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Multi-author Reviews

Metabolic regulation – physiological and medical aspects

This multi-author review is dedicated to Dr. R. Jäger in Mainz.

The editors wish to thank Professor Dr. G. Wegener for having coordinated this review.

Editorial

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This multi-author review is based on an International Workshop on Metabolic Regulation held in the Fachbereich Biologie of the Johannes Gutenberg-Universität Mainz*. The workshop brought together workers from different fields, ranging from metabolic biochemistry and molecular biology, comparative and medical physiology, to clinical research.

We should like to thank all the participants for their enthusiastic attendance and valuable contributions, and the University of Mainz, particularly Vice President Dr. Dagmar Eißner, for support. Most of all we thank Dr. Rudolf Jäger, since the idea of this workshop was his, and he shouldered most of the organization. Dr. Jäger has meanwhile retired after having served the University of Mainz for more than 30 years as research worker, teacher and administrator. He has done much to foster research and to create an atmosphere in which science can flourish. We are pleased to dedicate these proceedings to him.

All the participants of the workshop have in common a broad interest in the basics of metabolic regulation. This is reflected in the topics we have chosen: from swimming frogs and flying locusts to humans running a marathon, from the regulation of carbohydrate and lipid metabolism and its pathological deviations as encountered, for instance, in the 'metabolic syndrome', to the metabolic requirements of immune cells, tumour cells and germ cells. Many problems in very different systems turn out to share basic similarities, so a multidisciplinary approach and selection of apt model systems, as shaped by evolution, can greatly advance our understanding of metabolic regulation.

We have grouped the contributions under three headings and will briefly put them into context.

1. Metabolic regulation in exercise

Metabolism during exercise appears to be more complex than envisaged some years ago. Exercise metabolism seems to be coordinated and integrated by signals that originate in the nervous system, in addition to the motoneuronal signals that control contraction and relaxation. This series of papers starts with a discussion by Krause and Wegener about how the reactions that provide adenosine triphosphate (ATP) for contraction are regulated in vertebrate skeletal muscle. The focus is on white muscle, which is specialized for rapid and powerful contraction but is easily fatigued. The turnover of ATP during exercise is fuelled by phosphocreatine and from the conversion of glycogen to lactate. Since these fuels are stored in the muscle and the products are largely accumulated in the tissue, working white muscle can be regarded as a closed system in which reactions can easily be followed.

Muscle glycolysis has been studied for about a century, but the majority of studies have been performed under nonphysiological conditions (using isolated muscle, electrical stimulation, anaesthesia etc.). In the present paper the results of recent studies in which unrestrained frogs swam are discussed. Under these conditions, the regulation of the rate of glycolysis appears to be more complex than previously envisaged. The content of the

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glycolytic activator fructose 2,6-bisphosphate ($F2,6P_2$) in the leg muscles of thoroughly rested frogs is very low, but swimming triggers a more than a 10-fold increase in the content of $F2,6P_2$ in muscle. However, after a bout of exhaustive swimming, and some recovery, further exercise does not elicit a change in the content of $F2,6P_2$, even though all glycolytic intermediates and effectors of phosphofructokinase (PFK), including $F2,6P_2$, had returned to their respective preexercise levels, and the rate of glycolysis is increased. This suggests that muscle has some kind of 'metabolic memory' of whether the system that generates $F2,6P_2$ has previously been activated. The molecular basis of this phenomenon is unknown, but the nervous system is most likely involved. It is also not clear whether this observation can be generalized to species other than frogs. Frogs appear, once again, to be suitable experimental animals for fundamental studies of the biochemistry of metabolic regulation in muscles. Frogs have served science in this way for centuries, and it is sad to note that, owing to regulations and bureaucracy which tend to 'throw the baby out with the bath water', it is now almost impossible to do work on frogs, although it is generally agreed that, due to their amazing reproductive potential, common frogs are still numerous in places where their habitats have not been destroyed.

The second paper (Wegener) deals with insect flight, a highly specialized form of exercise that is performed by skeletal muscle that differs in several aspects from its vertebrate counterpart. However, insect flight muscle does not and cannot produce anaerobic end-products such as lactate and H^+. As a consequence, insects fatigue normally only when they run out of fuel. Insect flight muscles are fully aerobic, and a high steady state rate of aerobic metabolism is readily obtained during normal insect flight.

From measured levels of phosphocreatine, creatine and ATP, it is possible to estimate the free contents of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) (for example, those not bound to protein). Nuclear magnetic resonance (NMR) spectroscopy allows such experiments to be carried out on the intact insect during very brief (gated NMR) or prolonged intervals of flight. While flight causes only a very small decrease in the content of ATP in flight muscle, it brings about high fractional increases in the content of inorganic phosphate (P_i), free ADP and, in particular, free AMP. These metabolites are potent activators of regulatory enzymes, particularly PFK, but the latter enzyme also requires $F2,6P_2$ as an activator to account for the massive increase in glycolytic flux at the start of flight. However, the content of $F2,6P_2$ is decreased in prolonged flight in the locust, and this could be part of a mechanism to decrease glycolytic flux in order to facilitate the oxidation of fat which is the preferential fuel during prolonged flight. The content of $F2,6P_2$ in flight muscle appears to be under direct neuronal control through octopamine, which is a neurotransmitter (neuromodulator) similar in structure and function to catecholamines in vertebrates. It is not known whether an analogous mechanism exists in vertebrates.

Newsholme and Blomstrand discuss an intriguing aspect of central nervous system (CNS)-muscle interaction, central (mental) fatigue during prolonged exercise for which, previously, there had been no satisfactory biochemical explanation. Fatigue can originate at different levels: in the muscle, at the nerve-muscle interface and in the CNS. Central fatigue can undoubtedly arise during exercise in athletes but may also occur, possibly to a much greater extent, in patients during infections after injury, major surgery or burns. The hypothesis is put forward that central fatigue is caused, at least in part, by an increased uptake of tryptophan into the brain as a consequence of an exercise-induced increase in the free plasma concentrations of this amino acid (that is, the tryptophan not bound to albumin). The latter is caused by an exercise-induced increase in the plasma level of fatty acid which displaces tryptophan that is bound to albumin. Tryptophan is the precursor of serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter known to promote sleep and, possibly, fatigue. In the bloodstream, tryptophan competes with branched chain amino acids (BCAA) for entry into the brain. Exercise increases the free concentration of tryptophan and hence the plasma concentration ratio free tryptophan/BCAA, and thus the rate of entry of tryptophan into the brain is increased. Supplementation with BCAA during exercise should prevent a change in this concentration ratio and hence prevent an increase in the brain 5-HT level. Evidence that supplementation with branched chain amino acids during exercise can decrease mental fatigue is presented.

Another aspect of the possible interactions between the CNS and exercising muscle is discussed by H.-V. Ulmer. Well-trained athletes can control their energy expenditure precisely to match that which can be made available from oxidation of fuels. If they fail to do so, they will either fatigue too early or fail to use their potential to the fullest. Optimal planning and execution of a given exercise regime requires anticipation of the objective that has to be reached (teleoanticipation) and a feedback system from the working muscle that keeps the CNS informed of the degree of metabolic exertion that the body is experiencing. There is no doubt that such a system exists, but little is known about how it is organized or what its basic physiological, biochemical and psychophysiological mechanism(s) are.

2. Regulation of carbohydrate and lipid metabolism

Considerable evidence supports the important proposal that, under conditions of 'carbohydrate stress' (defined here as a reduction in the glycogen store in the liver),

fatty acids are mobilized from adipose tissue, and their rate of oxidation by muscle increases; this, in turn, decreases the rate of glucose utilization and oxidation. Conversely, when carbohydrate stress is removed (e.g. by refeeding a starved subject), the rate of fatty acid release by adipose tissue is reduced. Consequently, the plasma level of fatty acids and hence their rate of oxidation is decreased, and the rate of glucose utilization by the muscle increases. This is controlled by the increase in the concentration of insulin after feeding, since insulin inhibits the lipase in adipose tissue; indeed, it is one of the few agents that inhibit lipolysis. These responses stabilize the blood glucose concentration and conserve glucose. This interpretation of the role of insulin is based on the application of metabolic control logic: the pathway for fatty acid oxidation in muscle starts with the flux-generating step, lipolysis in adipose tissue, and this is the key process to be controlled by insulin, as discussed by Newsholme and Dimitriadis.

Schrezenmeir discusses clinical and metabolic aspects of the 'metabolic syndrome' (syndrome X), a common health hazard in western societies. The metabolic syndrome combines several conditions that occur together in many patients: these include abdominal obesity (leading to the well-known 'apple shape'), non-insulin-dependent diabetes (insulin resistance), hypertension, alterations in the concentration of some blood lipoproteins and atherosclerosis. One major clinical problem is to identify as early as possible those people who are at risk of developing the metabolic syndrome before they actually show pathological effects in their glucose metabolism. It is intriguing that this appears to be feasible using not the glucose tolerance test but a test in which subjects are fed a standardized meal. The test discloses an altered response in fat metabolism. 'Triglyceride high responders' seem to prefer meals high in fat; they show high plasma levels of triglycerides and free fatty acids after a meal. This could be part of a vicious cycle in which resistance to insulin, and eventually impaired rates of insulin secretion, are mediated by the elevated levels of free fatty acids in the postprandial state. The mechanism(s) causing a substantial proportion of the population to react as 'high responders' is not known, but an altered insulin processing in the beta cells of the endocrine pancreas could be involved, as well as a predisposition to increased numbers of cells in the hypothalamus that produce the neuropeptide galanin, which induces an increase in energy and particularly fat intake.

Becker and colleagues discuss some aspects of the metabolism of trehalose in insects. Trehalose is a non-reducing, hence non-toxic disaccharide of glucose and functions as the main blood (haemolymph) sugar in many insects; its concentration is usually maintained at a much higher level than that of glucose in vertebrate blood. Trehalose is synthesized exclusively in the fat body, and the rate is under hormonal control from the corpora cardiaca, a neurohaemal organ that is connected to the insect brain and produces and releases neuropeptides, some of which function as hypertrehalosaemic hormones. Synthesis of trehalose in the fat body poses similar problems of metabolic regulation as does maintenance of the blood glucose level in humans (see Newsholme and Dimitriadis). At the cellular level, the synthesis of haemolymph sugar requires suppression of glycolysis but stimulation of gluconeogenesis. As in the liver, $F2,6P_2$ appears to be pivotal in this process, in contrast to previous hypotheses. The signalling pathway from the neuropeptides to $F2,6P_2$ has not yet been elucidated, but cyclic-AMP (cAMP) seems not to be involved.

Trehalose is hydrolyzed by trehalase, and this process is regulated in various insects organs, most notably in the flight muscle. In locust flight muscle trehalase is bound to membranes, and its activity increases more than 10-fold upon flight, but the molecular mechanism by which this is achieved is completely obscure. It has been possible to separate fractions of trehalase that differ in their response to detergents, and this may open up a fresh approach to solving the long-standing problem of how trehalase activity is regulated.

Schmidt and Kamp provide a critical account of the 'Pasteur effect', particularly in animals that are adapted to temporary anaerobiosis. The Pasteur effect, a classical problem of metabolic regulation, describes the inverse relationship between glucose consumption and respiration. The molecular basis of the Pasteur effect is thought to rest with the regulatory properties of the key glycolytic enzyme PFK. When challenged by hypoxia/anoxia, facultative anaerobes markedly depress their metabolic rate; that is, they do not compensate for the reduced 'efficiency' of anaerobic ATP production by an increased anaerobic glycolysis. This has been taken to indicate that hypoxia-tolerant animals possess a 'negative Pasteur effect', and phosphorylation of PFK has been proposed as a mechanism to inhibit PFK during anaerobiosis. Schmidt and Kamp take a closer look at several invertebrates and show that most of them react to hypoxia with increased glycolytic flux in the initial phase of anaerobiosis, that is, a Pasteur effect. The authors also show that phosphorylation of purified PFK causes an activation rather than an inhibition of this enzyme. They finally extend the concept of the Pasteur effect to include glycogen as a substrate and adduce evidence that changes in metabolites that bring about the Pasteur effect at the level of PFK would have a similar effect on glycogen phosphorylase.

At the centre of Hofer's contribution is the evolution of regulatory mechanisms via enzymatic interconversions. Protein phosphorylation and dephosphorylation modulate protein function, and play an important role in the regulation of crucial physiological processes. A large

number of reactions in any cell are regulated by protein phosphorylation, and an intriguing question is how the specificity of protein interconversion is brought about. Protein kinases show great promiscuity with respect to their substrates if assayed in vitro, which is difficult to reconcile with the requirement for precise molecular signalling. Protein kinases all belong to one large family of proteins, and Hofer discusses evolutionary aspects of these proteins, especially with respect to their catalytic specificity. He has chosen as an example the PFK of the parasitic nematode *Ascaris suum*, which is activated by phosphorylation. *Ascaris* lives in the intestine; its energy metabolism is therefore restricted to the anaerobic degradation of carbohydrate. This simplified organization makes *Ascaris* a suitable experimental animal for the detailed study of the molecular aspects of the interrelation of kinases and their substrates.

3. Metabolism of fast growing cells and specialized tissues

In the first paper of this series Newsholme discusses the role of high rates of glutamine utilization in some cells of the immune system and in tumour cells, which is considered to be important not only in generating energy but also in providing optimal conditions for precise changes in the rate of key synthetic processes (e.g. synthesis of purine and pyrimidine nucleotides). The significance of such high rates of utilization for the animal as a whole is discussed in relation to conditions such as sepsis, trauma and major burns.

Conventional biochemical methods of extracting frozen tissues can only give overall metabolite contents of tissues and organs; they do not provide information about where the metabolites are actually located in cells and tissues. Müller-Klieser and his colleagues (Schwickert et al.) present a method for mapping metabolite contents in tissue slices at a microscopic level, thus complementing biochemical information about tissue metabolite contents with information about their location. They have applied their method to biopsies of human cervical cancer, which proved heterogeneous with respect to distribution of glucose, ATP and lactate. The correlation of high local lactate content with the tendency for metastasis in these cancers observed by the authors deserves further study.

The energy metabolism of tumour cells has long been of key interest for biochemists and physiologists because tumour growth depends critically on provision of substrates and oxygen. P. Vaupel discusses the relationship between key bioenergetic parameters and oxygen in experimental mouse sarcoma located under the skin of the foot, which makes them readily accessible not only to conventional biochemical methods and O_2-sensitive needle electrodes but also to non-invasive and non-destructive NMR spectroscopy. The latter method is particularly useful because it allows the selective representation of compounds that are freely mobile in the cytosol (as opposed to metabolites that are bound to cellular structures). Only the free fraction of a metabolite can act as substrate or effector on an enzyme. It turns out that the bioenergetic viability of a tumour depends to a large extent on its size, which is inversely correlated with oxygen supply (tissue pO_2) and the ratio of high-energy phosphate to P_i. In tumours of up to 350 mm^3 ATP content is relatively well preserved, but in larger tumours ATP content declines, that of P_i increases markedly and intracellular acidosis becomes evident. These processes correspond to a critical median pO_2 of about 10 mm Hg.

The following contribution by Busse and Vaupel extends the investigation of tumour metabolism towards intervention with the goal of clinical application. The authors apply temporary overheating to selectively damage tumour tissue and to monitor the effects of hyperthermia on metabolic parameters such as content of energy-rich phosphates, degradation of purine nucleotides (particularly ATP) and accumulation of degradation products.

The degradation of ATP is likely to have deleterious effects on tumour cells because it increases thermosensitivity due to an increased acid load and to the generation of reactive oxygen species that can damage membrane lipids, protein and DNA. There is good evidence that these mechanisms are crucial in the selective damage to tumour cells that is brought about by hyperthermia.

Weyel and Wegener report on metabolic effects of anoxia on adult insects, an experimental system that, prima facie, bears no relation to the preceding study by Busse and Vaupel in which experimental tumours were exposed to metabolic stress. Yet the lack of oxygen causes a rapid and almost complete breakdown of ATP in insect tissues. In contrast to mammalian cells (tumour cells included), where such a process would be lethal, insect tissues can endure this situation for several hours and fully recover. The tolerance of anoxia in these animals is the more surprising as insects, like mammals, have high metabolic rates and are strictly dependent on aerobic ATP production. Insects are hence useful experimental animals for studies aimed at identifying physiological and metabolic properties that are correlated with a high tolerance of anoxia or, more specifically, can recover from a complete breakdown of their cellular energy status. During anoxia more than 99% of the ATP in the flight muscle is broken down, and the main product is AMP; very few of the nucleotides are further degraded, and there is no evidence for generation of significant reactive oxygen species during post-anoxic recovery.

Purine nucleotides are important building blocks for nucleic acids that are required in large quantities when

germ cells are produced. Hoeger and colleagues introduce an invertebrate that shows remarkable metabolic adaptations for this purpose. The body cavity of the marine worm *Nereis* contains large coelomic cells, eleocytes, which contain ADP and AMP at much higher concentrations than ATP. The authors provide evidence by means of ^{31}P NMR spectroscopy and biochemical analysis that these nucleotides are stored in large vacuoles in the cells. During sexual maturation of both males and females, nucleosides are released from the eleocytes into the coelomic fluid, where they are taken up by developing spermatozoa and oocytes. Little is known about how these processes are regulated and coordinated during the life cycle of the worms.

Spermatozoa have only one purpose, to fertilize eggs, but to do so they may have to compete with many other sperm cells, travel actively for considerable distances or wait and survive for long periods until an egg is available. Spermatozoa are thus highly specialized cells, the capacities of which are in some respects reduced, and yet they perform functions (such as vigorous directed movement) usually seen only in whole animals. The structure and physiology of sperm have been shaped by evolution to fit a wide variety of modes of reproduction encountered in the animal kingdom. Kamp and colleagues adduce examples, from annelid worms and teleosts to birds and mammals, to outline how these adaptations and specializations may be exploited for the study of basic questions of metabolism and cell function. One particularity of spermatozoa arises from the fact that the ATP-producing structures are often distant from ATP-consuming motile structures. Another interesting feature is that spermatozoa, although fully developed, may have to be kept inactive for long periods. This requires a depression of energy expenditure which must then be released when the cells are triggered to become functional.

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