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Physiological Media in Studies of Cell Metabolism

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Abstract—Changes in cell metabolism accompany the development of a wide spectrum of pathologies including cancer, autoimmune, and inflammatory diseases. Therefore, usage of inhibitors of metabolic enzymes are considered a promising strategy for the development of therapeutic agents. However, the investigation of cellular metabolism is hampered by the significant impact of culture media, which interfere with many cellular processes, thus making cellular models irrelevant. There are numerous reports that show that the results from in vitro systems are not reproduced in in vivo models and patients. Over the last decade a novel approach has emerged, which consists of adaptation of the culture medium composition to that closer to the composition of blood plasma. In 2017−2019, two plasma-like media were proposed, Plasmax and HPLM. In the review, we have summarized the drawbacks of common media and have analyzed changes in the metabolism of cells cultivated in common and plasma-like media in normal and pathological conditions.

INTRODUCTION

The main condition for obtaining reliable results from biomedical research in vitro is the correct cultivation of cell lines [1]. Most of the work in this field is based on the use of various human, mammalian, and other eukaryotic cell lines. At the same time, many authors, especially those who study cell metabolism, often face problems directly related to the cultivation of cell lines. Sometimes, these problems are due inability of certain cell lines to be cultivated normally in standard (widely used throughout the scientific world) media: DMEM, MEM, RMPI, DMEM-F12, etc. In other cases, the chosen research vector stimulates the authors to look for an opportunity to bring the experimental format as close as possible to real in vivo conditions, i.e., to modify the composition of culture media to the composition and concentrations of extracellular metabolites in tissues.

Various tumor cell lines have become the main cell cultures for modern biomedical science. Various types of cancer comprise the dominant group of studied pathological processes due to their wide prevalence, complexity, and diversity of molecular mechanisms of occurrence. Notworthy, the tumor is a complex, 3-dimentional, dynamic, and heterogeneous structure within the body, which includes mixed populations of both normal and cancerous (differentiated and undifferentiated) cells that are closely interrelated with each other and have unique features. Despite this, almost all studies are performed with homogeneous cancer cells (lines) in the form of a monolayer (2D culture).

These studies have made a huge contribution to understanding the basic cellular processes and mechanisms of carcinogenesis and many other pathologies. However, it cannot be denied that the laboratory models used by many researchers do not reproduce all the processes of a real tumor.

One of the factors determining the difference between processes in laboratory cell models and tissue cells is the different percentage of oxygen around the cells. Most researchers work at a normal atmosphere that contains 21% oxygen with 5% CO_2 added (bringing the proportion of oxygen to 18.6%) [2]. The percentage of oxygen around the cells is lower than this value because of limited diffusion through the culture medium layer. The oxygen level in the tissues is estimated as 3.4−6.8% [2, 3], and the oxygen level in tumors is reduced compared to normal tissues [2]. Thus, the usual conditions of cultivation represent hyperoxia, which can affect many redox-dependent processes and signaling pathways mediated, among others, by transcription factors HIF-1, NF-kB, etc. [2].

Another factor is the geometry of cell culture. The vast majority of research is done in 2D cultures. In the last decade, many researchers have begun discussing the use of 3D cultures (spheroids, organoids), which consist of cells more in conditions more closely approximating the real conditions, thus providing more reliable results [4]. Although these cultures partially recreate the microenvironment of cells, the complexity of working with them and their sensitivity to

factors such as the quality of reagents, the professionalism of the experimenter, etc., sharply limit their use.

Finally, the third factor is culture media because the composition and concentrations of metabolites can affect the growth and differentiation of cells, the status of signaling pathways, and, as a consequence, the results of the experiment. In recent years, new cultivation media have appeared that mimic the composition of human blood plasma and are becoming widespread.

The purpose of this review is to show examples of violations of various cellular processes in cells cultured in traditional media and to present literature data on changes in metabolism that which occur during the transition to new plasma-like culture media.

EXAMPLES OF THE INFLUENCE OF NONPHYSIOLOGICAL COMPOSITION OF CULTURE MEDIA ON PROCESSES IN CELLS

Traditional (nonphysiological) nutrient media for cell cultures have been used since the 1950s [5−7]. DMEM, RPMI, and other media were developed to saturate cell cultures with the necessary nutrients and to maintain stable and maximally rapid proliferation of cancer cells for a long period. The vast majority of experiments related to the study of cell metabolism were carried out in these media [8].

Many classical media lack some natural metabolites that are present in human/animal blood plasma, and the concentrations of others often significantly differ from those in vivo. An example is the concentration of glucose. In the initial composition of the DMEM medium, the glucose concentration was 1 g/L corresponding to the physiological value (5.5 mM). However, its level was increased to 4.5 g/L, which corresponded to the conditions of hyperglycemia. This medium is most often used for cultures of attached cells, and most researchers do not pay attention to the importance of choosing an adequate glucose concentration. It is worth noting that the increase in the glucose concentration leads, at least, to an increase in the production of reactive oxygen species (ROS) due to an enhancement of the expression levels of some NADPH oxidase (NOX) isoforms (the data are summarized in review [9]). In turn, activation of the Nrf2/ARE protective cascade in response to oxidative stress leads to an increase in the expression level of NADPH-quinone oxidoreductase (Nqo1) [10] and enhances glycolysis [11]. An abnormally high glucose level can also trigger endoplasmic reticulum stress and a subsequent unfolded protein response [12]. Thus, the use of culture media with elevated glucose levels leads to non-physiological processes in the cells.

The most known justification for the use of high-glucose media is the Warburg effect, i.e., enhanced glycolysis, which is characteristic of most tumor cells, and its separation from the Krebs cycle [13]. Many of the cancer cell lines used are characterized by a high level of glucose uptake and its conversion into pyruvate and then into lactate [14]. However, it has been found that the glucose level in the tumor microenvironment is not increased, but decreased by 3−10 times [15, 16]. The Bisroy group has proposed an elegant approach to modeling these conditions, which consists of the constant replenishment of glucose in the medium on a Nitrostat device [17]. The authors found that cancer cell lines differ in levels of oxidative phosphorylation and, as a consequence, sensitivity to biguanides (including metformin). It is noteworthy that these differences are masked when using a standard environment.

As shown by Balsa et al. [18], a high glucose content reduces the activity of the oxidative phosphorylation system, while a decrease in its level increases the respiratory activity of mitochondria. The assembly of respirasomes (respiratory supercomplexes) takes place under conditions of normal or reduced glucose content in the culture medium, and the ATF4 transcription factor plays a key role in this process. It is worth noting that this factor is activated in response to integrative stress, which is closely related to amino acid levels and endoplasmic reticulum stress [19].

Pyruvate is another component of culture media, whose high level of which affects cellular metabolism. It is known that many cancer cell lines depend on glutaminolysis, the process of converting glutamine into glutamate and then α-ketoglutarate (a metabolite of the TCA cycle). Considering that in vitro glycolysis in tumor cells is directed to the production of lactate rather than to the Krebs cycle, glutamine becomes one of the main carbon donors for the cycle. Accordingly, inhibitors of glutaminase, which catalyzes the key stage of glutaminolysis, are considered as potential anticancer drugs [20]. It has been reported, however, that the presence of pyruvate in the medium markedly reduces the sensitivity of cells to glutaminase inhibitors [21]. It is noteworthy that the concentration of pyruvate in culture media (e.g., 1 mM in DMEM) significantly exceeds its concentration in blood plasma (0.05−0.1 mM [22]). Although pyruvate-free media are also actively used, many researchers consider this supplement as a nutrient that increases the rate of cell growth, rather than as a possible regulator of metabolic and signaling pathways.

Some scientific groups tried to develop more physiologically based media to increase the reliability of the experimental results. For example, a special Brain-Phys medium was created with a reduced concentration of neuroactive ions and amino acids compared to that in classical media [23]. This specialized medium allowed researchers to study the electrical activity of neurons. Almost simultaneously, another group of scientists developed the SMEM medium with concentrations of amino acids, pyruvate, and vitamins close

to those in human blood to study the metabolism of glioblastoma and breast cancer cells [24, 25]. However, this medium did not contain many blood components, i.e., carnitine, metabolites of the TCA cycle, etc.

These attempts to resemble as close as possible to the in vivo conditions are also driven by the fact that the in vitro data are not always reproduced in in vivo experiments including the data obtained from patients. An example of such a discrepancy in cellular metabolism studies is very strong antiproliferative activity of glutaminase inhibitors against various types of cancer lines in vitro and the absence of antitumor activity of these compounds in vivo, as is shown by the example of pancreatic cancer [26].

PLASMA-LIKE CULTURE MEDIA

The Tardito group, which earlier developed the SMEM medium [27], and the Sabatini and Cantor group [28] continued work on improving the composition of culture media. They proposed two similar culture media, HPLM (Human Plasma-Like Medium) and Plasmax, the composition of which mimics human blood plasma. These media were based on a standard mixture of EBSS inorganic salts (Earle's Balanced Salt Solution) with added vitamins and polar metabolites present in plasma at concentrations $2~\mu M$ and higher [27, 28]. The concentration of glucose in these media was 1 g/L (as in the initial version of the DMEM medium), and the pyruvate concentration was $0.05{-}0.1$ mM. Another important additive was lactate, which is normally present in human blood at a concentration of at least 1 mM [22] and is an important nutrient for cells of many tissues [29, 30]. Moreover, these media contained both nonproteinogenic amino acids and intermediates of the Krebs cycle, i.e., citrate, acetate, carnitine, and acetylcarnitine, which are involved in the metabolism of fatty acids and acetyl coenzyme A (Ac-CoA) and are important for maintaining an active oxidative phosphorylation system [31–33].

The authors emphasized the importance of unique metabolites in the composition of these media. For example, uric acid (the final metabolite of purine catabolism) regulates the biosynthesis of pyrimidine nucleotides by inhibiting uracyl monophosphate synthase. Cantor et al. showed that the cytotoxic activity of 5-fluorouracil in cells cultured in the HPLM medium is lower than that in classical media [28].

Fetal bovine serum (FBS) is almost always added to standard laboratory media to $10{-}20\%$ concentration. It consists of numerous growth factors, hormones, and trace elements necessary to ensure the stable proliferation of a wide range of cell cultures. However, FBS introduces an indefinite and usually ignored pool of polar metabolites and lipids, concentrations of which may differ depending on the lot and origin of the serum, and this composition is rarely analyzed.

There are also specialized culture media that contain albumin, transferrin, insulin, growth factors, and/or peptides or protein hydrolysates instead of the serum [34]. They support cell growth but their use is severely limited because of the high cost and the need to optimize cultivation protocols. To reduce the effect of serum on the composition of metabolites in plasma, the authors of the Plasmax medium reduced its content to 2.5%, while the authors of the HPLM medium replaced the usual serum with dialyzed serum without polar metabolites.

Finally, it should be noted that experiments with physiological media simulating human blood plasma cannot always be directly extrapolated to animals. Thus, the Cantor group [28] revealed differences in the composition of mouse and human blood plasma. They demonstrated that mouse plasma has an order of magnitude lower concentration of uric acid (the importance of which was mentioned above), which cannot inhibit the biosynthesis of pyrimidine nucleotides at such a low concentration. It cannot be excluded that differences in the concentrations of other low-molecular compounds, hormones, and growth factors also cause significant changes in cell metabolism.

DIFFERENCES IN CELL METABOLISM WHEN CULTIVATING IN STANDARD AND PLASMA-LIKE MEDIA

The use of physiological media in biological research is just beginning but the results of several scientific groups have already been published, thus proving the relevance and expediency of using these media.

Physiological media have a significant effect on the metabolism of cancer cells in vitro. One of the metabolism-changing trace elements of the Plasmax medium is selenium, which is present in the form of selenite. Selenium-containing proteins, primarily glutathione peroxidase-4 (GPx4), protect cells from oxidative stress and, in particular, from lipid peroxidation (e.g., [35]). VandeVoorde et al. [27] have demonstrated that cells die by ferroptosis if seeded at a low density of classical media (e.g., DMEM-F12). The use of the physiological selenite-containing Plasmax medium prevents the death of even single cells. It should be emphasized that this approach may also be useful for laboratories engaged in clonal selection.

The use of physiologically based media affected the morphology and cell growth rate of some cell lines (as illustrated by the example of the MDA-MB-468 line) and the density of intercellular contacts [27]. Similar data were obtained by our group for the following cell lines: hepatocarcinoma Huh7.5, cervical cancer HeLa, and kidney of the green monkey Vero E6, which, when cultured in Plasmax medium, became more elongated and formed less dense contacts with each other [36].

It is known that glutamine catabolism is accompanied by the production of ammonium, which is toxic and slows down cell growth [37]. To avoid this problem, some groups of researchers add pyruvate for rapid cell growth. However, it has been found that pyruvate at the commonly used concentration (1−2 mM) can cause a pseudohypoxic phenotype in normoxia because of stabilization of the HIF-1α transcription factor [27]. The existence of feedback should be again noted because HIF-1α regulates the expression of key genes of glycolysis enzymes [38].

In addition, the example of triple-negative breast cancer demonstrated that cells cultured in a physiological medium consume 2−3 times less glutamine, leucine, isoleucine, serine, cystine/cysteine, and tyrosine and are characterized by different levels of consumption of other amino acids but similar levels of glycolysis and lactate production [27].

The most noticeable change in metabolism in classical media compared to that in plasma-like media is a disturbance of the urea cycle which is responsible for the disposal of toxic ammonia and its conversion into urea. In the commonly used DMEM-F12 medium, the concentration of arginine, the substrate of the key enzyme of the arginase cycle, increases by a factor of 10 (!) compared to the natural concentration (700 vs 64 µM). Using 13C-labeled arginine, Vande Voorde et al. [27] showed that the majority of this amino acid is converted into argininosuccinate, not ornithine (an arginase product). Consequently, the urea cycle flux occurs in the opposite direction in the DMEM-F12 medium. It is noteworthy that there is no such disturbance in the plasma-like media. Although the authors of this work have not investigated other classical culture media (e.g., DMEM), it is logical to assume that an increased level of arginine (398 µM) can cause a violation of the urea cycle. This casts doubt on the results of studies of conjugated metabolic systems obtained using classical media. This applies to processes associated with the urea cycle, i.e., ammonia utilization, NO production during the conversion of arginine to citrulline by NO synthases, and the metabolism of polyamines synthesized from ornithine. Indeed, according to our preliminary results, the levels of biogenic polyamines are significantly reduced in cells cultured in the Plasmax medium (data not shown). However, given the importance of this class of compounds in the processes of cell growth and differentiation [39−41], we can expect an altered relationship between this metabolic system and cell growth and function.

Physiological culture media affect also mitochondrial respiration (oxidative phosphorylation). We have shown on four different cell lines (Huh7.5, A549, HeLa, and Vero E6) that the replacement of classical media with Plasmax leads to a pronounced increase in the respiratory activity of mitochondria without a noticeable change in their mass [36]. It is worth noting that different standard culture media were used for each of these lines, i.e., DMEM, DMEM-F12, and MEM. A similar increase in the respiratory activity of cells has been described by other researchers for other cell lines, i.e., breast cancer MCF7, adenocarcinoma prostate LNCaP, and osteosarcoma SaOS2 [42, 43]. Moreover, in the latter work, an increase in respiratory activity is described in hypoxia conditions. This is of great importance because standard cultivation conditions in a normal atmosphere provide increased oxygen levels (or more correctly, hyperoxia); the oxygen level in tissues is in the range of 3.4−6.8% [2, 3]. Interestingly, increased respiration is accompanied by the formation of an extended mitochondrial network, which is noted for various human and animal cell lines [17].

It is also worth noting significant differences in the influence of the medium on glycolysis. Moradi et al. [42] reported a decrease in glycolysis in osteosarcoma, breast, and prostate cancer cells cultured in the plasma-like medium. According to our data, the effect depends on the cell line, i.e., the Plasmax medium does not cause a change in glycolytic activity in liver carcinoma cells Huh7.5, causes decreases in the HeLa and Vero E6 cells, and causes increases in the lung cancer cells A549 [36].

Another change that occurs in cells when using the physiological medium compared to various standard media is a sharp decrease in the mass (number) of lysosomes [36]. It is known that lysosomes are involved in the storage of some amino acids [44]; therefore, a change in their content should affect the cell metabolome. Moradi et al. [43] observed a decrease in the intensity of mitophagy, which also contributes to an increase in the length and branching of mitochondrial networks. We note, however, that there is still no literature data on changes in the activity of autophagy.

Finally, the replacement of classical media with physiological media also affects the redox status of cells. An increase in the respiratory activity of Huh7.5, A549, HeLa, and Vero E6 cells is accompanied by an increase in the production of ROS, in general, and superoxide anions in mitochondria, in particular [36]. This fact can be explained by the increased levels (but not the percentage) of electron leakage from the electron transfer chain in the mitochondrial respiratory complexes. These data are not consistent with the results of Moradi et al. [43], who revealed a decrease in the production of hydrogen peroxide in myofibroblasts of the C2C12 line cultured in plasma-like medium under normoxia conditions (21% oxygen). The authors attribute this effect to the possible increased activity and expression of antioxidant enzymes. In the same work, the difference in the sensitivity of cells to estradiol E2 and selective modulators of estrogen receptors was also demonstrated. Thus, when culturing C2C12 cells in the classical medium, estradiol E2 induced a decrease in the level of hydro-

gen peroxide but did not affect cells cultured in the physiological medium.

APPLICATION OF PLASMA-LIKE MEDIA IN BIOMEDICAL RESEARCH

Over the past few years, there have been examples of the use of plasma-like culture media in various fields of biology. Physiological media will allow one to investigate the causes and mechanisms underlying the resistance of some types of cancer cells to asparaginase. This enzyme is used to reduce the level of exogenous asparagine, the biosynthesis of which is disrupted in lymphoblastic leukemia cells. In the work of Chiu et al. [45], the physiologically based Plasmax medium was used to study the metabolism of amino acids (asparagine and glutamine) in stromal mesenchymal cells, including when cultured with acute lymphoblastic leukemia cells. It was revealed that the stromal cells in the presence of asparagine absorb both asparagine and glutamine from the medium, while in the absence of asparagine, stromal cells, on the contrary, secrete asparagine; in this case, about a quarter of this asparagine is synthesized from glutamine coming from the medium. Thus, stromal cells can use extracellular glutamine for the synthesis and secretion of asparagine necessary for blast cells, which reduces the therapeutic effectiveness of asparaginase.

It is known that in many cases, homozygous deletion of the methylthioadenosine phosphorylase (MTAP) gene takes place in glioblastoma cells, gastrointestinal tract tumors, leukemia, and other types of malignancies [46]. Since this enzyme is important for the metabolism of purines and biogenic polyamines, the absence of its encoding gene creates vulnerability for cancer cells. Thus, an increase in the intracellular concentration of the substrate of this enzyme, methylthioadenosine (MTA), has been described for these cells in vitro [47]. At the same time, high MTA concentrations reduce the sensitivity of cells to arginine-N-methyltransferase, which can be used for antitumor therapy. When using the physiological Plasmax medium, Barekatain et al. [48] showed that the above-described mutation does not cause the accumulation of MTA in cells but promotes its secretion. Moreover, secreted MTA can be absorbed by stromal cells *in vivo*, which has been demonstrated in the co-culture experiment with glioblastoma cells and macrophages. The authors hypothesized that differences in the levels of certain compounds (cysteine, methionine, etc.) in the physiological medium prevent the accumulation of MTA in the primary tumors of patients.

Continuing the study of the cytotoxicity and efficacy of antitumor drugs, Khadka et al. [49] revealed that the dependence on glutaminolysis processes, but not glycolysis, is reduced in Plasmax-cultured glioma cells with a deletion in the gene of enolase (ENO1, one of the glycolysis enzymes). These data correlate with the *in vivo* results, which have shown the absence of noticeable antiproliferative activity of the glutaminolysis inhibitor against tumors with ENO1 deletion [49]. At the same time, the glutaminase inhibitor is equally active against cells with and without this deletion if the cells are cultured in a standard medium. This is an excellent illustration of the fact that the use of the physiological media (that contain reduced concentrations of pyruvate and 2-oxoglutarate) in similar studies is advisable and allows for a more adequate assessment of the effectiveness of antitumor drugs. In addition, Khadka et al. [49] found several minor but no less interesting features of physiological media. These authors have shown that lactate in blood plasma, which is contained in Plasmax is apparently a poor carbon donor for the Krebs cell cycle, whereas pyruvate is a much more important component of the tricarboxylic acid cycle in cancer cells. It is worth mentioning the data obtained using classical media. It has been shown that the contribution of lactate as a carbon donor to the Krebs cycle and, as a consequence, maintaining the respiratory activity of cells strongly depends on the type of tissue and cells [29, 50]. Based on the above, we conclude that all these data require additional verification.

Bagshaw et al. [51] evaluated the effect of physiological and nonphysiological zinc concentrations on various aspects of the metabolism of rat aortic smooth muscle cells and endothelial cells (RASMC and RAENDO) cultured in the Plasmax medium. A violation of the expression of the genes for oxidative phosphorylation and fusion and dissociation (fusion-fission) of mitochondria was revealed during the treatment of cells with zinc sulfate in both types of cells. Expression of the *Mff* (mitochondrial fission factor) and *Mfn2* (mitofusin 2) genes increased. Using the Seahorse technology, the authors visualized mitochondrial networks and analyzed the mitochondrial function of these cell lines. It has been shown that increased zinc concentrations in comparison with physiological values induce an enhancement of mitochondrial fusion/dissociation processes, thus increasing both basic and maximum oxygen consumption. The results allow better understanding of how the use of, for example, biodegradable zinc-containing implants can affect vascular cells and the human cardiovascular system.

Rossiter et al., the developers of the PLM medium [52], also studied the effects in cells during the transition to the physiological medium. Using the CRISPR technology, they performed genetic screening of genes responsible for various metabolic pathways of chronic myeloid leukemia cells K562. The authors identified 525 genes, expression of which significantly increased during the transition to the HPLM medium. In addition, they were able to identify genes, the expression of which changed when the dialyzed serum was replaced with the common serum. The authors believe that one of the most significant results is the relationship between alanine aminotransferase (ALT/F2) and

mitochondrial pyruvate transporter (mitochondrial pyruvate carrier, MPC). In cells cultured in the physiological medium with dialyzed serum, increased levels of both proteins were observed, which may contribute to the synthesis of alanine from pyruvate.

One of the most intensively developing areas of biomedical research is immunometabolism [53]. It makes sense to carry out studies in the field of immunology using media with physiological concentrations of metabolites. For example, Laney-Greene et al. [54] have demonstrated that the transcription profile of T-lymphocytes in the physiological medium differs significantly from that in the standard RPMI medium. In addition, the level of their activation increases in response to contact with an antigen. It is also shown that these effects can mainly occur due to the increased concentration of calcium ions in the physiological HPLM medium. An increase in the expression levels of some genes of serine, arginine, asparagine, and proline metabolism (*AS1*, *PHGDH*, *PYCR1*, *GOT1*) seems to be associated with a significantly lower arginine concentration in the HPLM medium.

It should be noted that the work of our group was devoted not only to identifying changes in cellular metabolism but also to the effects of the medium on the replication of various viruses [36]. We have shown that Huh7.5, A549, and Vero E6 cells when cultured in the Plasmax medium support the replication of hepatitis C virus, influenza A virus, and coronavirus-2, which causes severe acute respiratory syndrome (SARS-CoV-2), although the replication activity of viruses decreases compared with reproduction in the same cells cultivated in classical media. The exact reasons for the decrease in virus replication levels are unclear. However, it can be assumed that the virus replication decreases from non-physiologically high levels, which are characteristic of superpermissive cell lines (Vero E6 and Huh7.5).

It has been previously shown that respiratory viruses and hepatitis C virus cause oxidative stress in infected cells [55, 56]. In the Plasmax medium, the ability of viruses to disrupt the redox status of cells was much more pronounced. The ROS levels were comparable to those for classical culture media, although the replication activity of viruses was an order of magnitude lower [36]. This fact can be explained by the presence in the physiological environment of not only pyruvate but also lactate, the ratio of which determines the ratio between NAD and NADH [57].

We can confidently expect the appearance of studies which will reveal new metabolic abnormalities caused by the use of classical/standard media. However, even the currently available information allows for the conclusion that the results of studies of the metabolic characteristics of certain cells need to be verified in physiologically based media. This is especially true for the study of biochemical processes in mitochondria and lysosomes, redox balance in the cell, and violations of these and other pathways in various pathologies and viral infections.

CONCLUSIONS

The development and use of physiological media are extremely important for understanding biological processes and interpreting data, especially when studying cell metabolism. These media allow the in vivo processes to be performed under simpler conditions, i.e., in in vitro systems. In addition, the use of nonstandard culture media devoid of one or more metabolites makes it possible to investigate the dependence of cell growth and the status of signaling cascades on metabolic pathways.

It is worth noting that the replication of viruses and their cytopathic effect directly depend on the metabolism of cells, which means they are also strongly influenced by the composition of the culture medium. A more complete understanding of the mechanisms of pathogenesis of various infectious diseases requires further studies of virus-mediated disorders in the host cell. It would be desirable for these studies to be based on models which simulate in vivo conditions as adequately as possible, thus not distorting natural processes. One of the available opportunities for significant improvement (an approximation to real conditions) of existing models is the use of physiologically based media.

Physiological variants of the medium can be useful and convenient for solving various tasks including the cultivation of primary cells, the production of biomolecules (including viral proteins), etc. It is worth noting that physiological media can be modified in a variety of ways to perform different tasks, i.e., simulation of various diets and pathological conditions of certain tissues, aging, and other processes. Perhaps, in the future, these media will contain metabolites at concentrations corresponding to the values in a particular patient, thereby bringing practical medicine closer to a personalized format.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies with the use of humans and animals as objects of research.

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