Methylglyoxal: From a putative intermediate of glucose breakdown to its role in understanding that excessive ATP formation in cells may lead to malignancy

Manju Ray*† and Subhankar Ray**

*Department of Biological Chemistry, Indian Association for the Cultivation of Science, Calcutta 700 032, India
**Department of Biochemistry, University College of Science, University of Calcutta, Calcutta 700 019, India

In the 1920s, methylglyoxal, a keto-aldehyde, was widely held as one of the key intermediates of glucose breakdown. But with the elucidation of Embden-Meyerhof pathway of glycolysis, this idea was rejected. However, in the 1970s and the 1980s the metabolic pathway for methylglyoxal in different organisms was established. Methylglyoxal has growth-inhibitory and anticancer properties and it had been generally assumed that these properties are interrelated. But recent studies have convincingly showed that methylglyoxal is tumoricidal. It inhibits mitochondrial respiration and glycolysis of exclusively malignant cells which critically reduces ATP level in these cells rendering them non-viable. We have obtained strong evidence that in malignant cells both mitochondrial complex I and the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, may be critically altered. Based on these results and considering the role of ATP in biological systems, a new hypothesis on cancer has been proposed, which suggests that excessive ATP formation in cells may lead to malignancy. Moreover, the reported anticancer property of methylglyoxal strongly suggests that methylglyoxal alone or in combination with some synthetic or natural product(s) should immediately be put to trial for the treatment of cancer.

Historical perspective

Methylglyoxal is a unique compound both chemically and biochemically. In spite of its small size it contains a reactive aldehyde group and a ketonic group, making it an acceptor of electrons. This compound has a chequered history in biology. In 1913, Neuberg and Kerb proposed a theory, relating to the metabolic sequence of alcoholic fermentation from glucose considering methylglyoxal as an intermediate1. The pathway they suggested was:

1 For correspondence. (e.mail: bcmr@iacs.ernet.in)

(1) Sugar (glucose) → Methylglyoxal → Pyruvic acid
↓
Acetaldehyde + CO₂
(2) Methylglyoxal + Acetaldehyde → Pyruvic acid
+ Alcohol

The insertion of methylglyoxal in the intermediary metabolic sequence leading to alcohol fermentation had first been made on the basis of chemical consideration. Biochemical arguments in favour of the proposal were also presented, such as (i) identification of a methylglyoxalase in animal tissues2,3 and (ii) repeated identification of methylglyoxal among the products of alcoholic fermentation. On the other hand, methylglyoxal was recognized by Meyerhof to be transformed at a faster rate than sugar to lactic acid by slices of liver and intestinal tissues4. So in the 1920s, methylglyoxal was widely held as one of the key intermediates of glucose breakdown. But in early 1930s, different phosphorylated intermediates of glycolytic pathway were identified. In 1932, Lohmann reported that GSH is the co-substrate of methylglyoxalase reaction5. He also showed that glycolysis could occur in the absence of glycolalase activity. He demonstrated that if muscle extract was dialysed to remove any glutathione and then the coenzymes of glycolysis were added, it could transform glycogen to lactic acid, though it could not metabolize methylglyoxal. Finally, with the elucidation of Embden-Meyerhof pathway, the idea that methylglyoxal was a key intermediate of glycolysis was abandoned6–9. However, several investigators time to time reported the formation of methylglyoxal in different biological systems. Meyerhof and Lohmann10 showed that triosephosphates could be converted to methylglyoxal non-enzymatically in the presence of acid and suggested that because the reaction mixture in which methylglyoxal had earlier been reported in biological systems all contained trichloroacetic acid, the appearance of methylglyoxal as a product of the test reaction was due to acid catalysed breakdown of triosephosphates which were also present in the reaction.
mixtures. This view was widely held up to 1950s. However in 1952, Entner and Doudoroff detected the production of methylglyoxal during glucose catabolism by iodoacetate-treated cells of Pseudomonas saccharophila\textsuperscript{11}. Methylglyoxal appeared to be produced from glyceraldehyde-3-phosphate under such condition and one mole of glucose was converted to one mole of pyruvate and one mole of methylglyoxal. They regarded this as a new phenomenon which revived the question of the biological role of methylglyoxal in glucose catabolism. However, no enzymatic reaction producing methylglyoxal from phosphorylated glycolytic intermediates was identified at that time and the role of methylglyoxal as an intermediate of glucose catabolism was forgotten again.

In 1960, Elliott isolated a crude enzyme fraction from ox plasma which is capable of converting aminoacetone to methylglyoxal\textsuperscript{12}. This was the first definite demonstration of an enzymatic formation of methylglyoxal from a specific metabolite. However, the specific enzyme which is responsible for this conversion could not be identified at that time.

In 1964, we find a report of methylglyoxal production during glucose catabolism by an Escherichia coli strain. This paper also described enzymatic conversion of glyceraldehyde-3-phosphate to methylglyoxal by this organism\textsuperscript{13}. No further report on this subject appeared until the 1970s. However, the literature published thereafter caused a revival of interest in the biochemical function of methylglyoxal in intermediary metabolism. This renewed interest stemmed from the investigation of gluconeogenesis from glycerol in E. coli mutants lacking triose phosphate isomerase (EC 5.3.1.1). Such mutants can produce dihydroxyacetone phosphate from glycerol but could not isomerize it to produce glyceraldehyde-3-phosphate\textsuperscript{14,15}. It was established that the enzyme methylglyoxal synthase (EC 4.2.99.11) which converts dihydroxyacetone phosphate to methylglyoxal was present in a variety of bacteria. The enzyme activity was extremely sensitive to inhibition by orthophosphate\textsuperscript{16}. These observations were soon confirmed independently\textsuperscript{17}. Methylglyoxal may also be formed during the catabolism of certain amino acids and other compounds\textsuperscript{18-20}. These results clearly indicate that methylglyoxal is indeed a normal metabolite and not an artifact generated during chemical analysis.

As is indicated above, one of the main arguments in support of the proposal that methylglyoxal might function as an intermediate in glycolysis is the widespread occurrence at high activity of an enzyme system (glyoxalase system) that converts methylglyoxal into D-lactate\textsuperscript{24}. Lohmann identified reduced glutathione (GSH) as the cofactor of this enzyme system\textsuperscript{5}. Racker elucidated that in this system glyoxalase I and II act in tandem to convert methylglyoxal into D-lactic acid\textsuperscript{21}. During 1970s, Cooper and his co-workers isolated the enzyme methylglyoxal synthase from a wild type strain of E. coli\textsuperscript{14-16}. This enzyme has subsequently been isolated and purified from a number of different bacteria\textsuperscript{8,22,23}. These were the first direct demonstrations of enzymatic formation of methylglyoxal in any biological system.

Establishment of metabolic pathway of methylglyoxal

The present phase of interest in the biological role of methylglyoxal began with the work of Szent-Györgyi. According to his hypothesis, methylglyoxal is capable of regulating cellular growth and in turn may act as a carcinostatic agent\textsuperscript{24}. In a subsequent section of this article, these growth inhibitory and carcinostatic actions of methylglyoxal are discussed in detail.

Szent-Györgyi and his co-workers were able to isolate methylglyoxal from biological samples\textsuperscript{25}. Thereafter, the pathways for methylglyoxal metabolism have been elucidated in mammalian system\textsuperscript{26-32}, yeast\textsuperscript{33-36} and protozoa\textsuperscript{37,38}. The enzymes participating in these pathways have been isolated, purified and characterized. Figure 1

**Figure 1.** Current status of methylglyoxal metabolism in mammalian system.
Reaction of methylglyoxal with biological molecules

Methylglyoxal being a highly reactive compound, has the potential to affect a wide variety of cellular processes. It primarily inhibits protein synthesizing machinery of the cells and it has been suggested that its growth inhibitory effect is mediated through inhibition of protein biosynthesis. Later studies have shown that methylglyoxal rapidly reacts with 7-methylguanosine residue of 16s and 23s RNA of ribosomes to inhibit protein synthesis.

Evidences of reactions of methylglyoxal with other biological molecules have also been speculated. Methylglyoxal reacts with free arginine, lysine in the cell pool and arginyl residues in proteins. It inhibits glycolytic enzymes. Methylglyoxal inhibits respiration specifically of the malignant cells. Methylglyoxal also reacts with protein thiol and free amino groups of various enzymes. Results obtained from experiments with tumour cells and data concerning the action of methylglyoxal on E. coli made Szent-Györgyi and coworkers to suggest that methylglyoxal preferentially blocks protein synthesis whereby cell proliferation was also arrested.

Turnover of methylglyoxal in biological systems

With the isolation and purification of different enzymes from various biological species, the metabolic pathway for methylglyoxal has been elucidated. Moreover, methylglyoxal has been isolated from various organisms. From these it is reasonable to assume that methylglyoxal is indeed formed in different organisms. However, it was difficult to quantitate directly and precisely the amount of this compound in the cell pool due to its high chemical reactivity and lack of suitable analytical methods. According to one school of thought, the accumulation of this metabolite in cells during its metabolic turnover is too small to produce any of the biological effects described above and the compound may have only limited physiological significance. However, recent studies have used several precise and sensitive procedures for the determination of methylglyoxal in biological systems. It has been observed by several investigators that d-lactic acid is always present in animal systems and is produced via methylglyoxal. D-Lactate levels were about one-sixth of d-lactate levels in rat liver. It has also been demonstrated that d-lactic acid level is higher than those of l-lactate in some animals and plants. In a relatively recent study, sources of carbon for d-lactate formation have been investigated in rat liver homogenate and by rat liver perfusion in situ. Of all the carbon sources tested, e.g. l-threonine, glucose, glyceral, acetone, etc., glyceral was found to be the
best substrate for D-lactate formation and glucose was the second most preferred substrate. However, in all these studies although significant D-lactate formation has been observed, cellular methylglyoxal level has been found to remain almost unchanged. When human red blood cells were incubated with 25 mM glucose to simulate hyperglycemic condition, there was increased D-lactate formation with concomitant increase in the level of methylglyoxal. The levels of D-lactate and methylglyoxal were significantly lower when the cells were incubated with 5 mM glucose.  

Growth inhibitory and anticancer properties of methylglyoxal

As early as 1958, the anticancer property of ketoaldehydes and their derivatives were first studied and effective response was obtained. Szent-Györgyi and his collaborators in their pioneering work on the biological role of methylglyoxal had put forward strong evidences for the anticancer and growth inhibitory effect of methylglyoxal. Együd and Szent-Györgyi showed that when methylglyoxal was injected into mice along with sarcoma 180 cells, no tumour developed and the mice remained completely healthy. At the same time, Apple and Greenberg with their remarkable experiments showed that methylglyoxal significantly inhibited tumour growth and in some cases produced indefinite survivors among mice bearing leukemia, lymphosarcoma, adenocarcinoma, sarcoma 180 and other varieties of tumours at daily dose level of approximately 80 mg/kg of body weight. Single dose of about 225 mg/kg of body weight significantly inhibited advanced leukemia and produced indefinite survivors among mice bearing either lymphosarcoma or carcinoma. Szent-Györgyi synthesized an ethylamine derivative of methylglyoxal and tested it for its ability to prevent spontaneous cancer in C57 mice by feeding the compound by mixing with drinking water. No cancer was observed during the first 16 weeks in mice which received the compound; while cancers developed as usual in the control groups. Similar therapeutic activity of methylglyoxal towards cancer bearing animals had also been obtained by other investigators. As mentioned before, of all the methylglyoxal catabolising enzymes present in cells, glyoxalase I is most powerful and ubiquitous. Several inhibitors of glyoxalase I had been synthesized and tested for their possible anticancer activity. It had been observed that appropriate concentration of some of these inhibitors caused several fold increase in methylglyoxal toxicity against L1210 leukemic cells.

In a relatively recent study with a wide variety of human post-operative tissue samples both normal and malignant, it has been observed that methylglyoxal completely inhibits the respiratory ability of specifically malignant cells. Moreover, ascorbic acid significantly augments the effect of methylglyoxal. Experiments with plant system have also yielded similar results. Treatment of malignant tissues of different plant species by optimal concentration of methylglyoxal in combination with ascorbic acid has shown the regeneration of normal buds and plantlets in high frequency.  

Methylglyoxal has been found to be growth inhibitory for a wide variety of proraryotic and eukaryotic cells. Significant growth inhibitory effect of this compound on germinating seeds, tumour cells, E. coli, S. cerevisiae, protozoa and other cells has been observed. It has been suggested that the anticancer property of methylglyoxal is due to its growth inhibitory effect which is in turn mediated through the inhibition of protein synthesis. The polymerization step is inhibited at the ribosomal level. Studies on the incorporation of labelled precursors into DNA, RNA and protein showed that only incorporation into protein was affected rapidly enough to be considered as the primary locus of inhibition by added methylglyoxal. The inhibition of protein synthesis by methylglyoxal was reversed by the addition of equimolar amounts of cysteine suggesting that the inhibitory activity depends on the SH content of the dividing cells. Szent-Györgyi and others suggested that methylglyoxal is a physiological growth inhibitory substance and with glyoxalase enzyme system responsible for its catabolism, it may control cellular growth.

Tumoricidal effect of methylglyoxal, inhibitory effect of methylglyoxal on the energy metabolism specifically of malignant cells

As mentioned above, the growth inhibitory effect of methylglyoxal has been well documented. It has also been observed that malignant cells are more sensitive than normal cells to methylglyoxal. It has also been suggested that the anticancer property of methylglyoxal is due to its growth inhibitory property which is mediated through inhibition of protein synthesis and interaction of methylglyoxal with nucleic acids. However, it has not been investigated whether there is any qualitative difference between normal and malignant cells in the inhibitory effect of methylglyoxal on protein synthesis or its interaction with nucleic acids.

In 1991 Ray et al. had observed that methylglyoxal is tumoricidal. When Ehrlich ascites carcinoma (EAC) cells were incubated for 20 minutes in presence of 5 mM methylglyoxal, more than 90% of the cells became non-viable. Moreover, when these methylglyoxal-treated EAC cells were inoculated into healthy mice, no tumour developed. It had also been observed that ascorbic acid significantly augments the tumoricidal effect of methylglyoxal. In contrast, lactaldehyde, a
catabolite and a structural analogue of methylglyoxal could exert a protective effect on the loss of viability and transplantability of methylglyoxal-treated EAC cells (Figure 2)\textsuperscript{68}. On further investigation it had been observed that methylglyoxal inhibits the respiration of EAC cells and a wide variety of post-operative human malignant tissues and also of leukemic leukocytes; but when other experimental conditions were same, increased concentration of methylglyoxal had no effect on the respiration of various normal tissues and of leukocytes of healthy humans (Figure 3)\textsuperscript{68}.

The above-mentioned effect of methylglyoxal on the respiration and viability of malignant cells strongly suggested that methylglyoxal possibly interferes with the oxidative process and energy metabolism of malignant cells. In an extension of the above-mentioned studies, Ray \textit{et al.} had further observed that methylglyoxal inhibited the mitochondrial respiration of EAC cells and leukocytes from leukemic patients; whereas the respiration of mitochondria of liver and kidney of normal mice and leukocytes from healthy humans remained unaffected (Figure 4)\textsuperscript{37,88}. Methylglyoxal also inhibited aerobic glycolysis of whole EAC cells and leukemic leukocytes but had no effect on aerobic glycolysis of normal leukocytes (Figure 5). Moreover, this inhibition of glycolysis had been found to be due to inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by methylglyoxal\textsuperscript{87}. As a consequence of inhibition of both glycolysis and mitochondrial respiration, the ATP level of EAC cells and leukemic leukocytes had been found to be critically reduced\textsuperscript{87,88}. That malignant cells have defective mitochondrial function resulting in high aerobic glycolysis had long been proposed\textsuperscript{89-91}. The inhibitory effect of methylglyoxal on respiration, specifically of the malignant cells, provided an opportunity to understand precisely the possible alteration of mitochondrial functions in malignant cells.

With EAC cell mitochondria, and by using different respiratory substrates, electron donors at different segments of the mitochondrial respiratory chain and site-
RESEARCH ACCOUNT

Figure 4. Effect of methylglyoxal on mitochondrial respiration of some normal and malignant cells. The direct oxygen tracing of some typical experiments are shown here. Tracings represent the effect of methylglyoxal on α-oxoglutarate, ADP-stimulated mitochondrial respiration of EAC cells (a), normal mouse liver (b), human leukemic leukocyte (c) and normal leukocyte from healthy donors (d). The numbers along the traces represent mg-atoms oxygen consumed per minute per mg of mitochondrial protein. M: mitochondria; MG, methylglyoxal; 1-OG, α-oxoglutarate. Further details of experimental procedures and results are available in refs 87, 88.

Figure 5. Effect of methylglyoxal on L-lactic acid formation by EAC cells, leukemic leukocytes and leukocytes from healthy donors. Values are mean (±SEM) of five different samples from five different mice/patients/donors. Further details of the experimental procedures and results are available in refs 87, 88.

Figure 6. Components of mitochondrial electron transport chain and coupled phosphorylation and the site of inhibition by methylglyoxal. The broken arrows indicate the sites of inhibition. CCCP, carbonyl cyanide m-chlorophenylhydrazone; cyt, cytochrome; TMPD, N,N,N',N'-tetramethyl-p-phenylene-diamine; TTFA, thienoyl trifluoroacetate.

specific inhibitors, it was observed that methylglyoxal inhibits the electron flow through complex I of mitochondria of this cell (Figure 6). Moreover, lactic acid similarly to its effect on whole EAC cells could exert a protective effect on the inhibition of EAC cell mitochondrial respiration by methylglyoxal. The above-mentioned studies were also performed with human leukemic leukocytes and similar results were obtained.

In summary, the results of all these studies indicate: i) methylglyoxal inhibits the respiration of a wide variety of human post-operative malignant tissue slices and also of EAC cells and human leukemic leukocytes, but ii) it has no inhibitory effect on the respiration of human normal (non-malignant) tissue slices, leukocytes of healthy donors and also of the slices of different tissues from normal animals, iii) methylglyoxal inhibits aerobic glycolysis of EAC cells and leukemic leukocytes but it has no effect on glycolysis of normal leukocytes, iv) methylglyoxal inhibits mitochondrial respiration (at the level of complex I) of EAC cells and leukemic leukocytes but has no inhibitory effect on mitochondrial respiration of normal leukocytes and of liver and kidney of normal mice, v) as a result of inhibition of both mitochondrial respiration and glycolysis, ATP levels of EAC cells and leukemic leukocytes have been found to be critically reduced, vi) lactic acid, a metabolite of methylglyoxal can significantly protect both cellular and mitochondrial respiration of EAC cells and leukemic leukocytes against the inhibitory effect of methylglyoxal, vii) methylglyoxal inactivates GA3PD of EAC cells, leukemic leukocytes and a wide variety of malignant post-operative tissue samples, whereas it has little inactivating effect on this enzyme from similar normal sources (Table 1).
Table 1. Effect of methylglyoxal on GA3PD from human malignant and benign tumours and normal tissues

<table>
<thead>
<tr>
<th>Organ or tissue/histological diagnosis</th>
<th>Specific activity of GA3PD (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without methylglyoxal</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
</tr>
<tr>
<td>Cervix uterus/squamous cell carcinoma</td>
<td>1.45 ± 0.25</td>
</tr>
<tr>
<td>Breast/infiltrating duct carcinoma</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>Colon/mucin secreting adenocarcinoma</td>
<td>1.75 ± 0.13</td>
</tr>
<tr>
<td>Blood (leukocyte)/chronic myeloid leukemia</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>Benign tumour</td>
<td></td>
</tr>
<tr>
<td>Fibroid, uterus/leiomyoma</td>
<td>1.50 ± 0.16</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Uterus (5)</td>
<td>0.96 ± 0.18</td>
</tr>
<tr>
<td>Colon (3)</td>
<td>1.25 ± 0.14</td>
</tr>
<tr>
<td>Blood (leukocyte) (5)</td>
<td>0.54 ± 0.04</td>
</tr>
</tbody>
</table>

For each sample, the results are the average of at least 3 individual assay ± SD. The datum from each sample (patient or donor) was used to calculate the mean ± SEM for n number of donors (presented in the table). The numbers in the parentheses indicate the number of patients/donors. Details of the assay of the enzyme, incubation condition and results are described in ref. 93.

Excessive ATP formation may lead to malignancy: a new hypothesis on cancer

All these results convincingly suggest that in malignant cells both complex I of mitochondrial respiratory chain and the glycolytic enzyme GA3PD are critically altered and methylglyoxal acts at these altered sites to elicit inhibition of mitochondrial respiration and aerobic glycolysis in these cells. As a consequence of this inhibition, ATP level in malignant cells is critically reduced, rendering these cells non-viable. We may recall here that mitochondrial oxidative phosphorylation is the principal contributor of ATP to the cellular energy pool and most of this mitochondrially-generated ATP is due to oxidation of NADH by mitochondrial complex I. Moreover in glycolysis, one of the two ATP generating steps is catalysed by the combined action of the enzymes GA3PD and glycero phosphate kinase; and this process can be considered as replica of mitochondrial oxidative phosphorylation. Based on the above-mentioned experimental finding and considering the role of ATP in biological systems, a new hypothesis on cancer has been proposed which suggests that excessive ATP formation in cells may lead to malignancy.

In biological systems, ATP is the principal contributor of energy necessary for anabolic reactions and is also a precursor of RNA and DNA. Active transport of sugars and amino acids into cells is also driven by hydrolysis of ATP. So if the above-mentioned alterations of mitochondrial complex I and GA3PD of cells favour excessive ATP formation which the corresponding cells cannot utilize through their normal metabolic activity, then it is quite likely that this excess ATP will initiate all the ATP-consuming anabolic reactions and the cells will grow, multiply and ultimately may turn malignant.

Recent observations on the effect of methylglyoxal on mitochondrial respiration of cardiac tissues and of other organs of normal animals of different species have further provided strong evidence in support of the proposed hypothesis. These experiments have shown that methylglyoxal inhibits mitochondrial respiration of cardiac tissues of different species but it has no effect on mitochondrial respiration of other organs of normal animals. Moreover, similar to the effect of methylglyoxal and lactaldehyde on mitochondrial respiration of malignant cells, methylglyoxal inhibits the electron flow through complex I of the mitochondrial respiratory chain of cardiac tissue, and lactaldehyde can protect against this inhibitory effect of methylglyoxal. These effects are strikingly similar to the effects on malignant cells, which suggest an essential similarity between cardiac mitochondria and malignant cellular mitochondria. In order to carry out its normal activity, heart has to produce an enormous amount of energy in the form of ATP, therefore there is a balance between the formation and breakdown of ATP in heart tissue. But if mitochondria of other normal organs are transformed in such a manner that they produce excessive ATP which they cannot utilize through their normal metabolic activity, then as stated above it is quite likely that this excess ATP will trigger all the ATP-consuming anabolic reactions which will stimulate growth and may ultimately turn the cell malignant.

Recent experimental evidences have further suggested that ATP may act as a mitogen. It has been observed that extracellular ATP and to a lesser extent adenosine, an ATP metabolite, acts synergistically with platelet-derived growth factor to induce DNA synthesis resulting in cell proliferation in osteoblast-like cells. Moreover it has also been suggested that cellular ATP level is an important determinant for apoptic cell death.

Interrelationship of glycolysis and mitochondrial respiration in malignant cells

The results obtained from different studies as described above also illuminate the interrelationship of two ATP generating processes, glycolysis and mitochondrial respiration in malignant cells.

One of the earliest observations regarding the biochemical characteristics of malignant cells was that they produce lactate via glycolysis at an abnormally high rate even in presence of oxygen, which normally inhibits this
process. Warburg hypothesized that this characteristic was of central importance in the malignant transformation and that it is due to one or more defects in the capacity of the malignant cellular mitochondria to carry out oxidative phosphorylation. However, some normal tissues, e.g., brain, retina, intestinal mucosa, leukocytes have also been shown to carry out aerobic glycolysis, and there are several types of malignant cells which do not show elevated aerobic and/or anaerobic glycolysis. Despite these limitations, increased glycolysis is a common feature of many malignant cells. Moreover, a close relationship between maximal rate of glycolysis and growth rate has also been observed in these cells.

On the other hand, whatever may be the reason for the high glycolysis, the impression that the oxidative metabolism of malignant cells is quantitatively or qualitatively defective has not been substantiated by experimental results. Mitochondria of malignant cells have a full complement of tricarboxylic acid cycle enzymes, functional respiratory complex and also exhibit tightly-coupled oxidative phosphorylation. The overall oxidative rate of the malignant cells is well within the range that has been displayed by most normal cells. Moreover, in some instances it has been observed that the oxidative rates are higher in malignant cells compared to that of the normal counterpart.

On the other hand, studies on the inhibition of glycolysis of malignant cells by methylglyoxal have indicated that methylglyoxal inactivates GA3PD of EAC cells and this inactivation is largely responsible for the inhibition of glycolysis of these cells by methylglyoxal. The GA3PD catalysed reaction is the only point in glycolysis where NADH is formed. Methylglyoxal also inactivates GA3PD of a wide variety of malignant post-operative tissue samples, whereas, methylglyoxal has little inactivating effect on this enzyme from similar normal sources. This suggests that in malignant cells GA3PD may be critically altered. Recently, several articles have appeared in the literature which have further substantiated the involvement of GA3PD with high glycolytic capacity and other aberrations of malignant cells. By using cDNA probe many of these studies have observed in several types of malignant cells an enhanced expression of a protein which is identical to the subunit of GA3PD. The presence of a novel isoform of this enzyme has also been indicated in a malignant prostate cell line. Moreover, in EAC cells, the catalytic activity of GA3PD is higher compared to this enzyme from other normal cells (unpublished observation from the authors' laboratory). A higher activity of GA3PD (either due to higher activity of the protein or due to the overexpression of the enzyme as stated above) will enhance NADH formation. It is likely that a portion of this NADH pool will be transported to mitochondria and will be oxidized by complex I. Another portion of this NADH pool will be oxidized by the catalytic action of L-lactic dehydrogenase with the concomitant reduction of pyruvate resulting in the formation of a higher amount of L-lactic acid (enhanced glycolysis) which has been observed in many malignant cells.

All these results strongly support the hypothesis that excessive ATP formation by enhanced glycolysis and mitochondrial respiration is responsible for malignant aberrations. Moreover, this putative excessive ATP formation is due to the altered GA3PD and mitochondrial complex I of malignant cells.

Excessive ATP formation can explain several features of malignancy

In recent years, the use of many modern techniques have vastly improved our knowledge regarding the differences in the properties of normal and cancerous cells. Many new concepts have also emerged regarding the characteristics of cancerous cells. However, most of these are essentially broad biological in nature and do not distinguish between a normal and a cancerous cell in terms of specific biochemical properties. Though the recent studies of viral origins of cancer, oncogenes, protooncogenes, tumour suppressor genes, etc. have vastly extended our knowledge in cancer biology, a critical examination indicates that no unique and specific difference which can explain malignant aberrations is apparent from the results of all these studies.

In contrast, the proposition that excessive ATP formation is responsible for malignancy is based on a unique and a specific biochemical property of malignant cells, the rationale of which has been discussed above. Moreover, if excessive ATP formation is the cornerstone of malignant aberration, then it can explain some important features of malignant cells and also some malignancy-related aberrations.

Metastasis of malignant cells

Malignant cells do not remain localized, instead they invade surrounding tissues, get into the circulatory system of the body, and set up areas of proliferation away from the site of their original appearance. This spread of malignant cells and establishment of secondary areas of growth is called metastasis. This phenomenon can be readily explained by the possible increased motility of malignant cells due to enhanced ATP level of these cells. It is well known that proteins responsible for cellular movement use the energy derived from the hydrolysis of ATP.

Pain in cancer patients

The prevalence of intractable pain in cancer patients is well-known. There are a number of physiological factors
for the presence of pain in cancer patients, e.g. bone destruction, luminal obstruction, infiltration or compression of nerves. Recent studies have indicated that ATP is possibly involved in generating pain signals. Released from the cytoplasm of the damaged cells into the extracellular space, ATP can stimulate sensory C-fibre nerves running into the spinal chord, many of which are receptors for painful stimuli (nociceptors). It seems possible that during the process of metastasis of cancerous cells, a portion of the excess ATP these cells possess may be released in the extracellular space, resulting in the painful stimuli.

**Increased glucose uptake by malignant cells**

It is well known that malignant cells are capable of transporting glucose inside their compartment at a much faster rate than normal cells. As mentioned before, this phenomenon can be readily explained by higher amount of ATP the malignant cells might possess, because ATP provides the energy necessary for the active transport of metabolites.

**Some directions for future research**

We outline here some directions for future research based on the proposal that altered mitochondrial complex I and GA3PD favour excessive ATP formation which subsequently turns cells malignant.

**Measurement of turnover of ATP**

It is reasonable to measure and compare the formation and turnover of ATP in normal and malignant cells. Because normal and leukemic leukocytes are excellent comparable source materials to identify any possible alterations in the cellular metabolism of malignant cells, the proposed study of measuring the formation and turnover of ATP can begin by using normal and leukemic leukocytes. Moreover, these cells can readily be obtained from human sources.

**Identification of a specific altered component in mitochondrial complex I of malignant cells**

Isolation and resolution of mitochondrial complex I from malignant cells, and cardiac and other normal cells and identification of a specific component which interacts with methylglyoxal is also of urgent necessity. If the specific component can be identified, then the possible alteration(s) in that component in precise molecular terms can be understood. In mitochondrial complex I, there are several proteins consisting of altogether 43 polypeptides and some non-protein components, e.g. FMN, non-heme iron, etc. Because methylglyoxal has been found to react with lysine and SH groups of proteins, it seems possible that methylglyoxal may react with a vital polypeptide of complex I which is similar in both malignant and cardiac cells, but this polypeptide is different from that of normal cells. This polypeptide may be responsible for the higher activity of complex I of cardiac and malignant cells, and this higher activity is responsible for stimulated NADH oxidation and increased ATP formation in the corresponding cells.

**Identification of alteration(s) and/or overexpression of GA3PD in malignant cells**

It seems rational to identify at the molecular level the putative alteration(s) of GA3PD in malignant cells which possibly make this enzyme different from that of other normal cells; to establish whether overexpression of GA3PD does indeed occur in all the malignant cells and to ascertain whether these differences can provide a biochemical basis for increased glycolysis of malignant cells.

**Therapy of cancer by methylglyoxal**

In a previous section we have presented the results of studies from different laboratories on the anticancer activity of methylglyoxal. Using both in vitro and in vivo experiments, these studies have convincingly demonstrated that methylglyoxal is a potent anticancer agent and it acts selectively against malignant cells, sparing the normal cells. Despite all these results it is very surprising and unfortunate that the potentiality of methylglyoxal as an anticancer drug including its human trials has not been seriously investigated. Szent-Györgyi suggested that the conditions for therapy are more favourable in man than in experimental animals. This lack of interest to use methylglyoxal as an anticancer drug has possibly stemmed from the widespread belief among the investigators working with this compound that methylglyoxal is toxic. In fact, many studies involving biochemical and biological effects of methylglyoxal suggest a possible toxic effect of this compound, and convincing evidence has accumulated that methylglyoxal is growth inhibitory. The high reactivity of methylglyoxal with many biomolecules has possibly assigned a toxic role of this compound in biological systems. It has also been suggested that methylglyoxal is responsible for many of the complications related to diabetes. Methylyglyoxal has also been found to be mutagenic and prolonged use of it increased the incidence of hyperplasia in experimental animals. Whatever may be the physiological relevance of all these reactions and toxic effects, the fact is that methylglyoxal is also well toler-
ated by both normal and tumour-bearing animals and its proven strong antitumour activity makes it a more potent candidate as a relatively harmless anticancer drug compared to other similar drugs that are now being widely used in the treatment of cancer.

Concluding remarks

Since the discovery in 1913 of glyoxalase enzyme system responsible for the breakdown of methylglyoxal in various organisms, many studies have been made to establish the biological role of methylglyoxal. Despite numerous publications resulting from all these studies, the true biological role of this potentially important biomolecule has remained elusive. Notwithstanding this limitation, important knowledge regarding the metabolism of methylglyoxal and its interactions with many important biomolecules and biological processes and functions has been accumulated in these intervening periods.

Moreover, these studies have clearly demonstrated that methylglyoxal is a potent anticancer agent and its effect can be substantially augmented in combination with other natural and synthetic compounds. Using the anticancer property of methylglyoxal, specially its inhibitory effect on the energy metabolism of exclusively malignant cells, a clear and very simple hypothesis has been proposed which can explain malignant aberration based on a specific biochemical difference. The hypothesis has been substantiated by further experimental evidences. All these studies strongly suggest that excessive ATP formation in cells may lead to malignancy and the two vital cellular components namely G3PD and mitochondrial complex I responsible for ATP formation are critically altered. Further biochemical characterization of these two components of malignant cells to identify the specific difference in precise molecular terms is of urgent necessity. Moreover, in order to save mankind from this deadly disease, methylglyoxal in combination with ascorbic acid and other synthetic inhibitors of glyoxalase I should be immediately put to trial for the treatment of cancer.

This article can appropriately be ended in an optimistic tone with a line written by Jesse P. Greenstein in 1947, 'It may well be, that once revealed, the explanation of the neoplastic transformation will be absurdly simple and measures of control will follow readily' (italics ours).
