ISSN 0006-2979, Biochemistry (Moscow), 2009, Vol. 74, No. 10, pp. 1059-1069. © Pleiades Publishing, Ltd., 2009.
Published in Russian in Biokhimiya, 2009, Vol. 74, No. 10, pp. 1301-1313.

REVIEW

Critical Evaluation of Toxic versus Beneficial Effects of Methylglyoxal

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Received January 15, 2009

Abstract—In various organisms, an array of enzymes is involved in the synthesis and breakdown of methylglyoxal. Through these enzymes, it is intimately linked to several other physiologically important metabolites, suggesting that methylglyoxal has some important role to play in the host organism. Several *in vitro* and *in vivo* studies showed that methylglyoxal acts specifically against different types of malignant cells. These studies culminated in a recent investigation to evaluate a methylglyoxal-based formulation in treating a small group of cancer patients, and the results were promising. Methylglyoxal acts against a number of pathogenic microorganisms. However, recent literature abounds with the toxic effects of methylglyoxal, which are supposed to be mediated through methylglyoxal-derived advanced glycation end products (AGE). Many diseases such as diabetes, cataract formation, hypertension, and uremia are proposed to be intimately linked with methylglyoxal-derived AGE. However methylglyoxal-derived AGE formation and subsequent pathogenesis might be a very minor event because AGE are nonspecific reaction products that are derived through the reactions of carbonyl groups of reducing sugars with amino groups present in the side chains of lysine and arginine and in terminal amino groups of proteins. Moreover, the results of some *in vitro* experiments with methylglyoxal under non-physiological conditions were extrapolated to the *in vivo* situation. Some experiments even showed contradictory results and were differently interpreted. For this reason conclusions about the potential beneficial effects of methylglyoxal have often been neglected, thus hindering the advancement of medical science and causing some confusion in fundamental understanding. Overall, the potential beneficial effects of methylglyoxal far outweigh its possible toxic role *in vivo*, and it should be utilized for the benefit of suffering humanity.

DOI: 10.1134/S0006297909100010

Key words: methylglyoxal, advanced glycation end products, glucose, cancer, antiviral

Methylglyoxal is a normal metabolite present in various organisms. Interest in the biological role of methylglyoxal began almost a century ago, but its metabolic pathways in different organisms have been elucidated only recently (for reviews, see [1-4]). An array of enzymes is involved in both the synthesis and breakdown of this metabolite. Through these enzymes, methylglyoxal is intimately linked either as a substrate or as a product to various important metabolites (Fig. 1). For this reason, it is easy to think that methylglyoxal should have some important role to play in the host organism. It is difficult to reject this view as being merely teleological.

Szent-Gyorgyi championed the idea that methylglyoxal is a natural growth regulator and can act as a strong anticancer agent [5]. Methylglyoxal has been found to possess strong activity against a number of pathogenic viruses [6]. In fact, the anticancer effect of methylglyoxal stemmed from the study of its antiviral effect [7]. Recently, it has been observed that methylglyoxal acts against a number of other pathogenic microorganisms. [8-10].

Szent-Gyorgyi and other investigators with remarkable experimental evidences showed methylglyoxal could arrest growth and/or destroy malignant cells without any apparent toxic effect to the host [11, 12]. At the same time, Apple and Greenberg showed significant curative effect of methylglyoxal on animals harboring a wide variety of malignant cells [13].

Despite these promising results, until recently neither academic researchers nor clinicians have made any attempt to translate this research potential to the benefit of humankind. On the other hand, recent literature abounds with the toxic effects of methylglyoxal. For

Abbreviations: AGE, advanced glycation end products; 3-DG, 3-deoxyglucosone; HbA1c, glycated hemoglobin.

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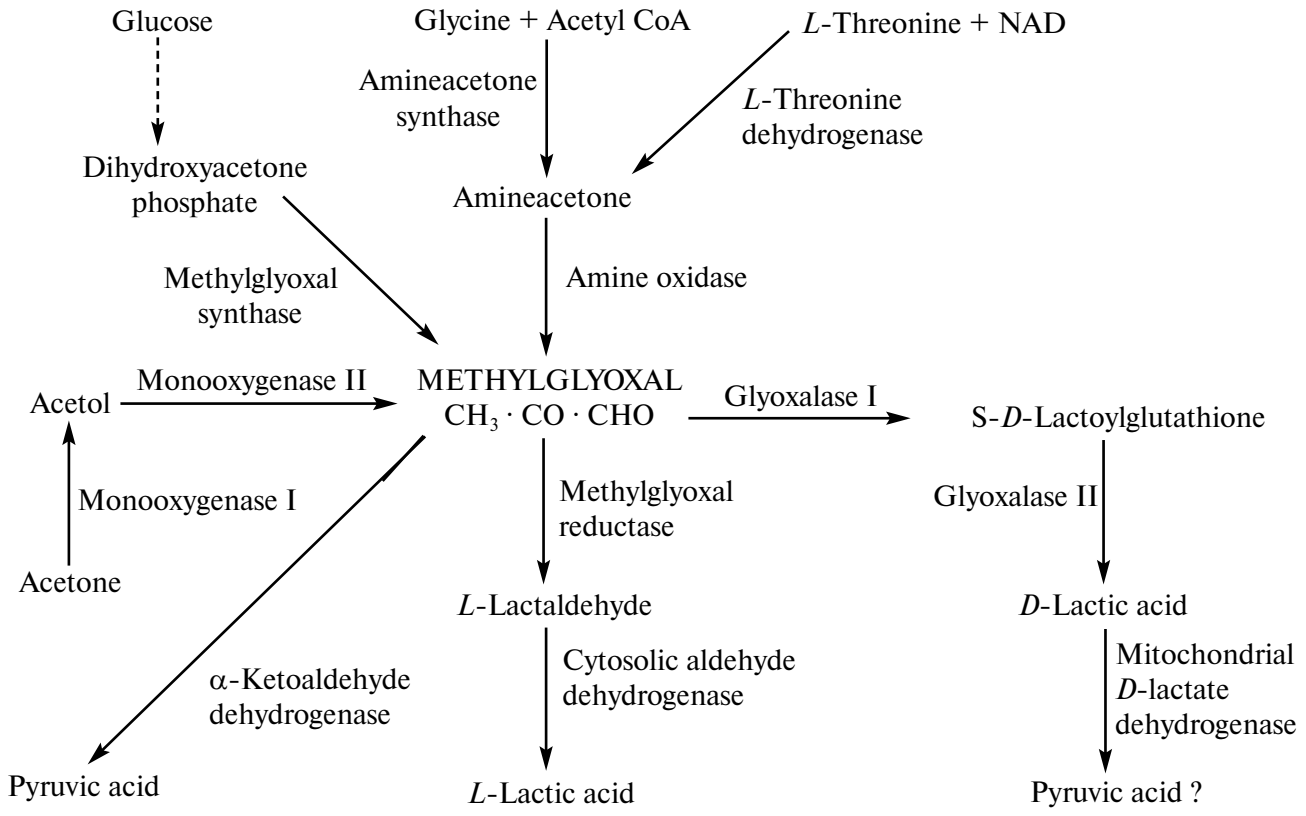


Fig. 1. Current understanding of methylglyoxal metabolism in mammalian systems.

example, from the mid 1980s numerous publications have appeared purporting many deleterious effects of methylglyoxal. It was proposed that this toxic effect is mediated mainly through advanced glycation end products (AGE) by reaction of the carbonyl groups in methylglyoxal with the amino group present in lysine and arginine and in terminal amino group of proteins. Moreover, it was also suggested that many diseases such as diabetes, cataract formation, hypertension, and uremia are intimately linked with methylglyoxal-derived AGE formation.

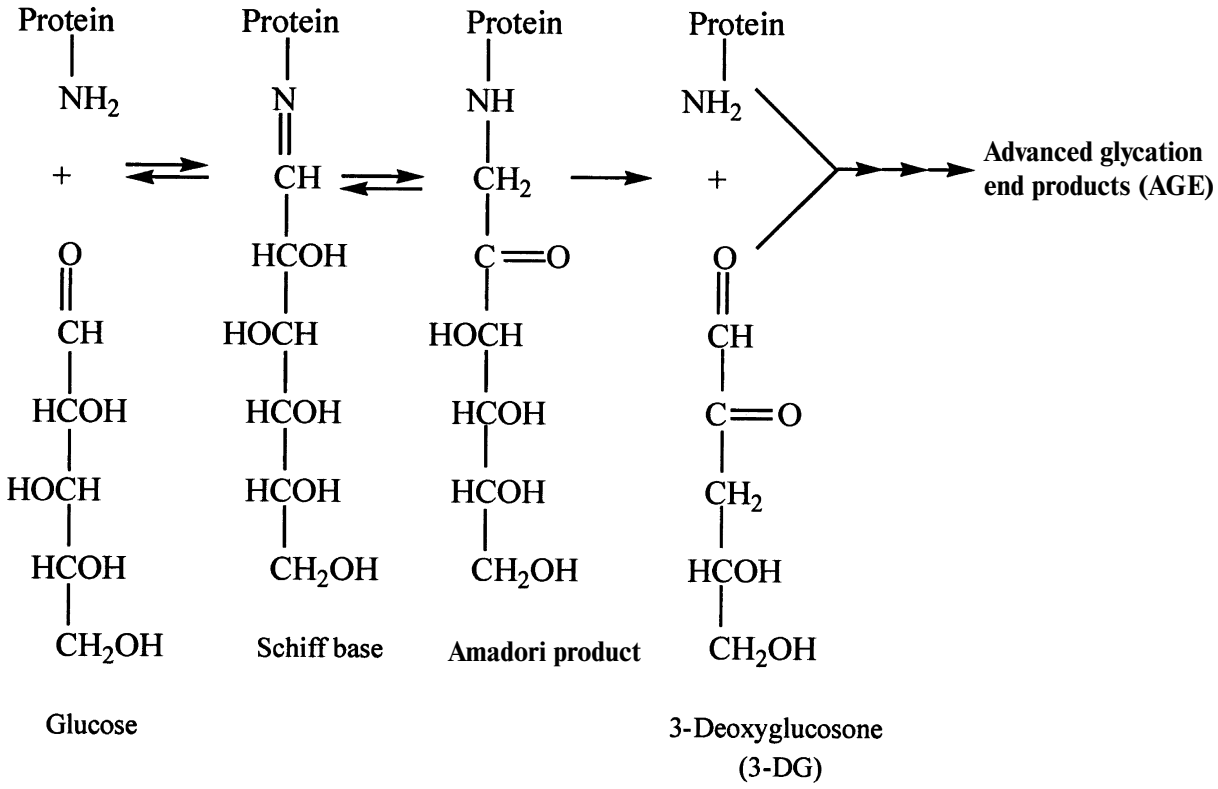
In this context, the observations and the conclusions of the potential beneficial effects of methylglyoxal have often been ignored, thus impeding the advancement of medical science and also causing some confusion in fundamental understanding. But, the present review shows that many of the purported toxic effects of methylglyoxal are nonspecific in nature. In several cases, the results of *in vitro* experiments under non-physiological conditions have been extrapolated to the *in vivo* situation, and there are some contradictory results as well. With this introduction, we now critically discuss the studies of different investigators on the role of methylglyoxal in different physiological phenomena.

AGE FORMATION BY METHYLGLYOXAL AND OTHER CARBONYL COMPOUNDS

Because methylglyoxal is supposed to be an important precursor of AGE, a brief description of AGE formation is pertinent here. Some examples of AGE are carboxymethyllysine, argpyrimidine, pentosidine, glucosepan, DOGDIC, MODIC, etc. The Maillard reaction or glycation is usually initiated by the reaction of reducing sugars with lysine and/or arginine side chains and N-terminal amino groups of proteins yielding Amadori compounds (aminoketoses) as primary products. A large number of compounds formed in later stages of this process are grouped together as advanced glycation end products [14-18]. Some of the different AGE compounds and their possible reaction pathways are described in Figs. 2 and 3.

The examples of chemical reactions shown in the figures indicate that methylglyoxal alone is not responsible for AGE formation. Several compounds (including glucose) that are vitally related to cellular metabolism and nutrition are also precursors of AGE.

a



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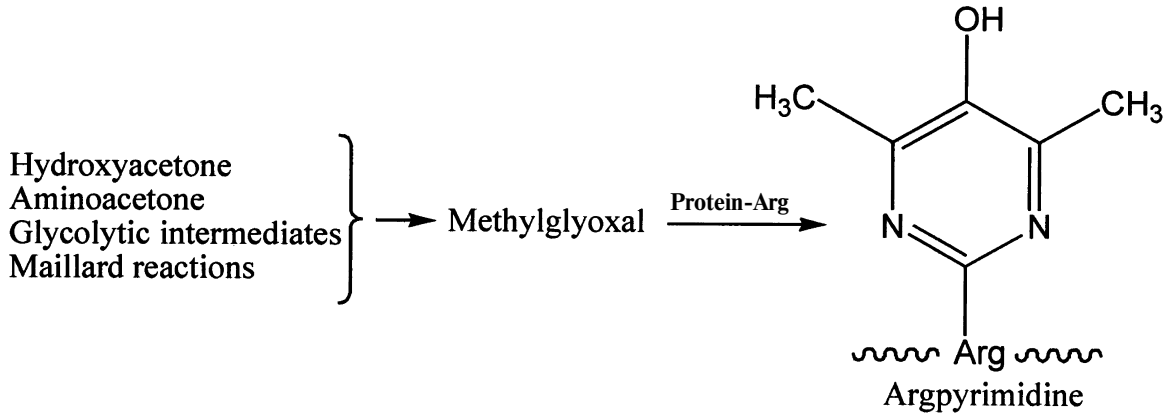

Fig. 2. a) Formation of AGE from glucose through 3-deoxyglucosone; b) argpyrimidine formation from methylglyoxal.

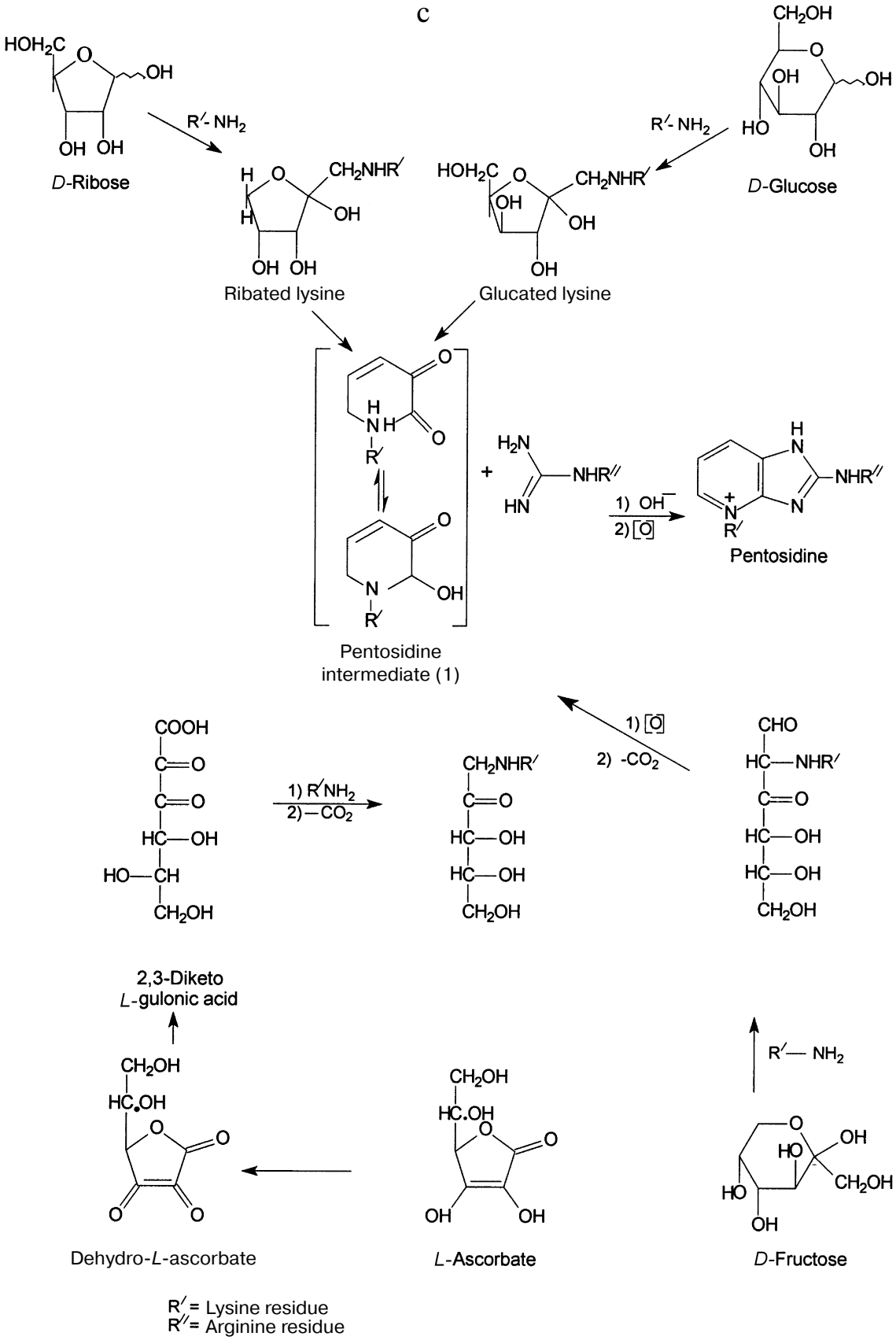

Fig. 2. c) Pentosidine formation from glucose, ribose, fructose, and ascorbic acid.

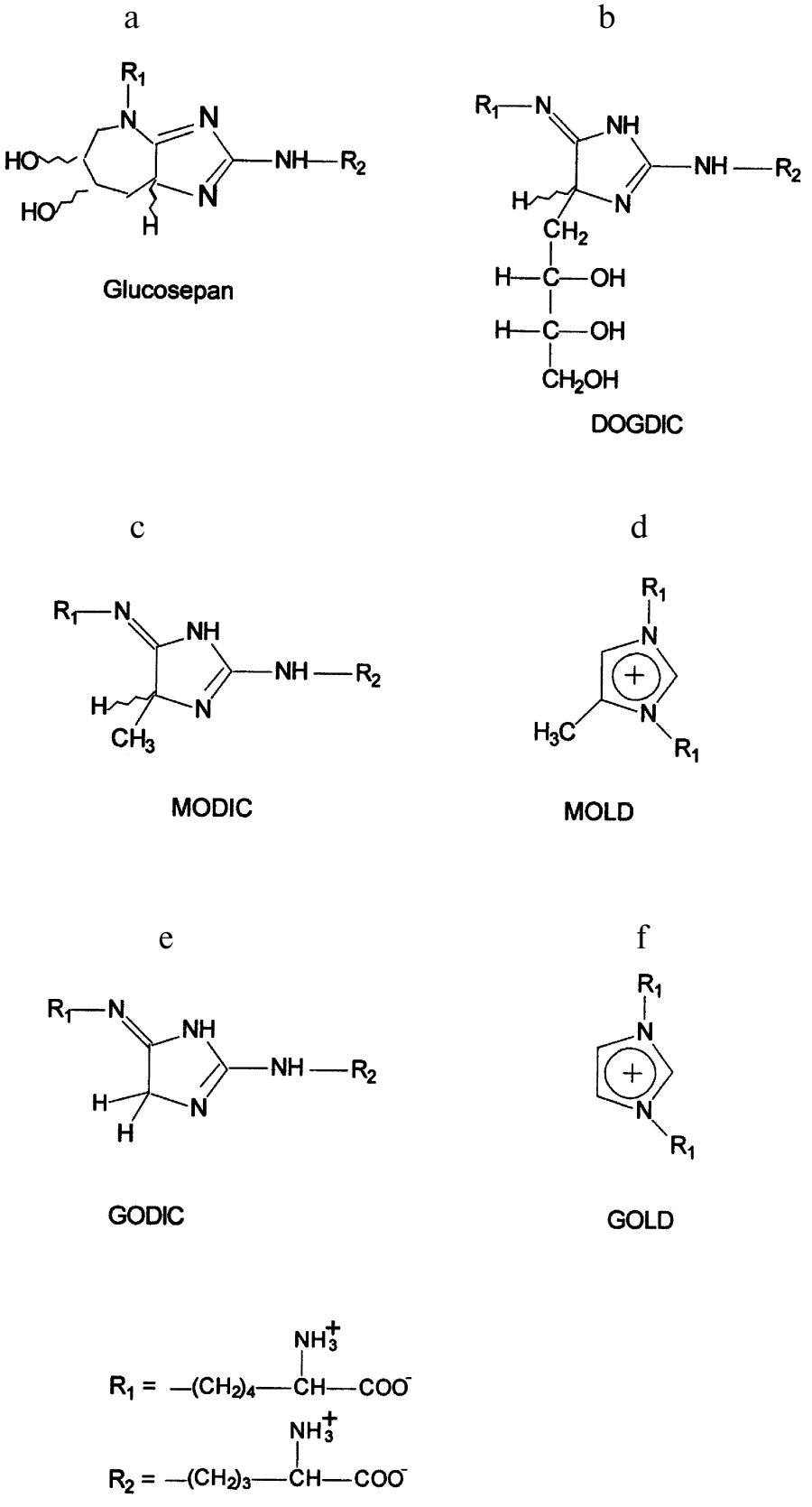

Fig. 3. Different AGE compounds derived from glucose (a), 3-deoxyglucosone (b), methylglyoxal (c, d), and glyoxal (e, f).

METHYLGLYOXAL LEVEL IN HUMAN BLOOD

Because methylglyoxal level in tissue and blood can influence methylglyoxal-derived AGE formation and might generate subsequent pathological conditions, different studies had measured methylglyoxal levels in whole blood and plasma of both normal subjects and diabetic patients. In the four studies that we cite here there is a considerable variation in the level of methylglyoxal in the plasma of normal subjects. We present here the values in nM: Odani et al. [19], 650 ± 160; Nemet et al. [20], 520 ± 42; Lapolla et al. [21], 194 ± 11; Beisswenger et al. [22], 123 ± 37. It is interesting to note that the increase in methylglyoxal level (189 ± 39 nM) in diabetic patients of one study [22] is far below the normal level compared to two other studies [19, 20] and almost equal to another study [21]. These results corroborate the comment "there is no consensus on the physiological concentration range of methylglyoxal yet" [23]. However, it is likely that different methods used in different studies yielded different results. In a recent paper, the specificity and sensitivity of different methods for the estimation of methylglyoxal and other dicarbonyl compounds have been evaluated [24]. It has been suggested that derivatization by 1,2-diaminobenzene and detection by gas chromatography and mass spectrometry is the most suitable method. This claim needs validation in different laboratories, and only after standardizing the method of methylglyoxal measurement we can arrive at any definite conclusion.

In vivo IDENTIFICATION OF AGE

Very few relevant AGE have been detected *in vivo*. Attempts have been made to identify lysine-arginine and lysine-lysine cross-links of carbonyl compounds in human materials. These are glucosepan (derived from glucose), DOGDIC (derived from 3-deoxyglucosone (3-DG)), MODIC and MOLD (derived from methylglyoxal), and GODIC and GOLD (derived from glyoxal) (Fig. 3) [17].

Accurate quantification of these compounds indicated that glucosepan was the dominant compound in plasma proteins of normoglycemic subjects (median 17.1 pmol/mg protein). In diabetic subjects the levels of glucosepan were significantly higher (median 29.2 pmol/mg protein). MODIC levels were found to be in the same range (3.9-4.1 pmol/mg protein) in both diabetic and non-diabetic groups. A significant correlation was apparent between glycated hemoglobin (HbA1c) values and the glucosepan, although MODIC levels were found to be almost independent of HbA1c concentration. In a separate and similar study of the levels of AGE in human skin collagen and glomerular basement membrane in relation to age and diabetes, it was observed that glucosepan is by far the most dominant compound [18].

In a relatively recent publication reporting *in vitro* experiments, it has been shown that human hemoglobin was modified by methylglyoxal, forming the hydroimidazolone derivative of arginine residues. Specific arginine residues that were modified in hemoglobin were also shown. These results suggested to the authors that this type of modification might be *in vivo*, rendering physiological relevance to their study [25]. However, as mentioned above, no correlation was found between HbA1c and methylglyoxal-derived AGE *in vivo* [17].

INVOLVEMENT OF METHYLGLYOXAL IN VARIOUS PATHOLOGICAL CONDITIONS

As mentioned above, methylglyoxal has been implicated with several pathological conditions. Besides diabetes, some examples are cataract formation, diabetic retinopathy, familial amyloidotic polyneuropathy, mood disorder, etc. However, a critical survey of the literature indicates that some important metabolites and derivatives of these metabolites might be involved with these pathological processes, and the role of methylglyoxal might be very minor.

Cataract formation and methylglyoxal. For example, metabolism of methylglyoxal and the formation of methylglyoxal-modified proteins have been linked with the development of senile and diabetic cataract due to significantly increased concentration of methylglyoxal in the lenses of diabetic subjects and subsequent glycation-derived AGE formation [26-29]. However, it has been shown that incubation of bovine and human lens protein digest with 5 mM glucose-6-phosphate lead to the formation of fluorescent yellow pigments and cross-links similar to those reported in aging and cataractous human lenses [30]. Moreover, the protein LM-1 was originally discovered and isolated from acid hydrolysate of crystallin from human cataractous lens. It was found to increase with age and in diabetic patients, and its levels correlated with the overall degree of lens pigmentation. However, it was shown that methylglyoxal is not involved in its formation as a cross-link involving lysine residue [31]. Studies on the role of fructose in glycation and cross-linking of proteins suggested that a significant proportion of human ocular lens proteins had reacted with fructose *in vivo* [32, 33]. Interestingly, it was also found that chemical modification of α-crystallin by methylglyoxal enhanced its chaperon function. The authors concluded that post-translational modifications by methylglyoxal might be a protective mechanism against environmental and metabolic stresses on eye lens [34].

Methylglyoxal and diabetic retinopathy. In diabetic retinopathy, there is selective loss of intramural pericytes. In studies on the role of AGE in the loss of pericytes

through apoptosis, it has been observed that chronic exposure to methylglyoxal-modified BSA [35] or methylglyoxal-modified fibronectin [36] or methylglyoxal alone [37] or methylglyoxal-derived hydroimidazolone [38] lead to a significant increase in pericyte apoptosis. On the other hand, surveys were conducted to assess the biological risk factors for diabetic retinopathy, and it was concluded that high blood glucose level [39, 40] and high systolic blood pressure [40, 41] are major risk factors for this disease. To understand the mechanism of pathogenesis of diabetic retinopathy, elevated levels of extracellular carbonic anhydrase have been implicated in one of the studies with diabetic retinopathy [42]. Another study proposed a model for diabetic retinopathy implicating the perturbation of redox regulation by glutaredoxin in hyperglycemia [43].

Using a microwave irradiation quenching technique and enzymatic estimation, retinas of diabetic rats were studied *in vivo*. It has been observed that polyols are increased dramatically; this indicates that glucose metabolism downstream of hexokinase is not elevated. Rather, metabolism upstream from glucose, such as the sorbitol pathway and polyol pathway, are increased [44].

These studies suggest that methylglyoxal may not be involved in diabetic retinopathy and, if it is involved at all, it plays a very minor role.

Methylglyoxal and familial amyloidotic polyneuropathy. In adipose tissue of three familial amyloidotic patients, argpyrimidine was found to be present at a concentration of 162.40 ± 9.05 pmol/mg protein. It was concluded that it was a methylglyoxal-derived AGE product. But no significant difference was observed in either glucose or methylglyoxal concentration between the sample patients and the control subjects [45]. From these results one may, however, easily and rightly conclude that "methylglyoxal-derived AGE formation" is just a coincidence, and methylglyoxal plays no role in this disease.

Peritoneal dialysis and methylglyoxal. Peritoneal dialysis (PD) and hemodialysis are common treatments for patients with reduced or absent renal function. It was suggested that long term PD leads to peritoneal injury. Glucose degradation products in heat-sterilized or non heat-sterilized peritoneal dialysis fluid (PDF) contribute to the bio-incompatibility of this fluid. It has been suggested that not only AGE but also 1,2-dicarbonyl compounds were formed during heat sterilization of glucose-based PDF. Glyoxal and methylglyoxal already present in the dialysis fluid reacted with peritoneal matrix proteins, accounting for the gradual loss of peritoneal membrane function that is often observed in patients subjected to PD [46, 47].

However, another study where the effects of individual glucose degradation products (GDP) on apoptosis of cultured human neutrophils and peripheral blood mononuclear cells were investigated showed that in contrast to the effect of methylglyoxal, glucose and other GDPs accelerated cellular apoptosis. Among the different GDP found in the heat-sterilized PDF, 3,4-dideoxyglucos-3-ene (3,4-DGE) accelerated cellular apoptosis even at a concentration of 25 μM. In contrast, no cytotoxicity was observed following the addition of methylglyoxal, acetaldehyde, formaldehyde, or 3-DG at concentrations found in PDF. It was concluded that 3,4-DGE is the main proapoptotic factor [48]. Other investigations also corroborated this finding [49, 50].

In vivo VERSUS *in vitro* EFFECT

A search in the literature on the biological effects of methylglyoxal indicates that the results of many *in vitro* experiments have been extrapolated to the *in vivo* situation (for a discussion and references, see [51]). However, it is obvious that *in vivo* and *in vitro* conditions can show vastly different results. It was shown that only 5 mM glucose could completely inactivate malate dehydrogenase, a vital enzyme present in both mitochondria and cytosol [52]. Obviously *in vivo* malate dehydrogenase remains active despite the presence of glucose. The same authors showed that fructose was a superior glycating agent to glucose, and 5 μg/ml α-crystallin, a lens protein, can completely protect from this inactivation. Even aspirin had a moderate protection against this glycation.

In a study of yeast protein glycation, enolase 2 was identified as the primary methylglycation target in yeast. Two other glycolytic enzymes, aldolase and phosphoglycerate mutase, and three heat shock proteins were also glycated. However, in the same paper the authors showed that despite the activity loss of the enzymes in a glycation-dependent way, glycolytic flux and glycerol production remained unchanged [53]. These results suggest that despite the methylglyoxal-derived glycation of protein the organism has excellent resilience for maintaining cellular homeostasis. In a very recent publication on protein glycation of yeast enolase, marked differences between *in vivo* glycated enolase and purified enolase glycated *in vitro* were revealed [51].

Human retinal pericytes, when incubated with high glucose (30 mM), did not undergo apoptosis despite accumulation of methylglyoxal. However, treatment with a combination of high glucose and an inhibitor of glyoxalase I, the enzyme primarily responsible for the breakdown of methylglyoxal, resulted in apoptosis. Interestingly, overexpression of glyoxalase I in these pericytes protected the cells against this inhibitor plus high glucose, suggesting the critical involvement of glyoxalase I in cellular defense [54]. This study also suggests that a very high concentration of methylglyoxal is needed to induce apoptosis in retinal pericytes. Moreover, glyoxalase I when present can act as a defense mechanism of the cells even in diabetes, which in turn might increase the methylglyoxal level.

In vivo TOXICITY OF METHYLGLYOXAL

Previous studies by Apple and Greenberg [13] and Egyud and Szent-Gyorgyi [11] on methylglyoxal treatment of cancer-bearing animals showed that cancer cells were destroyed without affecting the host. Moreover, these methylglyoxal-treated animals also produced healthy liters [11]. However, very few studies have been made on the effect of methylglyoxal administration to animals. In one such study, there was a report of toxicity on administration of methylglyoxal to rats [55]. However, a detailed toxicity study, both acute and long term with four species of animals, both rodent and non-rodent with different doses of methylglyoxal through oral, subcutaneous, and intravenous routes showed no adverse effects on the animals [56].

SOME POTENTIAL BENEFICIAL EFFECTS OF METHYLGLYOXAL

Antimalarial activity of methylglyoxal. Recently antimalarial activity of methylglyoxal has been reported [8]. Proliferation of the malaria parasite, *Plasmodium falciparum*, was inhibited by methylglyoxal with IC_{50} of only 0.2 mM. Dihydroxyacetone was also antiproliferative to the parasite but with IC_{50} of around 3 mM. A 4-day treatment of uninfected erythrocytes with 2 mM methylglyoxal neither caused any morphological abnormality nor cell lysis, showing that methylglyoxal does not at random produce any toxic effect. Moreover, only 15% inhibition of rabbit glyceraldehyde-3-phosphate dehydrogenase was observed on incubation of 2.5 mM of methylglyoxal for a period of 2 h, corroborating the earlier result on the effect of methylglyoxal on this enzyme [57]. Moreover the antimalarial activity of methylglyoxal [8] was corroborated by an earlier report of antimalarial activity of S-*p*-bromobenzylglutathione diethyl ester, an inhibitor of glyoxalase I, which is responsible for the breakdown of methylglyoxal. The median inhibitory concentration was around 5 μM [58].

Activity of methylglyoxal against *Staphylococcus aureus*. In a very recent publication, Mavric et al. have shown that the active principle of the well-known antibacterial property of New Zealand manuka (*Leptospermum scoparium*) honey is methylglyoxal [10]. The minimally inhibitory concentration against *St. aureus* was 1.1 mM. The amount of methylglyoxal in manuka honey was found to be 38-761 mg/kg. It has also been shown that the amount of methylglyoxal present in an antibacterial wound gel, a pharmaceutical product, was around 310 mg/kg.

Effect of methylglyoxal and AGE on mycobacterial infection. Apoptosis and activation of macrophages play an important role in the host response to mycobacterial infection. Elevated levels of methylglyoxal and AGE during mycobacterial infection of macrophages, leading to apoptosis and activation of the macrophages, have been demonstrated. Moreover, global gene expression profiling of methylglyoxal-treated macrophages revealed a diverse spectrum of functions induced by methylglyoxal, including apoptosis and immune response. These observations suggested novel intervention strategies against infectious diseases in which methylglyoxal and AGE play critical roles [9].

Antiviral effect of methylglyoxal. The antiviral activity of methylglyoxal has been briefly mentioned in the introductory section. To elaborate, in 1957 de Bock et al. screened the growth inhibitory effect of a number of compounds towards a strain of influenza virus. In experiments with chicken embryo, they showed that a number of α-ketoaldehydes were active, and methylglyoxal almost topped the list [6]. The antiviral activity of methylglyoxal has also been found against New-Castle disease and influenza [59], foot and mouth disease [60], and other viruses by other investigators [61]. Surprisingly, no study on the antiviral effect of methylglyoxal was pursued thereafter.

ANTICANCER EFFECT OF METHYLGLYOXAL

The anticancer effect of methylglyoxal has been briefly mentioned in the introductory section. It appears that the most remarkable positive effect of methylglyoxal arose from the studies of anticancer effect of methylglyoxal. As early as 1958, the anticancer effect of methylglyoxal was first studied and effective response was obtained [7]. These studies stemmed from the study of antiviral effect of methylglyoxal. Because virus is one of the causative agents of cancer, it was thought that methylglyoxal might have some anticancer effect. Szent-Gyorgyi and his collaborators in their pioneering work on the biological role of methylglyoxal put forward strong evidence for the anticancer and growth inhibitory effect of methylglyoxal [5]. Egyud and Szent-Gyorgyi showed that when methylglyoxal was injected into mice along with sarcoma 180 cells, no tumor developed and the mice remained completely healthy [11]. They even produced healthy offspring. However, the duration of the treatment was short, and methylglyoxal treatment began just after tumor inoculation. They also found that in tissue cultures methylglyoxal was more sensitive to malignant cells than to normal cells.

Apple and Greenberg showed that methylglyoxal significantly inhibited tumor growth and in some cases produced indefinite survivors among mice bearing leukemia, lymphosarcoma, adenocarcinoma, sarcoma 180, and other varieties of tumor at daily dose level of approximately 80 mg/kg of body weight. A 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 [13]. The *in vivo* anticancer effect of methylglyoxal was augmented in the presence of glyceraldehyde [62]. Similar therapeutic activity of methylglyoxal towards cancer-bearing animals has also been obtained by other investigators [12, 56].

Numerous studies have shown that methylglyoxal acts against a wide variety of tumors. For the sake of brevity, we mention here only some of these studies. Methylglyoxal is toxic to human neuroblastoma cells in a dose-dependent manner above concentration of 0.15 mM with a LD_{50} of approximately 1.25 mM. At the concentration of 5 mM methylglyoxal, the viable cells were only 6% [63]. When exogenous methylglyoxal was added to human leukemia HL-60 cells in culture, inhibition of growth and toxicity was induced; the LD_{50} was 0.24 mM. Methylglyoxal, however, did not induce any toxicity in differentiated cells, i.e. neutrophils under similar culture conditions [64]. Methylglyoxal produced an apoptotic response of human MCF7 breast and RKO colon cancer lines. However, overexpression of glyoxalase II inhibited the methylglyoxal-induced apoptotic response of the cells. Glyoxalase II acts in tandem with glyoxalase I for the breakdown of methylglyoxal to D-lactate. Likewise, cells deficient in glyoxalase II were hypersensitive to methylglyoxal-induced apoptosis [65]. Methylglyoxal was also able to induce severe (>99%) cell death in 24 h in human prostate cancer cells [66]. The activities of glyoxalase I and/or glyoxalase II were found to be higher in several cancer cell lines [67-69]. However, there are contradictory reports too [70].

Experiments with plant system had also yielded similar results [71, 72]. 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 [71]. Methylglyoxal can also induce differentiation of plantlets from calluses and can act as a complete substitute for kinetin in this regard [72].

Of all the methylglyoxal catabolizing enzymes present in cells, glyoxalase I is most potent and ubiquitous. Several inhibitors of glyoxalase I have been synthesized and tested for their possible anticancer activity. It has been observed that appropriate concentration of some of these inhibitors caused several-fold increase in toxicity against malignant cells [73]. The polyphenol curcumin was found to hamper the growth of breast cancer (JIMT-1, MDA-MB-231), prostate cancer PC-3, and brain astrocytoma 1321N1 cells. However, no effect on growth or vitality of human primary hepatocytes was observed. It was also observed that the growth inhibitory effect of curcumin was mediated through inhibition of glyoxalase I [74].

Methylglyoxal has been found to be tumoricidal. When Ehrlich ascites carcinoma (EAC) cells were incubated in the presence of methylglyoxal, more than 90% of the cells became non-viable. Moreover, when these methylglyoxal-treated EAC cells were inoculated into healthy mice, no tumor developed. It was also observed that ascorbic acid significantly augmented the tumoricidal effect of methylglyoxal [75]. On further investigation, it was observed that methylglyoxal inhibited the respiration of EAC cells, a wide variety of human post-operative malignant tissues, and also of leukemic leukocytes. But when other experimental conditions were the same, increased concentration of methylglyoxal had no effect on the respiration of various normal tissues and leukocytes of healthy humans [75, 76]. The inhibitory effect of methylglyoxal on the respiration of a wide variety of malignant human post-operative tissues supports the studies of Apple and Greenberg [13, 62].

Studies on the glycolysis and mitochondrial respiration of EAC cells and normal and leukemic leukocytes suggested that mitochondrial complex I and glyceraldehyde-3-phosphate dehydrogenase are altered specifically in malignant cells, and methylglyoxal acts on these altered sites to elicit its anticancer effects [57, 75-78]. Based on these results the new hypothesis that excessive ATP production may lead to malignancy was proposed [79].

As mentioned above, Apple and Greenberg showed remarkable curative effect of methylglyoxal on mice bearing a wide variety of cancers [13, 62]. From these results, it was logical to immediately investigate the efficacy of methylglyoxal in treating cancer patients. However, the first paper that reported the testing of methylglyoxal in treating cancer patients was as late as 2001 [80]. The study was extended to a larger number of cancer patients, giving promising results [81, 82]. It was found that methylglyoxal is quite effective in treating cancer patients who were suffering from a wide variety of cancers. Moreover, methylglyoxal is apparently devoid of any toxic effect, in contrast to other anticancer drugs that are now widely used. However, these studies need validation with greater numbers of cancer patients in different centers.

That methylglyoxal is effective against so many different types of malignant cells lends support to the view that different types of malignant cells have common and specific altered site(s).

CONCLUDING REMARKS

This review has critically evaluated both the reported toxic and beneficial effects of methylglyoxal and suggests that the potential beneficial effects of methylglyoxal far outweigh its possible toxic effects. It has not been proved that methylglyoxal significantly contributes to the suggested deleterious effects on the host. Compared to the voluminous *in vitro* work, the *in vivo* studies are very limited. Moreover, many related carbonyl compounds, which are vital for cellular metabolism and nutrition,

might also be linked with similar effects in the organism. Can we dispense with these nutrients? Any compound or even a metabolite has some potential adverse effect on the host however small this effect may be. The effect of a particular compound must be judged by balancing the benefit and adversity. The reported activity of methylglyoxal against a number of pathogenic microorganisms and its anticancer properties raise the question whether methylglyoxal is a natural defense of the host especially for mammals. However, due to the mindset of a large of number of researchers the potential beneficial effects of methylglyoxal are not being translated resulting in a serious deterrent to the advancement of medical science. The primary effects of methylglyoxal, which can be translated to the benefit of suffering humankind, are its activities against a number of pathogenic microorganisms and its anticancer effects. We appeal to academics, clinicians, research administrators, and pharmaceutical companies to translate this research potential to the benefit of humankind.

The authors thank the Council of Scientific and Industrial Research, Department of Science and Technology, Government of India, and Lifecare Innovations Pvt. Ltd. for financial assistance in preparing the manuscript. We thank Dr. Soumen Bera for his assistance in preparing the figures.

REFERENCES

1. Cooper, R. A. (1984) *Annu. Rev. Microbiol.*, **38**, 49-68.
2. Ray, M., and Ray, S. (1998) *Curr. Sci.*, **75**, 103-113.
3. Kalapos, M. P. (1999) *Toxicol. Lett.*, **110**, 145-175.
4. Ewaschuk, J. B., Naylor, J. M., and Zello, G. A. (2005) *J. Nutr.*, **135**, 1619-1625.
5. Szent-Gyorgyi, A. (1979) *Ciba Found. Symp.*, **67**, 3-18.
6. De Bock, C. A., Brug, J., and Walop, J. N. (1957) *Nature*, **179**, 706-707.
7. French, F. A., and Freedlander, B. L. (1958) *Cancer Res.*, **18**, 172-175.
8. Pavlovic-Djuranovic, S., Kun, J. F., Schultz, J. E., and Beitz, E. (2006) *Biochim. Biophys. Acta*, **1758**, 1012-1017.
9. Rachman, H., Kim, N., Ulrichs, T., Baumann, S., Pradl, L., Nasser, E. A., Bild, M., Rother, M., Kuban, R. J., Lee, J. S., Hurwitz, R., Brinkmann, V., Kosmiadi, G. A., and Kaufmann, S. H. (2006) *PLoS ONE*, **1**, e29.
10. Mavric, E., Wittmann, S., Barth, G., and Henle, T. (2008) *Mol. Nutr. Food Res.*, **52**, 483-489.
11. Egyud, L. G., and Szent-Gyorgyi, A. (1968) *Science*, **160**, 1140.
12. Conroy, P. J. (1979) *Ciba Found. Symp.*, **67**, 271-300.
13. Apple, M. A., and Greenberg, D. M. (1967) *Cancer Chemother. Rep.*, **51**, 455-464.
14. Grandhee, S. K., and Monnier, V. M. (1991) *J. Biol. Chem.*, **266**, 11649-11653.
15. Niwa, T., Katsuzaki, T., Miyazaki, S., Miyazaki, T., Ishizaki, Y., Hayase, F., Tatemichi, N., and Takei, Y. (1997) *J. Clin. Invest.*, **99**, 1272-1280.
16. Wilker, S. C., Chellan, P., Arnold, B. M., and Nagaraj, R. H. (2001) *Anal. Biochem.*, **290**, 353-358.
17. Biemel, K. M., Friedl, D. A., and Lederer, M. (2002) *J. Biol. Chem.*, **277**, 24907-24915.
18. Sell, D. R., Biemel, K. M., Reihl, O., Lederer, M. O., Strauch, C. M., and Monnier, V. M. (2005) *J. Biol. Chem.*, **280**, 12310-12315.
19. Odani, H., Shinzato, T., Matsumoto, Y., Usami, J., and Maeda, K. (1999) *Biochem. Biophys. Res. Commun.*, **256**, 89-93.
20. Nemet, I., Turk, Z., Duvnjak, L., Car, N., and Varga-Defterdarovic, L. (2005) *Clin. Biochem.*, **38**, 379-383.
21. Lapolla, A., Reitano, R., Seraglia, R., Sartore, G., Ragazzi, E., and Traldi, P. (2005) *Mol. Nutr. Food Res.*, **49**, 685-690.
22. Beisswenger, P. J., Howell, S. K., Touchette, A. D., Lal, S., and Szwergold, B. S. (1999) *Diabetes*, **48**, 198-202.
23. Chang, T., and Wu, L. (2006) *Can. J. Physiol. Pharmacol.*, **84**, 1229-1238.
24. Thornalley, P. J. (2008) *Drug Metab. Drug Interact.*, **23**, 125-150.
25. Gao, Y., and Wang, Y. (2006) *Biochemistry*, **45**, 15654-15660.
26. Kumar, M. S., Reddy, P. Y., Kumar, P. A., Surolia, I., and Reddy, G. B. (2004) *Biochem. J.*, **379**, 273-282.
27. Haik, G. M., Jr., Lo, T. W., and Thronalley, P. J. (1994) *Exp. Eye Res.*, **59**, 497-500.
28. Chellan, P., and Nagaraj, R. H. (1999) *Arch. Biochem. Biophys.*, **368**, 98-104.
29. Riley, M. L., and Harding, J. J. (1995) *Biochim. Biophys. Acta*, **1270**, 36-43.
30. Monnier, V. M., and Cerami, A. (1981) *Science*, **211**, 491-493.
31. Tessier, F., Obrenovich, M., and Monnier, V. M. (1999) *J. Biol. Chem.*, **274**, 20796-20804.
32. Szwergold, B. S., Kappler, F., and Brown, T. R. (1990) *Science*, **247**, 451-454.
33. McPherson, J. D., Shilton, B. H., and Walton, D. J. (1998) *Biochemistry*, **22**, 1901-1907.
34. Nagaraj, R. H., Oya-Ito, T., Padayatti, P. S., Kumar, R., Mehta, S., West, K., Levison, B., Sun, J., Crabb, J. W., and Padival, A. K. (2003) *Biochemistry*, **42**, 10746-10755.
35. Denis, U., Lecomte, M., Paget, C., Ruggiero, D., Wiernsperger, N., and Lagarde, M. (2002) *Free Radic. Biol. Med.*, **33**, 236-247.
36. Liu, B., Bhat, M., Padival, A. K., Smith, D. G., and Nagaraj, R. H. (2004) *Invest. Ophthalmol. Vis. Sci.*, **45**, 1983-1995.
37. Kim, J., Son, J. W., Lee, J. A., Oh, Y. S., and Shinn, S. H. (2004) *J. Korean Med. Sci.*, **19**, 95-100.
38. Fosmark, D. S., Torjesen, P. A., Kilhovd, B. K., Berg, T. J., Sandvik, L., Hanssen, K. F., Agardh, C. D., and Agardh, E. (2006) *Metabolism*, **55**, 232-236.
39. Cikamatana, L., Mitchell, P., Rochtchina, E., Foran, S., and Wang, J. J. (2007) *Eye*, **21**, 465-471.
40. Varma, R., Macias, G. L., Torres, M., Klein, R., Pena, F. Y., and Azen, S. P. (2007) *Ophthalmology*, **114**, 1332-1340.
41. Wong, T., and Mitchell, P. (2007) *Lancet*, **369**, 425-435.
42. Gao, B. B., Clermont, A., Rook, S., Fonda, S. J., Srinivasan, V. J., Wojtkowski, M., Fujimoto, J. G., Avery, R. L., Arrigg, P. G., Bursell, S. E., Aiello, L. P., and Feener, F. P. (2007) *Nature Med.*, **13**, 181-188.

43. Shelton, M. D., Kern, T. S., and Mieyal, J. J. (2007) *J. Biol. Chem.*, **282**, 12467-12474.
44. Ola, M. S., Berkich, D. A., Xu, Y., King, M. T., Gardner, T. W., Simpson, I., and Lanonue, K. F. (2006) *Am. J. Physiol. Endocrinol. Metab.*, **290**, E1057-1067.
45. Gomes, R., Sousa, S. M., Quintas, A., Cordeiro, C., Freire, A., Pereira, P., Martins, A., Monteiro, E., Barroso, E., and Ponces, F. A. (2005) *Biochem. J.*, **385**, 339-345.
46. Lapolla, A., Flamini, R., Lupo, A., Arico, N. C., Rugiu, C., Reitano, R., Tubaro, M., Ragazzi, E., Seragila, R., and Traldi, P. (2005) *Ann. N. Y. Acad. Sci.*, **1043**, 217-224.
47. Mortier, S., Faict, D., Schalkwijk, C. G., Lameire, N. H., and de Vriese, A. S. (2004) *Kidney Int.*, **66**, 1257-1265.
48. Catalan, M. P., Santamaria, B., Reyero, A., Ortiz, A., Egido, J., and Ortiz, A. (2005) *Kidney Int.*, **68**, 1303-1311.
49. Ortiz, A., Wieslander, A., Linden, T., Santamaria, B., Sanz, A., Justo, P., Sanchez-Nino, M. D., Benito, A., and Kjellstrand, P. (2006) *Curr. Med. Chem.*, **13**, 2695-2702.
50. Erixon, M., Linden, T., Kjellstrand, P., Carlsson, O., Ernebrant, M., Forsback, G., Wieslander, A., and Jonsson, J. A. (2004) *Perit. Dial. Int.*, **24**, 392-398.
51. Gomes, R. A., Oliveira, L. M., Silva, M., Ascenso, C., Quintas, A., Costa, G., Coelho, A. V., Sousa, S. M., Ferreira, A. E., Ponces, F. A., and Cordeiro, C. (2008) *Biochem. J.*, **416**, 317-326.
52. Heath, M. M., Rixon, K. C., and Harding, J. J. (1996) *Biochim. Biophys. Acta*, **1315**, 176-184.
53. Gomes, R. A., Miranda, H. V., Silva, M. S., Graca, G., Coelho, A. V., Ferreira, A. E., Cordeiro, C., and Freire, A. P. (2006) *FEBS J.*, **273**, 5273-5287.
54. Miller, A. G., Smith, D. G., Bhat, M., and Nagaraj, R. H. (2006) *J. Biol. Chem.*, **281**, 11864-11871.
55. Berlanga, J., Cibrian, D., Guillen, I., Freyre, F., Alba, J. S., Lopez-Saura, P., Merino, N., Aldama, A., Quintela, A. M., Triana, M. E., Montequin, J. F., Ajamieh, H., Urquiza, D., Ahmed, N., and Thronalley, P. J. (2005) *Clin. Sci.* (Lond.), **109**, 83-95.
56. Ghosh, M., Talukdar, D., Ghosh, S., Bhattacharyya, N., Ray, M., and Ray, S. (2006) *Toxicol. Appl. Pharmacol.*, **212**, 45-58.
57. Ray, M., Basu, N., and Ray, S. (1997) *Mol. Cell. Biochem.*, **177**, 21-26.
58. Thronalley, P. J., Strath, M., and Wilson, R. J. (1994) *Biochem. Pharmacol.*, **47**, 418-420.
59. Tiffany, B. D., Wright, J. B., Moffett, R. B., Heinzelman, R. V., Strube, R. E., Aspergren, B. D., Lincoln, E. H., and White, J. L. (1957) *J. Am. Chem. Soc.*, **79**, 1682-1687.
60. Ghizatullina, N. K. (1976) *Acta Virol.*, **20**, 380-386.
61. Baylor, M. B., and Egyud, L. G. (1967) *Virology*, **31**, 380-382.
62. Apple, M. A., and Greenberg, D. M. (1968) *Cancer Chemother. Rep.*, **52**, 687-696.
63. Webster, J., Urban, C., Berbaum, K., Loske, C., Alpar, A., Gartner, U., de Arriba, S. G., Arendt, T., and Munch, G. (2005) *Neurotox. Res.*, **7**, 95-101.
64. Ayoub, F. M., Allen, R. E., and Thornalley, P. J. (1993) *Leuk. Res.*, **17**, 397-401.
65. Xu, Y., and Chen, X. (2006) *J. Biol. Chem.*, **281**, 26702-26713.
66. Davidson, S. D., Milanesa, D. M., Mallouh, C., Choudhury, M. S., Tazaki, H., and Konno, S. (2002) *Urol. Res.*, **30**, 116-121.
67. Rulli, A., Carli, L., Romani, R., Baroni, T., Giovannini, E., Rosi, G., and Talesa, V. (2001) *Breast Cancer Res. Treat.*, **66**, 67-72.
68. Mearini, E., Romani, R., Mearini, L., Antognelli, C., Zucchi, A., Baroni, T., Porena, M., and Talesa, V. N. (2002) *Eur. J. Cancer*, **38**, 1946-1950.
69. Sakamoto, H., Mashima, T., Kizaki, A., Dan, S., Hashimoto, Y., Naito, M., and Tsuruo, T. (2000) *Blood*, **95**, 3214-3218.
70. Di, I. C., Angelucci, S., Pennelli, A., Zezza, A., Tenaglia, R., and Sacchetta, P. (1995) *Cancer Lett.*, **96**, 189-193.
71. Lieber, M. M. (1995) *Forntier Perspect.*, **4**, 35-41.
72. Roy, K., De, S., Ray, M., and Ray, S. (2004) *Plant Growth Regul.*, **44**, 33-45.
73. Creighton, D. J., Zheng, Z. B., Holewinski, R., Hamilton, and Eiseman, J. L. (2003) *Biochem. Soc. Trans.*, **31**, 1378-1382.
74. Santel, T., Pflug, G., Hemdan, N. Y., Schafer, A., Hollenbach, M., Buchold, M., Hintersdorf, A., Lindner, I., Otto, A., Bigl, M., Oerlecke, I., Hutschenreuter, A., Sack, U., Huse, K., Groth, M., Birkemeyer, C., Schellenberger, W., Gebhardt, R., Platzer, M., Weiss, T., Vijayalakshmi, M. A., Kruger, M., and Birkenmeier, G. (2008) *PLoS ONE*, **3**, e3508.
75. Ray, M., Halder, J., Dutta, S. K., and Ray, S. (1991) *Int. J. Cancer*, **47**, 603-609.
76. Biswas, S., Ray, M., Misra, S., Dutta, D. P., and Ray, S. (1997) *Biochem. J.*, **323**, 343-348.
77. Ray, S., Dutta, S., Halder, J., and Ray, M. (1994) *Biochem. J.*, **303**, 69-72.
78. Ray, S., Biswas, S., and Ray, M. (1997) *Mol. Cell. Biochem.*, **171**, 95-103.
79. Ray, S., and Ray, M. (1997) *Med. Hypothes.*, **48**, 473-476.
80. Ray, M., Ghosh, S., Kar, M., Datta, S., and Ray, S. (2001) *Ind. J. Phys.*, **75B**, 73-77.
81. Talukdar, D., Ray, S., Das, S., Jain, A. K., Kulkarni, A., and Ray, M. (2006) *Cancer Therapy*, **4**, 205-222.
82. Talukdar, D., Ray, S., Ray, M., and Das, S. (2008) *Drug Metab. Drug Interact.*, **23**, 175-210.