as much as 4% from xylitol without dilution of the specific activity in either.

It is concluded that methylglyoxal formation in rat liver cells is a metabolic process.

Szent Györgyi (1, 2) proposed that methylglyoxal interacts with highly active sulfhydryl groups that may participate in regulation of cell division in tissues and that this methylglyoxal—SH complex can arrest cell division in rapidly dividing cells. Methylglyoxal and similar aldehydes compounds exert significant effects on certain cancers by reducing their ascites fluid formation and prolonging the survival time of animals bearing those tumors (3, 4). Whether enzymatic methylglyoxal formation actually occurs in mammals has been controversial for many years (5–10). The isolation of methylglyoxal synthase from the enterobacteriaceae (11, 12) confirmed that methylglyoxal can be formed enzymatically from triose phosphates. This does not, however, prove the presence of this enzyme system in higher organisms, nor does it negate the possible occurrence of nonenzymatic conversion of triosephosphates in glycolyzing tissues. Riddle and Lorenz (10) observed that methylglyoxal formation from both DHAP and D,L-glyceraldehyde is accelerated by polyvalent anions at physiological pH values. In our investigation, glyceraldehyde-3-P and DHAP were used directly as substrates for methylglyoxal formation in dialyzed homogenates of rat liver to determine the characteristics of biological conversion of the triose phosphates to methylglyoxal in the presence of cofactors.

McKinnon and Martin (13) showed that glycolytic inhibitors could not inhibit lactate formation totally, thus indicating the presence of an alternative pathway in normal leukocytes. This was also thought to occur in perfused rat liver by Jakob et al. (14).

The characteristic accumulation of triose phosphates following the infusion of xylitol in the isolated rat liver (14) suggested the possibility that the pentitol xylitol could be a precursor for the formation of methylglyoxal. Xylitol is metabolized sequentially through the glucuronic acid-xylulose pathway, the pentose phosphate shunt and finally the glycolytic pathway in rat liver.

The present report describes the data that argue for the enzymatic formation of methylglyoxal in rat liver cells from xylitol or glucose in the presence and absence of metabolic inhibitors, and corroborates the methylglyoxal formation in homogenates.

RESULTS
Enzymatic Formation of Methylglyoxal from DHAP and Glyceraldehyde-3-P, and the Effect of Various Factors on Methylglyoxal in the Rat Liver Homogenate—To prove an enzymatic catalysis of methylglyoxal formation (Table I), the homogenate was heat-denatured at 60°C for 30 min. Denatured homogenate lost completely enzymatic activity of utilizing glyceraldehyde-3-P and DHAP. Lysine appears to enhance, to a relatively small magnitude, methylglyoxal formation with DHAP more than that with glyceraldehyde-3-P either in the absence of native homogenate or in the presence of heat-denatured homogenate, whereas glyceraldehyde-3-P and DHAP were significantly transformed to methylglyoxal by the native homogenate regardless of the presence of lysine (15 min).

When homogenates were incubated with triose phosphates rapid methylglyoxal formation was seen. This was not entirely proportional to the protein concentration. There was tradi-

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734, solely to indicate this fact.

† Recipient of a predoctoral fellowship from the Clayton Foundation for Research, Houston, TX, and to whom reprint requests should be addressed.
Methylglyoxal Formation in Rat Liver Cells

The rates of methylglyoxal formation from glyceraldehyde-3-P and DHAP was calculated according to the equation: 

\[ k_{obs} = \frac{v}{P} \]

where \( v \) is nanomoles of methylglyoxal formed per h and \( P \), the initial concentration of the substrates. The data suggested a constant \( k_{obs} \) of a first order reaction with both substrates. The analyses of \( k_{obs} \) of both glyceraldehyde-3-P and DHAP revealed that DHAP reacted about 1.7 faster than glyceraldehyde-3-P to form methylglyoxal for the amount of homogenate protein employed. However, the formation of methylglyoxal in either case tends to occur less with the larger amount of protein added to the reaction mixture. Although we acknowledged that if the rate constant of the interconversion between DHAP and glyceraldehyde-3-P is much larger than that of the conversion of triose phosphates to methylglyoxal, the kinetic constants of the two different substrates would not demonstrate a large difference, yet the possibility that DHAP was a direct substrate with \( k_{obs} \) 1.7 higher than that for glyceraldehyde-3-P seemed likely, because glyceraldehyde-3-P is primarily transformed to DHAP by triose isomerase. To further define the nature of the factor that influences the flow of methylglyoxal metabolism in the whole cell system, we compared the effect of the addition of GSH, NAD, phosphate ion, and imidazole to the dialyzed homogenate which retained only half of GSH originally present in the nondialyzed homogenates (Table III). About 90% of glyceraldehyde-3-P was recovered as DHAP after a 60-min incubation of the homogenate with 6.5 \( \mu \)mol of glyceraldehyde-3-P/mg of protein at 0°C. The temperature of the incubation mixture was then raised to 37°C after the addition of the various cofactors. A marked accumulation of methylglyoxal is evident

**Fig. 1.** Methylglyoxal biosynthesis from DHAP and glyceraldehyde 3-phosphate. a, effect of protein concentration on the biosynthesis of methylglyoxal; b, effect of substrate concentration on the biosynthesis of methylglyoxal. The reaction mixture (2 ml) contained 12 \( \mu \)mol of sodium phosphate buffer (pH 7.4), 280 \( \mu \)mol of KCl, 400 \( \mu \)mol of ATP, and 2.5 \( \mu \)mol of glyceraldehyde-3-P and 169 \( \mu \)mol of homogenate, or 3.0 \( \mu \)mol of DHAP and 48 \( \mu \)mol of homogenate and 30 \( \mu \)mol of lysine if indicated. The mixture was incubated at 37°C for 2 h.

**Table I**

Methylglyoxal formation in rat liver homogenates

Denatured homogenate was prepared by heating the native homogenate at 60°C for 30 min. The reaction mixture (2 ml) each contained: 12 \( \mu \)mol of sodium phosphate buffer (pH 7.4), 280 \( \mu \)mol of KCl, 400 \( \mu \)mol of ATP, 2.5 \( \mu \)mol of glyceraldehyde-3-P and 169 \( \mu \)mol of homogenate, or 0.3 \( \mu \)mol of DHAP and 48 \( \mu \)mol of homogenate and 30 \( \mu \)mol of lysine if indicated. The mixture was incubated at 37°C for 2 h.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Lysine (15 mm)</th>
<th>Methylglyoxal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glyceraldehyde-3-P</td>
<td>DHAP</td>
</tr>
<tr>
<td></td>
<td>( \mu )mol/mg protein</td>
<td>( \mu )mol/mg protein</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>0.42</td>
</tr>
<tr>
<td>Native</td>
<td>+</td>
<td>0.51</td>
</tr>
<tr>
<td>Homogenate</td>
<td>+</td>
<td>2.15</td>
</tr>
<tr>
<td>Denatured</td>
<td>+</td>
<td>2.48</td>
</tr>
<tr>
<td>Homogenate</td>
<td>+</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.72</td>
</tr>
</tbody>
</table>

**Table II**

Biosynthesis of methylglyoxal from glyceraldehyde-3-P and DHAP in rat liver homogenates

Approximately 5 \( \mu \)mol of glyceraldehyde-3-P or 3 \( \mu \)mol of DHAP were incubated for 60 min at 37°C with different amounts of rat liver homogenates (35 to 170 \( \mu \)g) which were previously dialyzed at 4°C for 5 h against 40 mm imidazole buffer. The reaction mixture (2 ml) each contained: 12 \( \mu \)mol of sodium phosphate buffer (pH 7.4), 280 \( \mu \)mol of KCl, 400 \( \mu \)mol of ATP, and 5 \( \mu \)mol of glyceraldehyde-3-P or 3 \( \mu \)mol of DHAP.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Methylglyoxal formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>nmol</td>
</tr>
<tr>
<td></td>
<td>( k_{obs} ) nmol</td>
</tr>
<tr>
<td></td>
<td>( k_{obs} ) nmol</td>
</tr>
<tr>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>50</td>
<td>220</td>
</tr>
<tr>
<td>85</td>
<td>260</td>
</tr>
<tr>
<td>170</td>
<td>250</td>
</tr>
</tbody>
</table>

\[ k_{obs} = \text{nmol of methylglyoxal formed}/h/nmol of initial concentration of the given substrates. \]

**Table III**

Methylglyoxal formation from DHAP in rat liver homogenates

Rat liver homogenates containing 14 mg of protein in 20 ml of 40 mm imidazole buffer at pH 7.0 were incubated with 90 \( \mu \)mol of glyceraldehyde-3-P at 0°C for 60 min. After incubation, 80 \( \mu \)mol of DHAP, 0.015 \( \mu \)mol of methylglyoxal and 0.15 \( \mu \)mol of D-lactate were formed and 3 \( \mu \)mol of glyceraldehyde-3-P remained. The resulting homogenate was divided into separate tubes with a final value of 2.4 ml, to study the effect of the addition of phosphate (12.8 \( \mu \)mol), NAD (4.0 \( \mu \)mol), GSH (3.2 \( \mu \)mol), or combinations of such additives. Subsequent incubation was at 37°C for the times indicated. The DHAP remaining and methylglyoxal or D-lactate formed were measured.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Metabolites</th>
<th>0 min</th>
<th>15 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>I</td>
<td>P</td>
<td>I</td>
</tr>
<tr>
<td>Control</td>
<td>3.62</td>
<td>2.80</td>
<td>1.45</td>
<td>1.56</td>
</tr>
<tr>
<td>NAD</td>
<td>0.23</td>
<td>0.25</td>
<td>0.11</td>
<td>0.25</td>
</tr>
<tr>
<td>GSH</td>
<td>0.81</td>
<td>0.94</td>
<td>2.64</td>
<td>3.18</td>
</tr>
<tr>
<td>NAD &amp; GSH</td>
<td>0.30</td>
<td>0.30</td>
<td>0.20</td>
<td>0.49</td>
</tr>
<tr>
<td>Control</td>
<td>0.09</td>
<td>0.16</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>NAD</td>
<td>0.26</td>
<td>0.11</td>
<td>0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>GSH</td>
<td>0.72</td>
<td>0.84</td>
<td>1.21</td>
<td>1.30</td>
</tr>
</tbody>
</table>

*P = phosphate buffer; I = imidazole buffer. A Values indicate the amount of metabolites shown before initiation of incubation at 37°C.
in the two fractions, one containing NAD and the other without factors added. On the other hand, the solution in the presence of GSH, regardless of added NAD, exhibited none or very little methylglyoxal formation, and a 2.5- to 3.5-fold rise of \( \Delta \)-lactic acid. The addition of phosphate and NAD promoted the utilization of glyceraldehyde-3-P. The level of \( \Delta \)-lactic acid was consistently low in the presence of phosphate relative to that formed in imidazole buffer.

**DISCUSSION**

The recognition of the methylglyoxal pathway of carbohydrate metabolism (15) has brought considerable attention to a new concept of the regulatory mechanism of glycolysis in cells. Recent isolation of methylglyoxal synthase from several different bacterial sources (11, 12, 15) prompted us to evaluate the methylglyoxal pathway in mammals. We investigated the biological formation of methylglyoxal, the availability of substrate for methylglyoxal synthase, and the factors influencing triose phosphate metabolism in the cell-free system.

Hopper and Cooper (15) have shown that phosphate may participate in regulation of the methylglyoxal pathway and glycolysis. In the studies reported here the comparison of phosphate and imidazole was made to determine the differential effect on the methylglyoxal pathway. The production of \( \Delta \)-lactic acid as the end product of methylglyoxal pathway was noticeably less in phosphate buffer than it was in imidazole buffer. The decreased formation of \( \Delta \)-lactic acid may correlate with an inhibitory effect of phosphate on methylglyoxal synthase as suggested by Hopper and Cooper (15). Alternatively, phosphate could be utilized as a stimulatory factor for glyceraldehyde-3-P dehydrogenase followed by an enhanced glycolysis, thus resulting in a low supply of the substrate for the methylglyoxal pathway. The rate constant of DHAP transformation to methylglyoxal, obtained in the presence of phosphate, is 1.7 higher than that of glyceraldehyde-3-P, suggesting glyceraldehyde-3-P transformation to methylglyoxal through DHAP. This finding is in agreement with the experiment (Table III) in which glyceraldehyde-3-P was converted gradually to DHAP at 0°C to the extent of the equilibrium prior to the accumulation of methylglyoxal. Under our experimental conditions, we cannot obtain any further evidence to support the implication that glyceraldehyde-3-P is not a substrate in the methylglyoxal pathway. It has been believed up till now that trioses or triose phosphates are transformed nonenzymatically to methylglyoxal (5, 6, 10); others believe (8, 9) that such conversion in the presence of tissue is enzymatic. As we discussed under "Results," the assay mixture containing glyceraldehyde-3-P, without the homogenates gave rise to only a small amount of methylglyoxal while methylglyoxal formation from DHAP was not observed.

It is confirmed that methylglyoxal is produced primarily in enzyme catalyzing process even in the presence of lysine. Nonenzymatic methylglyoxal formation in glycolyzing tissue has been suggested to occur by Riddle and Lorenz (10). It has been implied that triose phosphate liberates phosphate ion prior to the formation of methylglyoxal (5, 10). In their study, dihydroxyacetone or glyceraldehyde was incubated at 40°C over a period of 20 h in order to observe a substantial effect of nonenzymatic catalysis on methylglyoxal formation. This phenomenon should not occur under physiological conditions. Bonsignore et al. (6) postulated the possibility of the transformation of glyceraldehyde-3-P to methylglyoxal by nonenzymatic action of lysine at 20.0 \( \text{mm} \) or more. However, such concentrations of lysine are physiologically unlikely, and the interaction of lysine with glyceraldehyde-3-P was far less than that reported by Bonsignore et al. (7).

Our study in the whole cell system suggested that intact rat liver cells appear to possess very active methylglyoxal synthase activity. The observation that \( \alpha \)-ketoaldehyde dehydrogenase of intact rat liver cells catalyzes the conversion of methylglyoxal to pyruvate and L-lactic acid accumulation from methylglyoxal after 90-min incubation period indicated that a methylglyoxal pathway via pyruvate to L-lactic acid exists in rats.

All the cells studied in the presence of either glucose or xylitol with the addition of metabolic inhibitors, increases in methylglyoxal formation in both were observed. Lewis et al. (16), after similar studies without inhibitors, suggested that the methylglyoxal pathway in tumors was insignificant. They considered the occurrence of trace amounts of methylglyoxal an artifact since the radioactivity was nonspecifically absorbed by methylglyoxal. Our studies suggest that nonspecific radioactivity could not be associated with methylglyoxal or pyruvate used as carriers during the isolation of methylglyoxal and pyruvate from both substrates. The per cent conversion of methylglyoxal and pyruvate from both substrates should reflect the activity of the methylglyoxal pathway under the conditions employed. The most striking feature is the significant increase of methylglyoxal from xylitol in the presence of NaF and diamide. The possibility does exist that after 1 h incubation more GSH regenerates with glucose as substrate than that with xylitol. On the other hand, with two carbohydrates in the absence of the inhibitors, conversion to either methylglyoxal or pyruvate was fairly low, although xylitol gave rise to slightly higher rate of flux. The low accumulation of methylglyoxal could be attributed, in part, to a highly active glyoxalase activity with a consequent increase in \( \Delta \)-lactic acid level. Further, the pyruvate level was affected by the inhibitors in the same manner as the methylglyoxal level. In the case without the inhibitors, the route of methylglyoxal to pyruvate was low compared to that of methylglyoxal to \( \Delta \)-lactic acid.

After completion of our experiments, Grazzi and Trombetta (17) observed the formation of a methylglyoxal-inorganic phosphate enzyme complex with subsequent release of methylglyoxal on incubation of fructose bisphosphate aldolase (EC 4.1.2.13) with dihydroxyacetone phosphate. In addition Fodor et al. (18) reported the isolation of methylglyoxal from beef liver. Although these authors did not confirm that protein-bound methylglyoxal is truly a biological product, our experiments support the biosynthesis of methylglyoxal as a natural constituent of liver as implied by Fodor et al. (18).

**REFERENCES**


\(^{2}\) J. Sato, unpublished results.
Methylglyoxal Formation in Rat Liver Cells

Jung-Soo, Eun-Hong Bang, and Hyeon Yong Kim

MATERIALS AND METHODS

Animals and Chemicals:

Male Sprague-Dawley rats weighing 200 to 250 g were supplied by the Taiyo Breeding Laboratory, Houston, Texas. Glucose was obtained from Sigma Chemical Company, St. Louis, Missouri, and all other chemicals were analytical reagents grade and purchased from appropriate companies.

Preparation of Liver Homogenate: Homogenate was prepared from rat liver perfused with Hank's solution (without Ca2+ and glucose). After 5 minutes, the liver was excised, cut into small pieces (about 5 x 5 x 5 mm), and transferred into a 50 ml glass flask containing 10 ml of medium: HEPES buffer (pH 7.4), glucose (100 mM), and ATP (5 mM). The tissues were homogenized by sonication. The homogenate was then centrifuged at 10,000 g for 10 minutes at 0°C, and the supernatant was used as the source of tissue homogenate.

Measurement of Methylglyoxal Formation:

Incubation was performed at 37°C for 1 hour in a shaking water bath. The incubation medium contained 100 mM glucose, 10 mM ATP, 5 mM MgCl2, and 10 mM HEPES buffer (pH 7.4). The reaction mixture was incubated for 1 hour at 37°C. After incubation, the reaction mixture was centrifuged at 10,000 g for 10 minutes at 0°C. The supernatant was collected, and the methylglyoxal concentration was determined by the method of Szeto and Wong (1974). Methylglyoxal concentrations were determined by the method of Leary et al. (1975). Experimental procedures are described in the legends of the appropriate figures and tables.

RESULTS

The Activity of Methylglyoxal Synthetase in the Whole Liver Cell and the Participation of Methylglyoxal Synthetase Activity in the Methylglyoxal Formation in Rat Liver Cells

The activity of methylglyoxal synthetase in the whole liver cell was measured by the method of Szeto and Wong (1974). The activity of methylglyoxal synthetase in the liver homogenate was measured by the method of Leary et al. (1975). Experimental procedures are described in the legends of the appropriate figures and tables.

DISCUSSION

The activity of methylglyoxal synthetase in the whole liver cell was measured by the method of Szeto and Wong (1974). The activity of methylglyoxal synthetase in the liver homogenate was measured by the method of Leary et al. (1975). Experimental procedures are described in the legends of the appropriate figures and tables.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health (GM-18517) to J.-S. Jung.

REFERENCES


The activity of methylglyoxal synthetase was measured by the increase of intracellular methylglyoxal (IPG) detected by the method of Szeto and Wong (1974). The activity of methylglyoxal synthetase was measured by the method of Leary et al. (1975). Experimental procedures are described in the legends of the appropriate figures and tables.

In conclusion, the activity of methylglyoxal synthetase in the whole liver cell was measured by the method of Szeto and Wong (1974). The activity of methylglyoxal synthetase in the liver homogenate was measured by the method of Leary et al. (1975). Experimental procedures are described in the legends of the appropriate figures and tables.
TABLE 4

<table>
<thead>
<tr>
<th>Substance</th>
<th>Glucose</th>
<th>Pyruvate</th>
<th>Glucose</th>
<th>Pyruvate</th>
<th>Cell Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>Uptake</td>
<td>Metabolism</td>
<td>Uptake</td>
<td>Metabolism</td>
<td>Uptake</td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>8</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Note: The data represent the mean of at least three independent experiments. The radioactivity of methylglyoxal and pyruvate was determined by gas chromatography and/or liquid scintillation counting.

In late 1980, methylglyoxal was determined enzymatically and subjected to the same procedure by use of methylglyoxal. The percent conversion of activated dehydrase from glucose-6-phosphate to methylglyoxal in the same degree of activity was lower than methylglyoxal. However, the relative specific activity of methylglyoxal was close to that of glucose-6-phosphate. The labeled carbon of methylglyoxal was incorporated into methylglyoxal and pyruvate. The experiments were performed in the presence of [14C] glucose and [14C] xyaltol, and the resulting methylglyoxal and pyruvate were isolated and analyzed by gas chromatography and liquid scintillation counting. The data represent the mean of at least three independent experiments. The radioactivity of methylglyoxal and pyruvate was determined by gas chromatography and/or liquid scintillation counting.

REFERENCES
Methylglyoxal formation in rat liver cells.
J Sato, Y M Wang and J van Eys


Access the most updated version of this article at
http://www.jbc.org/content/255/5/2046.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/255/5/2046.citation.full.html#ref-list-1