Biochemistry (Moscow), Vol. 67, No. 12, 2002, pp. 1329-1346. Translated from Biokhimiya, Vol. 67, No. 12, 2002, pp. 1611-1631.

REVIEW

Molecular Mechanisms of the Cross-Impact of Pathological Processes in Combined Diabetes and Cancer. Research and Clinical Aspects

Ya. A. Aleksandrovski

Kantorforschungsgruppe, Zeppelinstr. 22, 76530 Baden-Baden, Germany; E-mail: Yakov.Alexandrovski@t-online.de

Received May 13, 2002
Revision received May 29, 2002

Abstract—The mechanisms of interaction and cross-impact of metabolic processes in a combined diabetes and cancer condition are discussed. A hypothesis is proposed whereby the processes responsible for destruction of the organism in the case of diabetes—long-term hyperglycemia and generation of methylglyoxal—may substantially impact tumor development. The hypothesis is based on the fact that both diabetes and carcinogenesis cause dysfunction of the vital cellular signal system regulated by the protein kinase C (PKC) family. Normalization of the PKC functional activity in the case of diabetes restrains development of diabetic complications and inhibits the processes of tumor growth and metastasizing in carcinogenesis. On this basis, an attempt is made to interpret both the detrimental and beneficial effects of diabetes on cancer. The resultant effect is determined by the type of tumor and the duration and level of hyperglycemia. The mechanisms of the impact of diabetes mellitus on cancer are analyzed to develop recommendations for combined cancer therapy options.

Key words: cancer, diabetes, protein kinase C, methylglyoxal

If the system is not contradictory it is incomplete.

(The philosophical aspect of the Gödel theorems)

Diabetes mellitus inhibits neoplasms and numerous types of malignant tumors. This mechanism of diabetes is not understood. Moreover, some characteristic features of cancer tumors would suggest quite an opposite effect.

In fact, malignant cells are capable of assimilating glucose highly intensively, using it as a vital energy source. A malignant tumor consumes sugar at a rate higher than that of its input, acting as a powerful pump, "draining" glucose from the body [1]. For instance, in mice with Ehrlich carcinoma, the concentration of glucose in the ascitic fluid proved lower than the sensitivity threshold of standard enzymatic test methods (about 1-10 μM) and increase almost to normal physiological values upon removal of cancer cells [1, 2]. According to some calculations, the cancer cells present in 5 ml of ascitic fluid could consume in 24 h more than 500 mg of glucose, while its content (in mouse blood and liver) is less than 50 mg [3].

Thus, *in vivo* tumors are in a state of permanent "glucose" starvation, realizing only a small proportion of their potential for glucose utilization [1]. Because the actual requirements of tumors for glucose are never met, there are grounds to believe that tumors are ready for assimilation of some extra substantial quantity of this carbohydrate. In its turn, long-term hyperglycemia *in vivo* is possible in principle via constant injection of saturated solution of glucose or due to pathology as diabetes mellitus.

In fact, numerous clinical and experimental data demonstrate that both by diabetes mellitus patients and animals with experimental diabetes, development of malignant neoplasm causes a substantial decrease in hyperglycemia down to normalization of sugar content in the blood. And, conversely, excision of, say, hard tumors causes development of hyperglycemia. Numerous cases of hypoglycemia occurring in both oncological patients and animals upon inoculation of malignant tumor can be accounted for by the same reason [1].

The above data give reasonable grounds to expect that the hyperglycemic condition either man-induced or a consequence of diabetes should be conducive to growth of malignant tumors. However, as mentioned at the

0006-2979/02/6712-1329$27.00 ©2002 MAIK "Nauka/Interperiodica"

beginning of the present paper, both experimental studies and clinical practice reveal an opposite effect for a number of tumors.

The above contradiction appears to be accountable for by the effect of insulin, whose deficiency primarily determines diabetic hyperglycemia. However, numerous experiments have demonstrated that for the majority of cancer cells, growth and consumption of glucose are insulin-independent processes. By and large, the capacity of insulin to affect the process of malignant tumor development, for instance, as growth factor is in evidence. However, the detrimental impact of diabetes mellitus on malignant growth cannot be solely interpreted as a result of lack of insulin in case of diabetes [1, 4-6].

Generally, if glucose is to be considered to be one of the main energy sources of tumor growth it is unlikely that disorder of carbohydrate metabolism *per se* determined by diabetes mellitus would strongly impact the "vital" metabolic pathways of cancer cells, inhibiting their growth and metastasizing.

The present communication proposes a working hypothesis of a possible scenario of interaction and cross-impact of metabolic processes determined by combined pathologies as diabetes and cancer. According to our concept, *the processes responsible for the destruction of the body in the case of diabetes damage not only the cells of healthy tissues*, *but also the cells of a neoplasm*. In other words, it is probable that the death of cancer cells can follow at least one of the numerous patterns for the destruction of the entire organism in the case of diabetes. The proposed approach in principle elucidates a number of mechanisms of detrimental impact of diabetes on cancer, but also permits discussing a possibility of brief and reversible triggering of those processes in oncological patients for therapeutic purposes.

I. DIABETES MELLITUS AND CANCER

Diabetes and cancer. The relationship between these two diseases has been investigated for decades. The issue has received great attention due to the fact that a considerable proportion of oncological patients show not only characteristic disorders of carbohydrate metabolism [7-10], but also appearance in some cases of diabetes mellitus as a result of cancer therapy [11]. According to a large body of available evidence, coincidence of the above diseases ranges from 10 to 30% and can reach considerable values in the case of liver cancer and endometrial or pancreatic carcinoma. The interrelationships between the above diseases have been looked into from several angles, the most important being the problem of the effect of diabetes mellitus on the origin and course of oncological diseases.

Numerous clinical data available regarding the above problem are often contradictory [12-18]. The differences in interpretation appear to be associated not only with the nature of the phenomenon itself, but also with generalization of some particular regularities revealed, without considering numerous forms of cancer, differences in the process of neoplasm malignancy, and different methods for therapy of diabetes mellitus. Of importance is also the fact that statistical studies based on a large body of data, normally covered a protracted period (several decades) and did not take into account differences in approaches to therapy of both diabetes and cancer according to the concepts of those diseases existing in particular periods.

For instance, in view of the fact that insulin affects carcinogenesis, the present-day therapy of diabetes mellitus recommends administering some essentially lower dosages of that hormone than was practiced before. Another illustrative example can be supplied: biguanide derivatives broadly used in the therapy of type 2 diabetes besides their opportunity to lower sugar level are similar to their analogs, bisguanylhydrazones, in having cytostatic properties, i.e., they can inhibit the process of cell proliferation [19-21].

Let us indicate one more probable source of erroneous concepts concerning coincidence of diabetes and cancer. Some authors that were busy with this problem did not take into account or did not point out in their papers the consecutive order of the manifestation of these pathologies (diabetes against the background of cancer or cancer against the background of diabetes), although statistically their coincidence is very unlikely (see further).

For example, the coincidence of pancreas cancer (as a primary illness) with diabetes mellitus is more than 60% [7], while there is no reliable relation between diabetes (as a primary illness) and pancreas cancer [18]. During recent years an increasing body of evidence has been obtained suggesting that the high coincidence rate of diabetes mellitus and pancreas cancer is not determined by the effect of diabetes on the process of induction of carcinogenesis. Most likely the carbohydrate disorder is the consequence of a primary oncological disease [22], whereas primary induced diabetes prevents induction of pancreas cancer (experiments with animals) [23-25]. This conclusion also can be confirmed with clinical observations: diabetes mellitus, which is characterized by relatively mild clinical manifestation, frequently is an early presenting sign of pancreatic cancer, indicating progress of a primary oncological process [26-28].

The large number of contradictory data and conflicting views need to be analyzed. In order to get a *general insight* into the interaction of diabetes mellitus and cancer let us neglect, in the first stage, the instances of the highest coincidence rate of these diseases. It well may be that the high coincidence rate of diabetes and oncological diseases is determined by interference of some obscure factors or mechanisms specific for any of the above-mentioned tumors.

Generally, analysis of numerous clinical and statistical studies on coincidence and the course of oncological diseases reveals that the bulk of data available in the literature suggest that *diabetes mellitus prevents oncological diseases and checks the process of malignant cell proliferation*. Let us see some figures.

According 22,971 autopsy reports and anamnesis from the deceased patients with and without diabetes mellitus as primary disease malignant tumors were found in 8.8 and 18% of cases, respectively [12].

The mortality rate from cancer in individuals free from disorder of carbohydrate metabolism is 12.7-20.4% and in diabetes patients, 7.1-9.5% [29].

The life-span of oncological patients with diabetes is longer [30, 31]. For instance, the percentage of mammary carcinoma metastases and diabetes patients who survived 18 months after the onset of hormone cancer therapy against similar patients who only had an oncological disease was, respectively, 64 against 16% [30].

The conclusion is well proved by animal experiments. Normally, alloxanic or streptozotocin diabetes which was manifested before or after inoculation of the tumor substantially handicapped its development. Against the background of long-term hyperglycemia, the experimental animals showed decline of weight and size of the hard tumor as well as reduction in the number of ascites, whereas the period of their survival increased. According to anatomical examination, in that case hard tumors were cankered by multiple necroses [1, 23-25, 32-41].

Some available evidence support clinical observations [30]: upon induction of alloxanic diabetes the size of rat mammary carcinoma declined by 50-80% [34] after 42 days. Another example. Carcinogenesis caused by injection of azo dye was sharply inhibited in alloxanic diabetes rats: tumor growth features were only revealed in one rat out of 14, whereas hepatoma incidence in controls was 90-100% [32].

As reported by numerous experimenters, the more severe diabetes, the heavier were depressed the processes of both origin and growth of tumors. For instance, heavy diabetic state handicapped origin of tumors of the liver and mammary gland induced by carcinogen in female rats. Similarly to the previous case, in a light diabetic condition, neither of the females developed mammary cancer, whereas hepatoma development was not depressed. Moreover, instead of mammary cancer, the subjects showed a very high percentage of tumor changes in the liver. In other words, a light form of diabetes prevented development of mammary cancer and promoted development of liver cancer [42]. The above findings demonstrate once again that diabetes may be conducive to development of certain malignant tumors.

Thus, the above evidence and concepts not only provide an insight into the issue stated in the title of the present communication, but also bring us to the point where it is appropriate to ask the traditional question: "Why does it happen so?"

As mentioned at the beginning of this paper, the answer to this question can be based on the assumption that the processes responsible for destruction of the body in case of diabetes may also depress malignant cells. Moreover, there are ample grounds to believe that cancer cells are to a much greater extent sensitive to pathological processes induced by diabetes.

Hence, in the first stage, it is necessary to find out what processes are primarily responsible both for the development of vascular complications in diabetes and cell proliferation. In the second stage, it is necessary to try to reveal the features in common between those processes because the answer must logically be found at the crossing point of the two pathologies.

II. PROTEIN KINASE C AS A UNIVERSAL REGULATOR OF PROCESSES OF TUMOR GROWTH AND DIABETIC COMPLICATIONS

The purpose of the present section is to state the major mechanisms responsible for development of (1) diabetic complications, (2) tumor growth and to attempt to (3) establish a relationship between them.

A reservation should be made from the outset. The objectives stated are a matter of profound independent study. Quite a number of problems, particularly, in oncology, are not yet understood. Consequently, it does not appear realistic to elucidate only the familiar aspects of each of the subjects under consideration and to present arguments in favor of a particular mechanism in this brief section. For that purpose, the problem can be approached in a different manner—first, an attempt can be made to reveal a single mechanism of pathology common of both diseases, and subsequently its importance for each of the diseases concerned can be determined.

The major stages of the mechanism of transmission of an external signal into the cell will be considered. After the signal molecule joins the receptor at the membrane surface, intracellular phosphoinositide-specific phospholipase C is activated through a number of mediators. This enzyme splits diphosphorylated derivative of phosphatidylinositol to yield two products: inositol triphosphate and diacylglycerol, which have a most important role to play in the subsequent development of response. Inositol triphosphate is a small water-soluble molecule, which provides liberation of calcium ions from their storage inside the cell. An increase in concentration of Ca^{2+} in the cell shifts equilibrium between the soluble and membrane-bound forms of intracellular protein kinase C (PKC, EC 2.7.1.37). As a result, the enzyme moves from cytosol to the inner surface of plasmatic membrane. The diacylglycerol localized in it

interacts with diffused enzyme and ensures its activation. The activated PKC phosphorylates the specific serine or threonine residues of various target proteins, which finally changes their functional activity in response to an external signal.

The above-described mechanism of signal transmission provides a basis for a number of processes. In fact, under the effect of vasopressin, glycogen is broken down in the liver. Also, acetylcholine stimulates secretion of amylase by pancreas cells, insulin hormone, and determines contraction of the smooth muscles. Three processes should be particularly stressed as follows: 1) activation of various somatic cells under the action of high glucose concentrations; 2) activation of neutrophils, and 3) processes of cell differentiation and proliferation. The former two are responsible for development of diabetic complications and the latter is responsible for malignant growth.

The above indicates that *regulation of PKC activity* is not only *common*, but at least, a *major* process responsible for both development of diabetic complications and tumor growth. This assumption naturally calls for some more detailed discussion, which is found below. Since PKC plays such an important role, let us consider some essential information about the enzyme concerned.

Repeated crossing of signal pathways initiated by different ligands form and maintain both intracellular homeostasis and functional activity of the cell. Signal transmission has an important role to play particularly in the processes of tumor growth and metastasis. Numerous findings over the last decade demonstrate that the PKC enzyme is a key molecule responsible for the development of a signal that is to determine the entire cell metabolism.

PKC is represented by a large multigene family of isoenzymes, which vary in their structure, expression in different tissues, type of activation and substrate specificity. As already noted, PKC catalyzes the reaction of phosphorylation of serine and threonine protein residues. Depending on the type of cofactor required for expression of its activity, the entire family of isoenzymes is conventionally divided into three classes.

The so-called conventional PKC-subclass (cPKC) consists of α, β, and γ isoenzymes manifesting Ca^{2+}- and diacylglycerol-dependent cofactor activity. The enzymes pertaining to this class are characterized by all the characteristic PKC properties.

The isoenzymes δ, ε, η, and θ form a so-called "novel" PKC subclass (nPKC), whose activation requires the presence of diacylglycerol as a cofactor but does not depend on the concentration of calcium ions.

Also identified are three atypical PKC isoforms (aPKC) λ, ζ, and ι, whose activity does not depend on the presence of any PKC cofactor.

There are grounds to believe that in addition to different localization and different mechanisms of activation and presence of PKC isotypes, of great importance to the development of cell response is *duration* of the effect on the enzyme. Presumably, brief activation of PKC initiates some processes which do not last long such as secretion or opening ion channels. By contrast, long-term activation of PKC may play a decisive role in the processes of cell proliferation, differentiation, and carcinogenesis [43, 44].

III. PROTEIN KINASE C AND PROCESSES OF MALIGNANT PROLIFERATION

It appears that there is no other field of research except oncology where the solution of purely scientific problems is so intimately associated with the pragmatic objective of finding a new effective method of cancer control. The history of this science abounds of cases where a sound theory remains only pure knowledge (if indispensable) without any application. A classic example is Warburg's theory. The numerous attempts to verify this theory have led to discovery of some fundamental properties of cancer cells. In particular, it has been revealed that disturbance of cell respiration is a consequence rather than cause of tumor development, and the rates of malignant proliferation for many tumors are directly dependent on the intensity of glycolysis. Nevertheless, that knowledge, unfortunately, still remains beyond the scope of practical medicine—no one has yet obtained a method for effective inhibition of glycolysis in tumors.

For that reason, investigation of the role of PKC isotypes in the processes of proliferation and malignant transformation of cells is of great interest particularly from the practical viewpoint as it has been revealed that regulation of the activity of those enzymes may markedly affect tumor growth [45-48]. Thus, among other things, it has become possible to find a carcinogenesis mechanism whereby the process of malignant proliferation can be affected.

Data on the activity and the role of PKC isotypes in malignant cells vary over a wide range depending on the tumor type. For instance, in tumor cells of the liver, the quantity of PKC-α isoform is substantially lower—in inverse proportion to the tumor size. At the same time, the levels of PKC-δ and PKC-ζ both in cytosol and in the membrane fraction of cancer cells were higher [49]. An increased expression of PKC-α in human mammary cancer cells is attributed to augmented tendency of the tumor to metastasizing [50], and declined cPKC activity both in cytosol and membrane is thought to be a biological marker of colorectal cancer [51]. Specific regulation of PKC isoenzyme activity can not only augment the action of cytostatics [52-54], but as already mentioned, directly affect tumor growth. The above is illustrated by data of the table.

Compounds capable of affecting PKC activity formed a new class of anti-cancer remedies that are cur-

PKC-isoforms whose activity regulation affects tumor growth

Kind of tumor cells	PKC	PKC regulation: activation (↑) suppression (↓)	Result of action	Literature
Metastatic human colon cancer cell lines KM12L4a	α	↑	"... induced resistance to multiple anticancer drugs ..."	53
Neuroblastoma cells SK-N-BE(2)	β1	↓	"... suppressed growth and enhanced the effect of anticancer agents ..."	54
Breast tumor cells MCF7	not determined	↑	"... marked reduction in the growth ..."	55
Human glioblastoma cell lines	α, ε	↓	"... no effect on proliferation ..."	56
	z	↓	"... blocked proliferation ..."	
Human fibrosarcoma cells HT 1080	α, γ, β1, but not β2 or δ	↓	"... stimulates locomotion and formation of crescent shape cells ..."	57
Rat liver epithelial tumor cells GP7TB	α	↓	".... success in suppressing tumor growth *in vivo* ..."	58
Prostate cancer cell lines PC3, PC3M, DU145	not determined	↓	"... inhibition of cancer cell growth ..."	59
Walker 256 carcinoma cells	α, β1, β2	↑	"... activation of PKC is a stop signal for tumor cell locomotion ..."	60
Human melanoma cells	α	↓	"... metastasis was suppressed by 75% ..."	61
Human pancreatic cancer cells	α	↑	"... induced block in cell proliferation ..."	62

rently at different stages of clinical trials [44, 48, 63-66]. As follows from data presented in the table, depending on the type of tumor, the action of the same effector can, in principle, both stimulate and inhibit tumor proliferation. The majority of compounds with the above properties are only known under code names, for instance, LY379196, Ro31-8220, A23187, H7, UCN-01, CGP 41251, or Go 7874, presumably for commercial reasons. A number of them are analogs of such natural PKC inhibitors as calphostin C [56], staurosporine, or bryostatin [67, 68]. The latter is characterized by a wide range of action that is manifested in initial activation and subsequent PKC inhibition. The literature cites examples of successful utilization of bryostatin in the therapy of some cancer types [68-70].

Lack of understanding of the role of PKC isoforms in the processes of carcinogenesis of various types of tumors is a serious obstacle in the search for new remedies with predetermined specificity. From that viewpoint, it should be noted that methods for gene therapy of malignant cells are very promising. Using oligonucleotides complementary to respective RNA/DNA [58, 61, 71, 72], or vectors encoding or expressing, respectively, different regions of PKC isoforms, it is possible to differentiate their individual contribution to tumor growth [54].

Let us consider some processes managed by the family of PKC isoenzymes and characteristic of carcinogenesis. A number of authors have indicated a relationship between the activities of PKC isoforms and such protein effectors as p53 or tumor necrosis factor (TNF-α). Both

these molecules belong to the class of tumor growth inhibitors. In fact, it has been revealed that the rate of p53 phosphorylation is inversely proportional to PKC activity, and application of PKC inhibitors augmented its antimetastatic effect [73]. For instance, inhibition of PKC-α, whose augmented expression is associated not only with progressive development of mammary cancer [50], but also with glioblastoma multiform, promoted activation of protein p53, which in turn augmented apoptosis of cancer cells [72].

A similar effect of inhibition of malignant growth was also recorded in experiments with malignant cells LoVo of the intestine. But in that case, PKC activation considerably augmented the antiproliferative action of TNF-α [74]. An opposite effect was observed in experiments with CMF-7 cells (mammary cancer): deceleration of apoptosis of tumor cells was attributed to decline of cytotoxicity of the necrotic factor under the action of PKC-activated PKC-η-isoform [75]. Generally, the number of processes induced in cancer cells by the interaction of PKC isoenzymes and TNF-α is extremely high. Available data indicate that the interaction of PKC and cytokine TNF-α is rarely determined by direct causal links, but is accomplished via a chain of numerous mediator molecules or processes. Hence, it appears to be impossible to regard any one of them as decisive.

Nevertheless, taking into account the subject of the present communication, phosphorylation processes of the first insulin receptor substrate-1 (IRS-1) under the effect of both activated PKC and TNF-α, merit special attention. The fact is that the level of IRS-1 phosphorylation determines its substrate properties in the reaction catalyzed by tyrosine kinase of the insulin receptor. The higher the phosphorylation level of this messenger molecule, the less is its ability to bring the insulin signal. According to modern concepts, the above processes are basic in developing insulin resistance in the cells of peripheral and adipose tissues, whose metabolism is largely determined by the action of insulin. That means that in some types of tumors, therapy methods, and metabolic processes of carcinogenesis *per se* may induce the condition of insulin resistance [76-79].

Let us illustrate the above by some examples. The already mentioned cancer cells CMF-7 show multiple changes in the expression of various PKC isoforms. It has been revealed that the concentration of IRS-1 in those cells is substantially lower and at the same time can be regulated by PKC-effectors such as bryostatin-1 or staurosporine [76]. According to other authors [80], in stomach cancer patients the TNF-α level was correlated to the level of insulin resistance.

Thus, analysis of molecular mechanisms of tumor growth on the PKC activity of isotypes has unexpectedly brought up the familiar problem of insulin resistance in carcinogenesis. Non-sensitivity of the cells to insulin is, at the same time, one of the main components of type 2 diabetes mellitus, determining the high level of hyperglycemia in this disease. Long-term hyperglycemia, in its turn, also causes the condition of insulin resistance and initiates various pathological processes that are characteristic of diabetes. We shall now consider the major processes that lead to a pathology defined in the literature as diabetic complications.

IV. EFFECT OF HYPERGLYCEMIA ON SOMATIC CELLS AND NEUTROPHILS. THE MECHANISM OF VASCULAR COMPLICATIONS IN DIABETES MELLITUS

The investigation of metabolic processes in different somatic cells (for instance, in the cells of endothelium, retina, or nerve cells) placed in solutions with high glucose content has demonstrated that both brief [81] and long-term hyperglycemia is responsible for stimulation of processes involving inositol phospholipids and diacylglycerol. In fact, PKC content declines in cytosol and the activity of this enzyme is augmented in the membrane; the membrane Na^+,K^+-ATPase is inhibited, the metabolism of arachidonic acid and a number of other processes is stimulated [82-84]. Information about the status of particular PKC isoform needs to be specified. Normally, hyperglycemia determined by diabetes promotes expression in different cells of virtually all the PKC isoforms. At the same time, some conflicting information has been obtained. We do not specify the available data [85-90] because today they are being intensively accumulated.

The hyperglycemia-induced changes in cell metabolism are largely associated with pathological processes concurrent with diabetes [91-94]. For instance, activated PKC can manage cell insulin resistance by phosphorylation of serine residues of the insulin receptor [91] and IRS-1 [77] and also through inhibition of phospholipase C—the post-insulin-receptor signal protein [95]; high concentrations of glucose and free fatty acids activate NAD(P)H-oxidase via PKC stimulation, promote formation of oxygen radicals [96] and, for example, vascular endothelial growth factor—cytokine, involved in the processes of development of vascular complications by diabetes (the PKC inhibitors handicap hyperglycemia-caused intensive generation of this molecule [97]), etc.

The present-day literature contains numerous similar examples. Similarly to carcinogenesis, it is premature to evaluate the contribution of any of them to the process of development of diabetic complications on the basis of modern knowledge. Notwithstanding, it is clear that PKC activation by hyperglycemia is, most certainly, the key event as the use of specific PKC inhibitors prevented or caused remission of early diabetic complications [86, 98, 99].

At the same time, attempts to interpret the entire complex of pathological processes regarded as diabetic complications on the basis of intracellular mechanisms alone appear to be doomed for failure. In fact, some substantial disorders in such regulatory extracellular systems as blood coagulation, fibrinolysis, kininogenesis, or complement system are also characteristic features of complications in diabetes [100-102]. Therefore, the pathological mechanisms responsible for the development of diabetic complications can be conventionally divided into two classes as follows: one class comprises *intra-* and the other *extra*-cellular processes.

Now let us try to define the mechanisms causing dysfunction of the above extracellular processes. For that, let us primarily point out two fundamental phenomena as follows.

1. The pathways of transmission of the signal into the cell as a result of interaction of neutrophil with the effector are determined by the above scenario involving participation of inositol phospholipids, diacylglycerol, PKC, etc. [103].

2. Diabetes mellitus patients show numerous changes in metabolism and functional activity of the above cells [104-119].

These data give grounds to believe [104, 120] that long-term hyperglycemia causes "excitation" of neutrophils that is identical or similar to the state of activation. It appears that in the case concerned the term "subactivation" better reflects the state of neutrophils and the multi-step process of their activation. Presumably, under long-term hyperglycemia only preliminary activation of the cells occurs, which is incomplete. This assumption is indirectly confirmed by numerous authors who point out a pronounced condition of neutrophil lower functional activity in diabetes mellitus patients.

Transition into the state "excitation" of polymorphonuclear leukocytes brings about substantial increase in the number of extracellular pathological processes destroying the entire organism. The above assumption is, in principle, debatable not only due to lack of direct evidence of activation of neutrophils with high concentrations of glucose but also due to some contradictory experimental data, even by the same authors. However, the causes of this seeming contradiction provide another piece of indirect evidence of intensive involvement of neutrophils in the development of vascular complications of diabetes.

In fact, unequivocal demonstration of neutrophil activation under the action of long-term hyperglycemia *in vivo* is as difficult as that *in vitro*. *In vivo*, and to be more exact in the blood, complete substitution by further portion of precursors of bone marrow occurs every 10-20 h, whereas *in vitro* polymorphonuclear phagocytes survive only for several hours. In both cases it is by far insufficient for transfer of neutrophils to a state of "excitation" by high glucose concentrations. However, being removed from the bloodstream, neutrophils do not die but live for another 96-120 h. Before irreversible migration to the tissues, neutrophils remain attached to the endothelium of smaller vessels for some time to develop a parietal marginal pool, which is in dynamic equilibrium with the circulating cells. The parietal pool is thus regarded as a variety of depots to urgently replenish the intra- and extravascular population of leukocytes [103].

Presumably, the maximum possible neutrophil lifespan both in the circulating and marginal pools is exactly the minimum period for its "excitation" by hyperglycemia. Because in that case the process of neutrophil activation is "slow" (3-5 days), occasional decline of the glucose level will significantly lessen the possibility of recording any deviations in their functional activity. In other words, a considerable relationship between the level of sub-activation of neutrophils and the level of hyperglycemia should be expected.

The above protracted period required for transfer of neutrophils into a state of excitation under the effect of long-term hyperglycemia is the major cause of certain contradiction of data available on the role of neutrophils in the process of development of diabetic complications. For instance, according to [104] the rate of generation of anion superoxide with non-stimulated neutrophils in diabetic patients almost three times exceeds the control values. The studies repeated after several years indicate some significantly smaller difference in this parameter [121]. The obvious difference in conclusions can be seen with respect to other parameters—adhesion capacity [122], chemotaxis [106, 123, 124], phagocytosis [107, 125], bactericidal potential [106, 107, 121, 125, 126], or the rate of oxygen consumption [109, 120, 127]. This wide range of variation of the concepts and data can be readily accounted for by the different case history of patients whose neutrophils were used in the experiments.

Let us now see if there are any accurate data available to make a reliable conclusion regarding the status of neutrophils in diabetes mellitus.

1. It has been demonstrated that the concentration of neutrophil elastase in the blood of diabetes patients is over 30% higher than normal [128-130]. According to the authors, the revealed fact unequivocally demonstrates activation of neutrophils in patients under study among other major causes of the development of vascular complications in diabetes.

2. A well-defined correlation between the augmented activity of alkaline phosphatase and the level of glycosylated hemoglobin in diabetes patients has been found [131]. If the latter value characterizes the case history of the patient over the last several weeks prior to measurements, i.e., the level of compensation of the disease over that period, increase in the activity of alkaline phosphatase is normally associated with activation of neutrophils. [132].

3. In about 40% of cases, diabetes patients reveal autoantibodies against myeloperoxidase [133]. This fact

clearly reflects the status of chronic activation of phagocytes and, as a consequence, sensitization of the organism by autoantigen released into the extracellular environment.

4. Luminol-dependent chemiluminescence is a major method of neutrophil activation. It is thought that the main causes of luminescence are the generation of oxygen-containing and other radicals in the systems of NAD(P)H-oxidase and myeloperoxidase, and also the processes associated with oxidation of arachidonic acid. Non-stimulated neutrophils from diabetes patients are characterized by high values of luminol-dependent chemiluminescence [120], generate a higher number of superoxide radicals per unit time, and more actively recover tetrazolium [104, 116]. These data also unequivocally indicate the state of activation of the leukocytes under study.

5. The proposed activation of PKC of neutrophils under the action of long-term hyperglycemia [120] must trigger a whole range of metabolic processes in the cell membrane, and in particular, promote increase in activity of NAD(P)H-oxidase [134, 135]. In turn, the activation of this enzymatic complex should be manifested in augmented rate of oxygen consumption by non-stimulated cells. In fact, non-stimulated neutrophils from diabetes patients exhibit substantial increase in the rate of oxygen consumption [120, 127].

The above data unequivocally indicate that: a) the neutrophils of diabetes patients are in a state of "excitation", which may, presumably, be regarded as that of subactivation, and b) the most plausible cause of functional stress of the cells is long-term hyperglycemia.

By and large, there are grounds to conclude that at a molecular level the major process responsible for development of diabetic complications is the activation of intracellular PKC by long-term hyperglycemia. Whereas common somatic cells (for instance, the cells of endothelium or retina) in that case show disorders of their own metabolism, transition into the state of "excitation" of such effector cells as phagocytes brings about an avalanche-like increase in the number of pathological processes destroying the entire organism.

In relatively compensated diabetes with moderate duration and level of hyperglycemia, the above pathological processes are much less severe. However, on account of the long duration of repair processes the injuries occurring during the brief disease decompensation periods can gradually accumulate in the body to form the clinical picture defined as vascular complications of diabetes mellitus.

With special reference to the objectives of the present study, one of the pathological processes involved in diabetes mellitus induced by "active" neutrophils calls for detailed consideration. This is the generation of methylglyoxal—a compound that can have an important role to play in the development of diabetic complications and in carcinogenesis.

V. METHYLGLYOXAL AND DIABETES MELLITUS

The augmented "respiration" of non-stimulated neutrophils from diabetes mellitus patients is actually a "protracted respiratory burst". In fact, the major energy source of neutrophils is glycolysis, and oxygen is not required by phagocytes for life support. As a result, all the respective processes are activated in the cell. There is an increase in the rate of glucose consumption and lactate synthesis [136], and the concentration of superoxide anion [104, 137] and hydrogen peroxide [138] increases. The processes of formation of phagolysosomes, trapping extracellular content and degranulation of azurophilic granules are activated. In fact, according to the data of [121] the intracellular activity of myeloperoxidase may decrease almost twofold in diabetes mellitus patients. As noted by other authors [139], the myeloperoxidase activity of leukocytes depends on the severity of the disease: the lowest values were recorded in patients with a severe form of diabetes mellitus. All specialists agree that lower activity of myeloperoxidase in phagocytes in this case is accounted for by release of the enzyme into the extracellular space. One required proof is already mentioned, sensitization of the organism by autoantigen and finding of respective autoantibodies in about 40% of diabetes mellitus patients [133].

Thus, neutrophils activated by hyperglycemia secrete various effector molecules into the bloodstream, in particular, myeloperoxidase and hydrogen peroxide. On the other hand, hyperglycemia initiates accumulation in the blood of ketone bodies to an extent directly proportional to the level of diabetes compensation. Moreover, round-the-clock monitoring demonstrates that the concentration of ketone bodies irrespective of the level of disease compensation, the number of insulin injections, or administration of sugar-lowering drugs invariably exceeds the normal [140]. Thus, the blood of diabetes mellitus patients provide conditions under which myeloperoxidase and H_2O_2 released from azurophilic granules promote the process of enzymatic oxidation of acetoacetate to form highly reactive α-oxoaldehyde—methylglyoxal.

Before discussing the possible mechanism of formation of methylglyoxal and its role in the development of pathological process in diabetes mellitus patients, the following reservation should me made. The nonenzymatic pathway of *in vivo* methylglyoxal formation from intermediate glucose metabolism compounds such as glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (the Embden–Meyerhof pathway) is commonly accepted. However, in diabetes mellitus a complementary (and, in our opinion, the main) source of methylglyoxal formation is the enzymatic process [20, 141-144].

In fact, in this pathology alone, i.e., due to disorder of carbohydrate metabolism, the blood of the patients demonstrates a substantial rise in the concentration of acetoacetate [145-148]. Methylglyoxal is also a product

of catalytic oxidation of acetoacetate under the action of oxygen or hydrogen peroxide in the presence of myoglobin or such enzymes as peroxidase, myeloperoxidase, lactoperoxidase, prostacyclin synthetase, or prostaglandin synthetase. The authors who demonstrated this emphasized that the process concerned may be the basis of vascular disorders in a number of diseases, and, in particular, in diabetes mellitus.

Our own [120] and literature experimental evidence available has led us to prove that under the effect of long-term hyperglycemia, neutrophils from the patients with diabetes mellitus may be transferred into a state similar to "excitation" and, hence, serve as a source of generation of both hydrogen peroxide and myeloperoxidase. Thus, there are ample grounds to believe that in case of a low level of diabetes mellitus compensation in addition to intensification of the already known metabolic pathways [20, 141-144], the enzymatic process of acetoacetate oxidation becomes a complementary and powerful source of biosynthesis of methylglyoxal in the body.

As shown in [149], the mean statistical concentrations of methylglyoxal in the blood of diabetes mellitus patients are virtually 6-fold (in type 1 diabetes patients) and more than 3-fold (in type 2 diabetes patients) higher than the normal (80 nM), averaging, respectively, 470 and 290 nM. The authors relate the data concerned to the diabetes mellitus type and also disease duration. However, in our opinion, the above values proved only augmented generation of methylglyoxal in diabetes mellitus patients. In fact, the current concentration of methylglyoxal must, primarily, depend on the level of compensation of the disease rather than duration or type of diabetes mellitus. This is demonstrated by the finding of positive correlation between the concentrations of glycosylated hemoglobin and methylglyoxal in the blood [150].

The potential mechanisms of possible pathological action of methylglyoxal on the body are diverse, which may explain why they are not yet accurately identified. Primarily, it should be pointed out that methylglyoxal can react with primary amino groups of diverse protein molecules. As a ketoaldehyde, methylglyoxal shows unique ability to specifically interact with molecules containing the guanidine grouping, for instance, arginine. Based on this property of methylglyoxal, let us try to characterize a number of important extracellular pathological processes forming characteristic features of diabetic complications.

The augmented concentration of methylglyoxal must, in principle, inhibit the biological activity of such proteins whose active center contains arginine. The presence of the above amino acid in the P1 position of active centers of antithrombin III and C1-inhibitor is not the only structural similarity between these two proteins. Being members of the same multigene family (serpine multifamily), they also show similarity in a wide range of homology of the DNA copies, the mechanisms of their action, and functional activity. Antithrombin III and C1-inhibitor ensure a fine mechanism of the regulation of a cascade of consecutive enzymatic reactions in the systems of blood coagulation, kininogenesis, and complement, profoundly involved in the process of formation of vascular complications of diabetes mellitus. The pattern of the revealed metabolic disorders in this case suggests that a probable cause of their origin is *concurrent* reduction in the activity of the above proteins.

A possibility of inactivation of both antithrombin III and C1-inhibitor under the action of methylglyoxal was demonstrated by us *in vitro* [120]. At the same time, there is evidence available of substantial decrease in the activity of antithrombin III in diabetes mellitus patients without changes in the concentration of this protein [151, 152]. These data are suggestive that under the same conditions the process concerned may occur with C1-inhibitor also, primarily, under heavy decompensation of diabetes mellitus, promoting a sharp increase in methylglyoxal concentration.

Inactivation of Cu,Zn-superoxide dismutase must be based on a similar mechanism. The deficiency of activity (but not concentration!) [153] of this enzyme in diabetic patients, which promotes accumulation of free radicals, has been recorded by various authors [137, 154]. In this case the level of inhibition of the activity of the enzyme concerned, which also contains arginine in its active center, was correlated to the concentration of glycosylated hemoglobin and, hence, to the level of decompensation of diabetes mellitus [153].

There are a number of other examples available of the detrimental effect of methylglyoxal on the organism in diabetes mellitus. However, the body of data available (the publications started appearing as recently as the end of the 1980s) is evidently insufficient for more accurate assessment of the role of methylglyoxal, providing only a general outline of future research.

At the same time, the capacity of methylglyoxal to inhibit the process of cell proliferation is long and well known.

VI. METHYLGLYOXAL AS AN INHIBITOR OF CELL PROLIFERATION

The property of methylglyoxal, an intermediate compound of carbohydrate metabolism, to effectively inhibit the process of cell division is well known. Thanks to this property methylglyoxal has become one of the key molecules in the theory of carcinogenesis proposed by Szent-Gyorgi [155]. According to this theory, the regulatory mechanism of the process of cell division is a function of interaction of its two antagonist components referred to as *retine* and *promine*. The role of *retine*, responsible for the state of cell rest is ascribed to methylglyoxal (although there existed different views [156]), and *promine* is associated with glyoxalase enzyme system or its cofactor—glutathione.

The criticism of this work is well stated in a number of publications [156, 157]. Although from the viewpoint of current concepts a number of aspects of the Szent-Gyorgi hypothesis have not been confirmed [157], it gave an impetus to a new trend in the search of cancer remedies as will be demonstrated below.

Over 40 years since the publication of the theory concerned, the role of the glyoxalase system and methylglyoxal in the processes of proliferation has received much attention. However, it is not yet understood whether methylglyoxal is an endogenous regulator of the process of cell division [155]. Nevertheless, the possibility of methylglyoxal having this function appears to be very attractive.

Practically, it implies development not only another therapy method but also the method for preventive cancer therapy based on the maintenance of different levels of methylglyoxal concentration in the cell. However, in contrast to the regulatory functions, depression of malignant growth can be attained at concentrations of methylglyoxal significantly exceeding physiological. This implies two options as follows: either exogenous methylglyoxal should be used or methods for manipulation of the processes regulating the concentration of this compound *in vivo* should be learned.

An injection of methylglyoxal at 40-100 mg/kg (and higher) to animals with different types of malignant tumor have revealed a possibility of its utilization as a cancer remedy [158-161]. Virtually all the types of tumors under study, both *in vivo* and in the cell culture [161-163], revealed a dose-dependent effect, i.e., an increase in concentrations of administered methylglyoxal intensified the process of degradation of tumor growth. In a number of cases malignant cell proliferation was completely depressed.

The possibility to regulate the concentrations of endogenous methylglyoxal appears more feasible via regulation of the activity of the glyoxalase enzyme system converting methylglyoxal to D-lactate. In fact, proceeding from the well-established concepts of the pathways of formation of methylglyoxal in the cell [141-144], it is difficult to accelerate the process of its biosynthesis in any possible way. At the same time, inhibition of the main pathway of catabolism of methylglyoxal will permit accumulation of this compound in the cell.

With that purpose, numerous alkyl-derivatives of S-glutathione were synthesized and tested [164-171] as well as a number of other compounds capable of playing the role of concurrent inhibitors of the first enzyme of the glyoxalase complex. Many of them were characterized by their ability to depress the growth of cancer cells *in vitro* and proved inefficient *in vivo* due to rapid degradation under the effect of a number of enzymes. However, in recent years, synthesis of new glutathione derivatives resistant to enzymatic hydrolysis and penetrating into the cell has been reported. Moreover, their toxicity in relation to some tumor types both *in vitro* and *in vivo* has been demonstrated [170, 171].

How is cell proliferation inhibited by methylglyoxal? Taking into account the high reactivity of methylglyoxal, it is highly probable that there are several underlying inhibition mechanisms. The possible consequences of the pathological effect of methylglyoxal in diabetes mellitus have been considered above. Let us now supply a few other examples reflecting the effect of methylglyoxal on the metabolism and structure of compounds whose functional activity is directly associated with processes of cell proliferation. Among those processes is inhibition by methylglyoxal of biosynthesis of polyamines essential for cell growth and differentiation [172-175] and also inactivation of a series of Arg-dependent enzymes ensuring glycolysis and respiration of cancer cells [176-180].

The capacity of methylglyoxal to modify chromatin, and, to be more particular, histones—proteins with a high content of lysine and arginine, which not only form complexes with DNA but also structurally organize genetic material during cell division—should specially be mentioned. According to the latest studies, hyperacetylation of histones caused, in particular, by the action of valproate, substantially checked both *in vivo* and *in vitro* growth of tumor cells and their metastatic process [181-184]. This explains why the fact of increased content of acetylated histones in diabetic rats should be taken into account [185].

Moreover, methylglyoxal can also exert a direct effect on the cell genetic material, generating "aggressive" hydroxyl radicals [186] to cause point mutation in the genes [187] or directly interacting with DNA base amino groups, and, primarily, with guanine [162, 188-190].

Thus, an increase in methylglyoxal concentration *in vivo* with injections or due to the inhibition of the glyoxalase system should promote suppression of malignant cell proliferation. It should be emphasized that injections initially increase concentrations of methylglyoxal in the extracellular space, whereas inhibition of the glyoxalase system causes accumulation of methylglyoxal primarily in the cell. However, as follows from the literature the cytostatic properties of methylglyoxal are manifested (to a varying extent) in both the above cases.

In fact, methylglyoxal exerts a considerable effect on the processes of malignant cell proliferation. For that reason, pessimistic forecasts [144] regarding the possibility of utilizing methylglyoxal in cancer therapy do not appear to be completely justified. Very promising in this respect are the latest findings on the use of inhibitors of glyoxalase I, which are stable *in vivo* [170, 171, 191]. Blocking the system of de-intoxication of the cell, these compounds promoted creation of local high concentrations of methylglyoxal, capable of considerably inhibiting malignant growth. The authors of [171] claim that in terms of

effectiveness, the therapeutic effect of the application of the above inhibitors was only comparable to the action of other cytostatics. As shown by one of the latest reports [192], the augmented expression of glyoxalase I in human leukemia cells prevented the antitumor agent-induced apoptosis.

As was shown above, diabetes mellitus is associated with augmented generation of methylglyoxal, so let us ask ourselves a seemingly queer question: "Could diabetes be used as a "generator" of high concentrations of methylglyoxal in cancer therapy?"

VII. CAN DIABETES MELLITUS BE USED IN CANCER THERAPY?

A negative answer to the heading question is almost evident. As the German proverb says, it is very dangerous to expel the devil with the help of Beelzebub (the son of the devil) even if the prospects of diabetes mobilization in the fight against cancer are very alluring. Indeed, otherwise, the organism will inevitably die either from cancer or from induced diabetes. Moreover, as follows from numerous experiments, manifestation of diabetes mellitus inhibits malignant growth only for a brief period, whereupon neoplasm development recurs at the original rate. Nevertheless, the investigation of the mechanism of diabetic complications development is suggestive that the answer is not necessarily negative.

Thus, the objective of the present paper was an attempt to find out why diabetes mellitus inhibits the development of neoplasm. It has been proposed that the inhibition of tumor growth in that case is a consequence of pathological processes responsible for development of diabetic complications. We have considered the main (in our opinion) mechanisms of these processes to find out the following.

1. The regulation of PKC activity is not only *common*, but at least a *major* process responsible for both development of diabetic complications and tumor growth.

2. Long-term hyperglycemia normally activates the majority of isoenzymes of the PKC family.

3. The regulation of the activity of PKC forms promotes suppression or activation of malignant growth depending on the type of cancer.

4. The duration of hyperglycemia transfers both somatic cells and neutrophils into a state of excitation, which can be regarded as a state of sub-activation.

5. The enzymatic oxidation of acetoacetate under the action of myeloperoxidase (which emerges from azurophilic granules of activated neutrophils) and hydrogen peroxide can serve as an extra strong source of methylglyoxal generation.

6. Methylglyoxal is an effective inhibitor of the processes of cell proliferation.

Hence, consideration of the possibility to use diabetes mellitus in the therapy of some particular types of tumors shows that it is not at all necessary to induce diabetes. It would be sufficient to find methods of triggering the above processes in the oncological patient during the course of therapy. In other words, it is necessary to learn to maintain *in vivo* the condition of long-term hyperglycemia and ensure augmented generation of methylglyoxal. Let us consider the existing options to compare them with the already known therapy methods.

VIII. HYPERGLYCEMIA AND CANCER THERAPY

Induction of hyperglycemia in oncological patients is no longer a new method for cancer therapy. Usually, development of short-term hyperglycemia is only used as part of an integrated multi-stage therapy. The essence of this approach consists in selective sensitization of the tumor to the action of various injury factors as thermo-, chemo-, and radiotherapy [193-195].

Hyperglycemia promotes intensification of glycolysis in cancer cells, owing to their particular preference for glucose. In its turn, the tumor accumulates excess of lactic acid, and, respectively, the tissue is acutely acidified. Increased acidity during the therapy period renders cancer cells more vulnerable, whereas in the normal tissue this parameter remains within normal values [1, 196, 197]. In a number of cases, this method considerably augments the effect of standard monotherapy [198-203]. For instance, post-therapy life expectancy data demonstrates that a 3-year survival in 69 rectal cancer patients who received hyperthermia–hyperglycemia–chemotherapy was 77%! [204]; and in experimental study on Guerin carcinoma rats, hyperglycemia and chemotherapy increased life expectancy by up to 6 times [203]. With combined use of hyperglycemia and hyperthermia 5 of the 20 experimental animals showed 100% disappearance of the tumors [196]. Clearly, the best results should be expected from the use of the above therapy pattern to control tumors with intensive glycolysis.

Thus, short-term hyperglycemia is a kind of adjuvant therapy, which is no therapy in the exact sense of the word, but only has a potency increase effect. In addition to the effect of acidification of cytosol of cancer cells, repeated injection of glucose concentrated solutions to oncological patients shows marked nitrogen-saving effect [1], reduces the rate of capillary blood flow to the tumor [195, 205], lessens catabolism of tissue proteins [1], positively changes the pharmacokinetics of the action of some cytostatics [206], and is strongly recommended by some physicians for practical use in the therapy of various types of malignant tumors, for instance, mammary cancer [207].

What effect on the tumor *in vivo* is exerted by long-term hyperglycemia of at least 75-100-h duration? That is

exactly the time required for prolonged activation of PKC isoenzymes under glucose effect [82]. Unfortunately, it is not easy to give an exhaustive answer to this question, for the respective data are lacking in the literature available. To be more exact, we have failed to find a description of experiments where induced hyperglycemia would be maintained in experimental animals for such a long period. At the same time, the term "long-term hyperglycemia" for many authors implies daily and brief (several-hour) induction of hyperglycemia recurring for several weeks [1]. Hence, let us analyze what theoretical answer could be expected.

Thus, long-term hyperglycemia, lasting at least 75-100 h, similar to carcinogenesis processes, cause dysfunction of the cell signal system controlled by PKC isotypes. Hyperglycemia activates the majority of PKC enzymes. The pattern of changes of PKC in tumors is determined by the cancer type. Changes in the activity of subcomponents of the PKC family exert a great effect on cell proliferation. Hence, hyperglycemia may both "compensate for" and "aggravate" dysfunction of PKC isoenzymes as determined by carcinogenic processes. This implies that long-term hyperglycemia may act as both a *promoter* and *inhibitor* of tumor growth, promoting development of some and suppressing the growth of other tumors. The result of this interaction is determined by the type of tumor, the stage of its development, and duration and level of hyperglycemia.

The above examples of detrimental effect of long-term hyperglycemia on neoplasm development as determined by diabetes mellitus may, strictly speaking, reflect the effect of both hyperglycemia and other diabetes-induced processes. To make sure that the contribution of hyperglycemia to those processes is decisive, examples are required where hyperglycemia also induced by diabetes mellitus would not suppress, but conversely, promote the development of cancer.

One of the latest reviews treats the interrelationship between diabetes mellitus and cancer in the following way: "... an association between diabetes and cancer was found over 100 years ago, the issue underwent different interpretations over the subsequent decades, and only modern, prospective, epidemiological cohort and case-control studies... have provided *reliable evidence of an increased* cancer risk in different patients, mainly in those with type 2 diabetes. This risk varies according to the tumor site: it is the greatest for primary liver cancer, moderately elevated for pancreatic cancer, and relatively low for colorectal, endometrial, breast, and renal cancers. The cause of the association is not clear ..." [14]. True enough, one could question the authors' view regarding "reliability" of some assertions. However, it is not about terms, but rather—as was justly mentioned—about contradiction of abundant data available on the subject (see Chapter I).

Discussing the mechanisms of interrelationship between diabetes mellitus and cancer an interesting question arises why the risk of cancer incidence is mostly recorded among type 2 diabetes patients. Presumably, on account of strict control and necessary daily therapy, the level of compensation of hyperglycemia in type 1 diabetes patients is much higher than that in type 2 patients. The above answer would be correct if only incomplete. Most likely, the main reason of this phenomenon lies in the fact that type 2 diabetic patients alone, and the bulk of these patients are *insulin-resistant*. Non-susceptibility of the organism to insulin is a condition that can hardly be treated with medication, and determines not only a stable increase in the glucose level for weeks and even months, but also a number of other pathological processes also controlled by PKC isotypes. It well may be that development of a similar picture of metabolic disorders under the effect of carcinogenesis or cancer therapy is the basis of insulin resistance (see Chapter III) or incidence of diabetes mellitus [7-11, 208, 209] in oncological patients.

IX. STARVATION AND IMMUNE STIMULATION—COMPONENTS OF CYTOSTATIC CANCER IMMUNE THERAPY

Our proposal to use endogenous methylglyoxal for suppression of malignant proliferation differs little from an already known therapy method based on its *in vivo* concentration regulation (see Chapter VI). However, there is some considerable difference. Seeking to intensify the processes of methylglyoxal biosynthesis, earlier authors primarily searched for inhibitors of its utilization systems. We are using a qualitatively different approach—utilization of the process of generation of methylglyoxal by diabetes. What is the essence of this process? As already mentioned, a major source of methylglyoxal generation in diabetes is, in our opinion, the enzymatic process of acetoacetate oxidation. A catalyst of this reaction is in all probability the enzyme myeloperoxidase, which is released into the extracellular space from the azurophilic granules of neutrophils as a result of their activation by long-term hyperglycemia (see Chapter IV).

Thus, in diabetes mellitus, particularly poorly compensated for (high concentrations of ketone bodies, activation of neutrophils), some conditions arise that ensure accumulation of methylglyoxal. In this case, the rate of ketoaldehyde formation even in relatively compensated diabetes mellitus patients is so high that the glyoxalase system, responsible for detoxication of the body from methylglyoxal and undergoing no considerable changes in diabetes [149], does not appear to cope with processing of methylglyoxal, which brings about the observed increase in its concentration.

Thus, upon learning to maintain *in vivo* favorable conditions for enzymatic biosynthesis of methylglyoxal from acetoacetate for the required period of time, consid-

erable suppression of malignant proliferation can be expected. In what way can these conditions be ensured without inducing artificial diabetes? Apparently, two chief components are needed as follows: *high concentrations of acetoacetate* and *activated neutrophils*.

Numerous studies of metabolism of ketone bodies both in humans and in animals have revealed that a single dosage of acetoacetate is rapidly metabolized without any considerable consequences for the body. In the course of multiple injections, utilization of acetoacetate proceeds increasingly slowly, and finally, the animals become ill with diabetes mellitus with all characteristic disorders [210, 211]. Hence, it is impossible to use exogenous acetoacetate in order to accumulate it in the body.

A classic method for inducing ketosis and maintaining it for a long time (a condition characteristic for uncompensated diabetes [212, 213]) is *starvation*. The acetoacetate concentration starts rising considerably as early as the third day and after a week it increases about 100-fold (which is about 1 mM) to remain at this level for virtually the entire starvation period [214, 215].

Thus, from the viewpoint of the one of the objectives set in the present study—maintenance *in vivo* of high acetoacetate concentrations without inducing diabetes—starvation is a fairly simple and harmless method that requires no medication.

Considering starvation as a natural component of a hypothetic method for cancer therapy, it should be emphasized that numerous attempts for treating cancer with starvation have been made over a long period of time. This approach is based on the fact of decrease in the incidence of neoplasm and inhibition of its growth proportionally to the level of nutritional insufficiency [216, 217]. In other words, the less full-value nutrition is received by the organism, the greater will be regress of the malignant tumor that affected this organism. But according to a leading specialist in this field [216], the above studies are mainly of scientific interest, but do not appear to be of practical usage. The reason is that due to constant deficiency of nutrients, the tumor-bearing organism declines, and, conversely, with resumption of full-scale nutrition, proliferation of the tumor progresses again.

In its effect on the process of neoplasm development, there is very much in common between starvation and diabetes mellitus [38, 218]. First, the greater is the organism affected by starvation or diabetes mellitus the greater the decline of the tumor that affected this organism. Second, both starvation and diabetes mellitus are characterized by high concentrations of acetoacetate. Third, attempts are known both to use starvation or diabetes mellitus as phenomenon (in experiment) in cancer therapy and opinions that reject such approaches. At the same time, periodic starvation as a factor preventing development of malignant tumor [219] can apparently be regarded as a fairly effective method of preventive cancer therapy.

Thus, there are grounds to conclude that, on one hand, starvation favorably affects the process of neoplasm inhibition, and, on the other hand, in accordance with the concepts of the present study, is feasible for the maintenance of high concentrations of acetoacetate in the body for a long time. Hence, starvation can be regarded as an essential but insufficient component for cytostatic cancer immunotherapy.

Another essential component of the hypothetic cancer therapy is stimulation of neutrophils. As already mentioned, activated neutrophils should ensure oxidation of acetoacetate to methylglyoxal in a quantity needed for inhibition of malignant cell growth. For that purpose, there are several standard methods available, the most common among which being the use of various adjuvants.

The application of adjuvants—stimulators of cell and humoral immune response in the therapy of oncological diseases similar to starvation therapy is a developing trend in *adjuvant cancer immunotherapy*.

There are dozens of adjuvants investigated [220]. They include polysaccharide compounds, snake poisons, some vitamins, remedies from shark liver, complete and incomplete Freund's adjuvants, influenza, pertussis, small-pox vaccines, etc. The most common and best studied in cancer therapy is the tuberculosis BCG (bacillus Calmette-Guerin) vaccine [221, 222], which is applied in combination with other remedies and therapy methods [223, 224].

Among the numerous characteristics of the BCG vaccine, similar to a number of other remedies (for instance, Freund's adjuvant or streptococcus remedies) their property of stimulating neutrophils should be noted. According to a number of studies both *in vitro* and in animals, the polymorphonuclear leukocytes are capable of specifically destroying or promoting destruction of cancer cells [225-228]. The concentration of neutrophils in oncological patients is increased, whereas their effector functions have declined [229, 230]. Generally, the effect of neutrophils on carcinogenesis is not yet completely understood. There are data available which also indicate the negative role of neutrophils [231], for instance, as promoters of tumor metastasis [232]. Nevertheless, there are grounds to believe that the activation of neutrophils observed in case of adjuvant immunotherapy exerts a positive rather than negative effect on the therapy process as a whole [233]. Similar therapy against the background of high concentrations of ketone bodies should ensure, due to activated neutrophils, ubiquitous development *in vivo* of sufficient quantities of methylglyoxal, an intermediate metabolite with strong cytostatic properties.

By and large, there is every reason to believe that a *combination* of two long-known methods of cancer therapy—*adjuvant immunotherapy* and therapeutic *starvation*—may bring about an advent of new and more effective method for therapy of oncological diseases.

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