Biochemical aspects of comparative nutrition

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In their excellent book, *Genetics and Metabolism*, Wagner & Mitchell (1964) point out that 'the magnitude of a biochemical reaction depends not only on the magnitude of a change in concentration of a component that enters the reaction but also on the existing operational state of the system'. For the purpose of the present review we might generalize this statement to say that the metabolic fate of ingested foodstuffs depends not only on the composition of the food but also on the physiological state of the animal. Further, because different animals can inhabit different environments the available metabolic choice may vary with the species. This review will briefly consider the way in which the interaction of animal and environment may modify the use of food for energy production. For this reason carbohydrate metabolism will be the main subject of consideration; lipids will be summarily dealt with and amino acids, which provide energy from the degradation of their carbon skeletons, will only receive mention where they impinge on carbohydrate metabolism.

Lipids

With few exceptions, only fats among the ingested foodstuffs may remain sufficiently undegraded after absorption from the intestinal tract for their dietary origin to be reflected in the composition of newly synthesized body components (McGuire & Gussin, 1967; Keith, 1967). Some animals have their own characteristic fatty acid synthesizing systems so that body lipids may reflect both diet and species. For example, analysis of the milk fats from eleven orders of mammals (Glass, Troolin & Jenness, 1967) revealed that lagomorphs synthesize high concentrations of $C_{8:0}$ and $C_{10:0}$, murids $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$ while perissodactyls synthesize from $C_{8:0}$ to $C_{12:0}$ saturated fatty acids. All the ruminants synthesize significant quantities of short-chain fatty acids while carnivores, in contrast, have little synthetic ability in the mammary gland and the milk of three marine carnivores was found to contain mainly $C_{16:1}$ unsaturated fatty acid probably reflecting a diet of fish and plankton. At the present time insufficient details are known about the metabolic pathways for specific comparisons to be made at the enzyme level. This also applies to the essential fatty acids. Finally it should be mentioned that insects, and probably most other invertebrates, require a dietary source of sterols (Clayton, 1964).

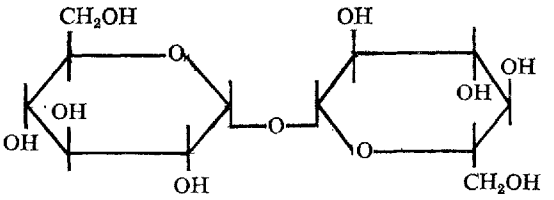
Carbohydrates

Unlike lipids, although carbohydrates may be absorbed into the intestinal mucosa as di- and oligo-saccharides, they pass into the bloodstream only as monosaccharides. If disaccharides are injected into the blood they are rapidly excreted in the urine (Datta & Ottaway, 1965).

Energy storage

Low energy. It is common knowledge that the usual carbohydrate energy store of animals is glycogen. Its structure does not vary greatly with the species or the organ from which it is isolated. Suggestions that diet, D-galactose in particular, may influence the degree of branching or chain length of glycogen have not been substantiated (Kjölberg, Manners & Wright, 1963) nor is there any evidence to support the suggestion that the residual liver glycogen of a starved rabbit differs from that of the fully fed animal (Kjölberg *et al.* 1963). Glycogen throughout the animal kingdom is similar in structure and properties with the possible exception of the Protozoa which have complex storage carbohydrates resembling starch, amylopectin and glycogen as well as a β-(1$\rightarrow$3)-glucan and a branched (1$\rightarrow$6)-linked poly D-galactose (Manners, Pennie & Ryley, 1967). The metabolic pathways of these compounds are not known.

Another carbohydrate, trehalose, appears to have a significant role as an energy store in insects (Sacktor, 1955; Clegg & Evans, 1961; Saito, 1963) and molluscs (Badman, 1967). It appears to be intimately connected with glycogen metabolism.



Trehalose (1(α-D-glucopyranosyl) α-D-glucopyranoside)

Clegg (1965) showed that the trehalose reserve of the dormant embryo of the brine shrimp, *Artemia salinia*, is converted into glycogen when dormancy is broken. In other animals it may act in a manner analogous to the glucose in human blood, maintaining a constant level of readily available energy. Control of the trehalose level may be simply enzymic or, perhaps, hormonal as well. Murphy & Wyatt (1965) suggested that in the silk moth, *Hyalophora cecropia*, the affinity of UDP-glucose for trehalose phosphate synthetase is greater than for glycogen synthetase. A high level of glucose in the blood-stream results in a rapid synthesis of trehalose followed by a slower synthesis of glycogen when a limiting trehalose concentration has been reached. In the cockroach, maintenance of a constant trehalose level appears to be under hormonal control (Steele, 1963). A similar sort of mechanism may apply in the oyster where the trehalose level alters relatively little although it only makes a significant contribution to carbohydrate storage in the summer months when the normally high level of glycogen falls to about 5% of its maximum level (Badman, 1967).

However, the situation may be more complex than this since some insects, such as the honey bee, *Apis mellifica*, may contain a variety of oligosaccharides (Maurizio, 1965) which could contribute towards an energy reserve. This means that they must be available for further metabolism and this has been demonstrated in vitro

using gut-free extracts of the thorax of mosquitoes, *Aedes sollicitans*, and blow-flies, *Phormia regina* (Van Handel, 1968). Among the insects the use of special sugars might be considered to be an adaptation to meet the excessive demands of flight metabolism. However, this can hardly apply to the oyster so that any suggestion as to adaptive significance must be treated with caution.

Another metabolic modification found in the tsetse fly, *Glossina mortisans*, is that proline, rather than a carbohydrate, serves as the haemolymph reserve for flight metabolism (Bursell, 1966). On the basis of isotope and enzyme studies Bursell suggests the metabolic pathway shown in Fig. 1. Proline is oxidized to glutamate

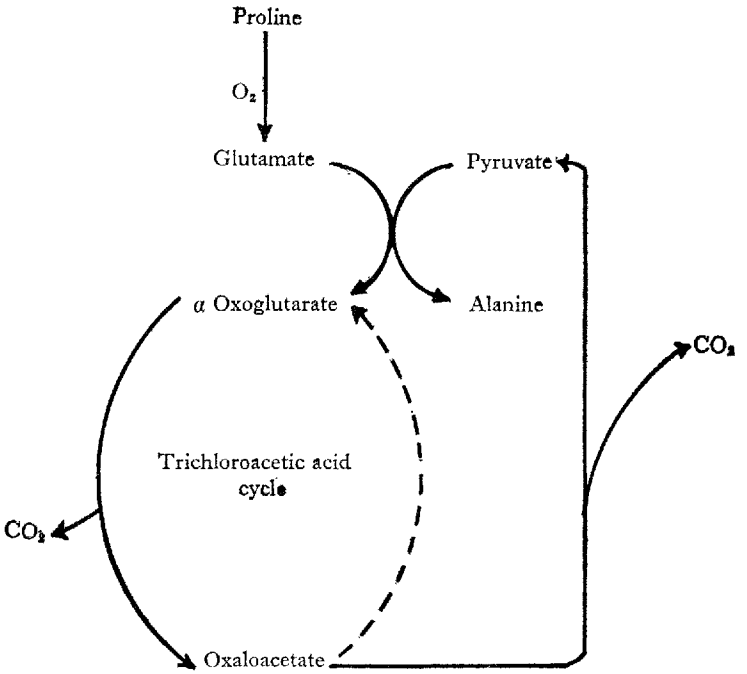
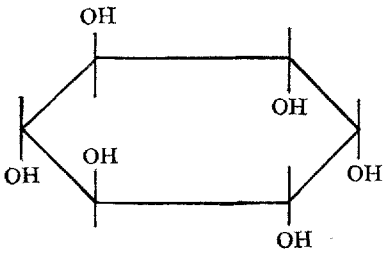


Fig. 1. Proposed pathway for the production of energy for flight metabolism in the tsetse fly, *Glossina mortisans* (Bursell, 1966).

which then transaminates with pyruvate to give α-oxoglutarate and alanine. The α-oxoglutarate is metabolized via the trichloroacetic acid cycle to give oxaloacetate which is then decarboyxlated to maintain the pyruvate level. The alanine simply accumulates. The process is quite efficient, yielding about 15 ATP for each molecule of proline oxidized.

A similar interlinked metabolism of amino acids and carbohydrates occurs in the common shore crab, *Carcinus maenas* (Huggins, 1966), but here the function appears to be control of the levels of tissue amino acids as part of a continuous adaptation of the osmotic pressure of the blood to varying estuarine conditions (Florkin & Schloffeniels, 1965).

A simple sugar, *myo*-inositol, is widely distributed in the tissues of most animals, and its function is normally associated with phospholipid synthesis.



Myo-inositol

In addition to being an essential growth factor for some animals, it has been suggested that *myo*-inositol may fulfil an analogous role to glycogen in shark muscle and, perhaps, in mammalian heart muscle where it occurs in relatively high concentrations.

High energy. Low-energy compounds such as carbohydrates are the raw materials for driving metabolic reactions. They suffer from the disadvantage that the necessary processing to provide energy, as ATP, takes a finite amount of time. This is particularly so for muscle where the change from a resting to an active state can create a demand for more energy than is instantly available from a quiescent glycolysis pathway or trichloroacetic acid cycle. This problem is overcome by the existence of high-energy compounds, phosphagens, which can instantly re-phosphorylate ADP. As Fig. 2 shows, they are all guanidino compounds derived from glycine, ornithine and taurine. Each has its own specific kinase:

e.g. for creatine, phosphocreatine + ADP $\rightleftharpoons$ creatine + ATP.

Phosphoarginine is found in most invertebrates from the Protozoa upwards. The echinoderms and tunicates have both phosphoarginine and phosphocreatine, sometimes together in the same animal, while all the chordates have phosphocreatine only (Thoai & Roche, 1964). The phosphagens derived from taurine are all peculiar to the Annelida and any of the phosphagens may occur in polychaete worms but, to date, only lombricine has been found in the Oligochaeta. It has been suggested (Moreland, Watts & Virden, 1967; Watts, 1968) that the marked evolutionary trend from arginine to creatine is because creatine has a unique selective advantage in being a dead end storage molecule which is not involved in any other metabolic reaction in the cell, whereas arginine has important roles as a protein amino acid and in the urea cycle. In times of food shortage an arginine lack could lead to depletion of the energy store and consequent decrease in the ability to survive.

The phosphagens and their kinases are distributed throughout many of the tissues of the body such as brain, thyroid, kidney, spleen and testis. Apart from obvious contractile tissues, the reactions for which phosphagens might act as an energy store are not known.

Glycolysis and related pathways of carbohydrate metabolism

As outlined in Fig. 3, the Embden Meyerhof pathway involves the breakdown

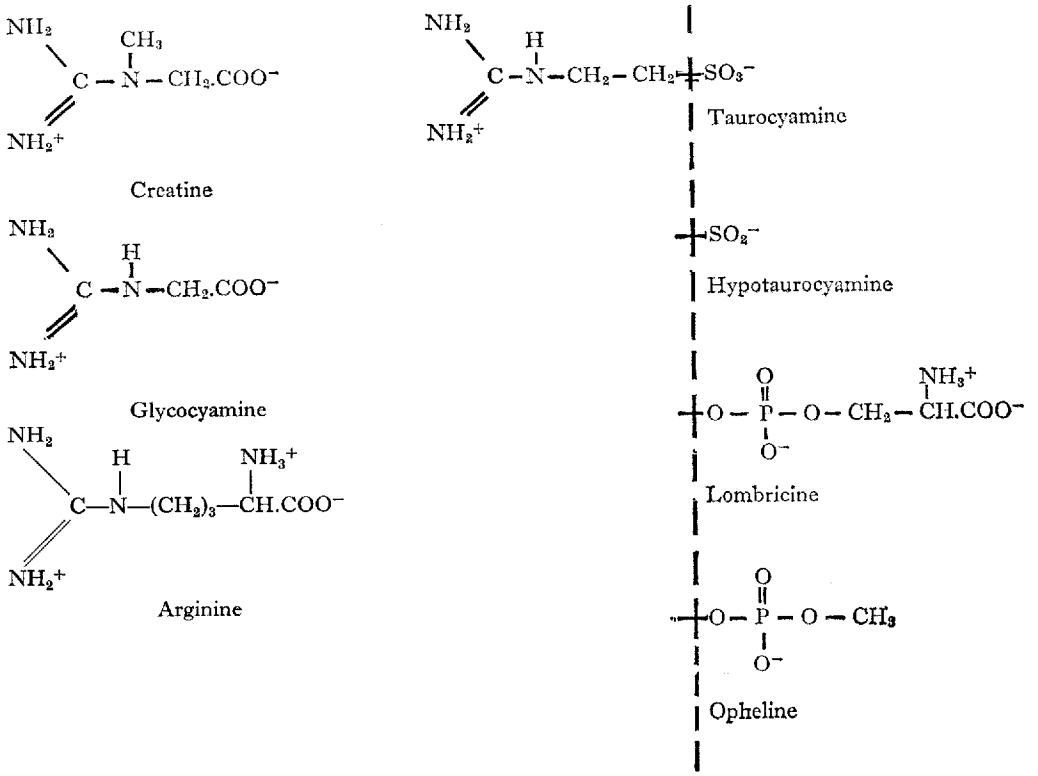


Fig. 2. Structures of the known phosphagen-forming guanidines which occur in nature.

of glucose to pyruvate. Under aerobic conditions the pyruvate is oxidatively decarboxylated to acetyl CoA which is further metabolized to CO_2 and water via the trichloroacetic acid cycle. The whole pathway is directed towards energy production (38 ATP from one glucose molecule). Further reactions (Fig. 3) convert pyruvate into malate and oxaloacetate and are important for the synthesis of intermediates in the trichloroacetic acid cycle, although, as will be seen later, the malic enzyme is more important for the generation of NADPH. Is is interesting to consider the importance of the trichloroacetic acid cycle in the metabolism of different groups of animals.

Vertebrates. Under anaerobic conditions, such as those produced in vertebrate muscle by excessive work, the trichloroacetic acid cycle can no longer operate but the glycolysis pathway is maintained by the conversion of pyruvate into lactate which regenerates NAD^+, essential for the further metabolism of glyceraldehyde 3-phosphate. The lactate is transported via the blood stream to the liver where it is reconverted into glycogen. Alternatively the lactate may be taken up by heart muscle or brain and reconverted into pyruvate under aerobic conditions and then metabolized via the trichloroacetic acid cycle. It is interesting that both heart muscle and brain contain a lactate dehydrogenase which differs from the main form in skeletal muscle in that it is inhibited by excess pyruvate and hence would work most

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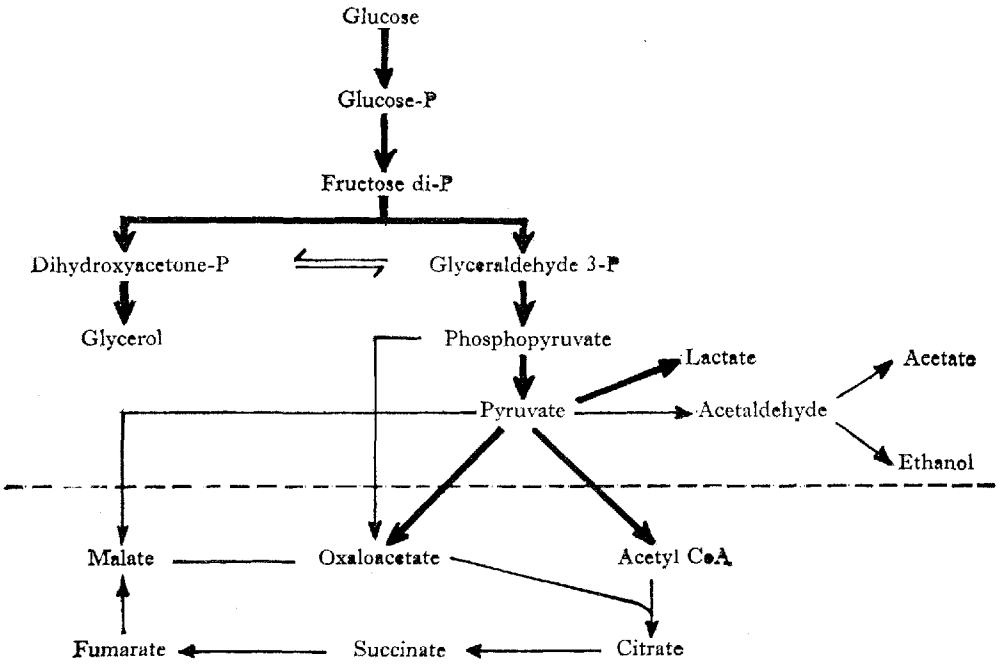


Fig. 3. Glycolysis and related pathways of glucose metabolism. The heavy arrows indicate the main routes in animals. The conversions of phosphopyruvate into oxaloacetate by phosphoenolpyruvate carboxylase and pyruvate into malate by the malic enzyme have little significance in the glycolysis pathway but have important roles in the reverse reaction during gluconeogenesis and fat synthesis respectively (see Figs. 5 and 6). The pathway to acetate and ethanol is characteristic of micro-organisms but also occurs in some invertebrates (e.g. nematodes).

efficiently in the direction of lactate oxidation under aerobic conditions favouring the further rapid metabolism of pyruvate. This might be seen as a safety device to ensure an energy supply to the heart and brain under conditions which might lead to a depletion of the blood glucose level. Under these conditions the energy yield of the glycolysis pathway in skeletal muscle falls to 2 ATP per glucose molecule. It is not surprising, then, that for most vertebrates lactate formation is only a short-term emergency device and prolonged anaerobiosis is lethal.

In a few adapted species a combination of physiological and biochemical mechanisms facilitates survival during a considerable period of low oxygen tension. Reptiles are noteworthy in this respect (Pfluger, 1875). Belkin (1963) has found that some turtles will survive for 15–18 h without oxygen. This is not just a reflection of the aquatic habit, as Bellamy & Petersen (1968) found that both terrestial, *Testudo tabulata*, and aquatic, *Podocnemys* sp., turtles maintained for 3 h in nitrogen with a pO_2 of about 5 mm Hg showed no significant changes in blood sodium, glucose or urea although the potassium level increased slightly and the lactic acid level by 70%. Sodium and potassium levels in the brain were little affected. It would seem that in these animals glycolysis alone can maintain some energy-requiring processes close to the aerobic level, and this finding is supported by in vitro studies (Reeves, 1963*a*;

Brodsky & Schilb, 1966). However, some economies have to be made and the normal gaits of the animals were impaired although the rapid protective movements of limb withdrawal could still be carried out.

If the trichloroacetic acid cycle is shut off the electron transport chain is not being used and the animals should be less sensitive to cyanide. This is indeed so. The minimum lethal dose of cyanide is about fifty times that for mammals. Death does not occur until 5–12 h after poisoning and seems to be at least partly due to disturbance of the sodium–potassium balance rather than just inhibition of the cytochrome chain (Bellamy & Petersen, 1968).

Among mammals hibernation affords protection against anoxia, and a hibernating hedgehog, *Erinaceus*, will survive for 1–2 h without oxygen in contrast to the active animal which is killed in 5 min (Biörck, Johansson & Schmid, 1956). Similar results have been found with other hibernating mammals.

An energy supply depending on anaerobic conditions obviously requires precise control of the glycolysis pathway, this can be achieved by feedback mechanisms, which will be mentioned later, or by direct control of the initial fuels, blood glucose and glycogen. In both reptilian and mammalian skeletal muscle it seems probable that the rate of glucose utilization is limited by the ability of the glycogen store to meet the energy demand. Only when the maximum rate of glycogenolysis is inadequate to provide the energy required is glucose fed directly into the glycolysis pathway and metabolized to pyruvate (Reeves, 1963*b*). The blood glucose is then replenished from the liver glycogen. This mechanism ensures reliability of energy supply with a minimum depletion of the blood glucose level. Regulation possibly involves hexokinase and phosphorylase, since the former enzyme is inhibited by glucose 6-phosphate which would result from glycogen breakdown (Crane & Sols, 1953).

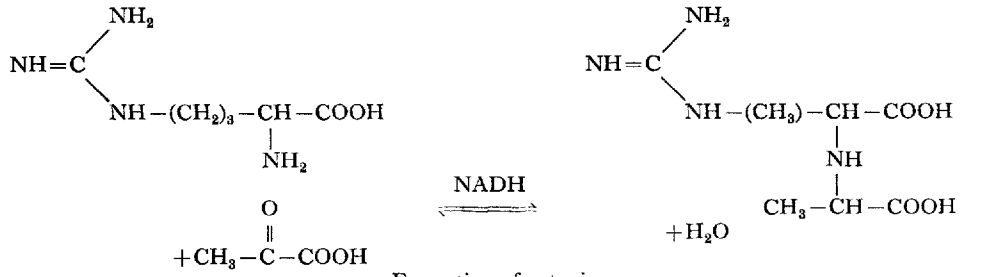
Recent in vivo studies on human subjects by Hultman (1967) indicate that the most efficient diet to replace muscle glycogen lost by working is carbohydrate. The glycogen level may be increased by 200–300% after strenuous exercise, so that the ability to carry out further work is increased by overnight resting. In contrast, diets

Table 1. *Anaerobic survival of some invertebrates* (*Bunge*, 1889)

	Anaerobic survival time
Leeches	
Hirudo medicinalis	3 days
Hamopsis	2 days
Clepsine	6 days
Nephilis	2 days
Flat worms	
(unspecified)	1–2 days
Worms	
Lumbricus sp.	2 days
Snails	
(freshwater unspecified)	10–15 h
Arthropods	
Asellus	1–5 h
Dytiscus	1–5 h
Hydrachace	1–5 h

rich in protein or fat resulted in poor glycogen resynthesis and a diminished work ability. These are, of course, short-term experiments and do not allow for the long-term replacement of protein. Fat mobilization appears to play no significant role in meeting such day-to-day energy demands if there is adequate carbohydrate in the diet.

Invertebrates. It has been known for many years that various invertebrates are resistant to anoxia (Table 1 and see also review by Slater, 1928). Cold-blooded animals with a lower metabolic rate might reasonably be expected to be able to make greater use of glycolysis as an energy pathway. At the same time this has resulted in some variation in the terminal stages of the pathway. Among some molluscs, lactate formation appears to be replaced by the formation of a novel compound, octopine, which results from the reductive condensation of pyruvate with arginine (Thoai, 1965).



Formation of octopine

It has been suggested that octopine is the equivalent of lactate without the disadvantage of acidifying the working muscle (Thoai, 1965). This is true to the extent that pK_2 of octopine must be much greater than the pK of lactate (cf. malonate, $pK_2 = 5{\cdot}7$) and hence buffer against any marked fall in pH below about 6·0. An alternative or additional advantage may be that octopine, like the α-glycerophosphate forming in insect muscle (see later) is retained inside the muscle cell to be used when aerobic conditions are restored.

More recently considerable attention has been paid to the metabolism of parasites, which have particular environmental and nutritional requirements and facilities. Nutrition presents little problem in that a ready supply of glucose and amino acids is available in the host tissues. In contrast to other animals which have a trichloroacetic acid cycle and may or may not use it, some parasites appear not to have such a functional cycle at all! Most helminth parasites studied may be essentially anaerobic (von Brand, 1966) and show a remarkable resistance to lack of oxygen although the presence of a small amount of oxygen will increase their time of survival in vitro (Rogers, 1948). Adaptation seems to involve at least three modifications of the trichloroacetic acid cycle and the terminal part of glycolysis.

In contrast to other animals, there has been controversy about whether helminths even possess a full complement of trichloroacetic acid cycle enzymes (von Brand, 1966). For example, no radioactive CO_2 was liberated from the tapeworm, *Hymenolepsis diminuta*, when incubated with [1–^{14}C]- or [6–^{14}C]-glucose, supporting the

idea that the trichloroacetic acid cycle is not important as an oxygen-dependent energy machine in these animals (Scheibel & Saz, 1966). However, recent enzymic and isotopic studies on *Ascaris lumbricoides* and *Haemonchus contortus*, other parasites thought to be essentially anaerobic (Saz & Weil, 1960; Seidman & Entner, 1961), suggest that at least some of their tissues contain a TCA cycle even if the enzymes are in very low amounts (Moon & Schofield, 1968; Oya, Kikuchi, Bando & Hayashi, 1965; Ward & Schofield, 1967*a*). The evidence suggests that evolution has led to the adoption of the trichloroacetic acid cycle as an anaerobic energy machine in these animals. This is achieved by making succinate the end-product of glycolysis rather than pyruvate or lactate. The point of departure (Fig. 3) is phosphopyruvate which is converted into oxaloacetate by phosphoenolpyruvate carboxykinase (Saz & Vidrine, 1959; Ward & Schofield, 1967*b*; Bueding & Saz, 1968). The oxaloacetate is then converted into fumarate and reduced to succinate. Unlike the lactate pathway for the anaerobic reoxidation of pyridine nucleotides, and incidentally lactate dehydrogenase activity is low in most of these animals (*Schistosoma mansoni* is an exception), the succinate pathway provides a mechanism for the simultaneous generation of ATP. This is possible because of the presence of a branched-chain electron transport system for carrying out oxidative phosphorylation. The details are not yet fully agreed upon but one scheme proposed for *Moniezia expansa*, a tape-worm, is shown in Fig. 4 (Cheah, 1967). NADH, α-glycerophosphate

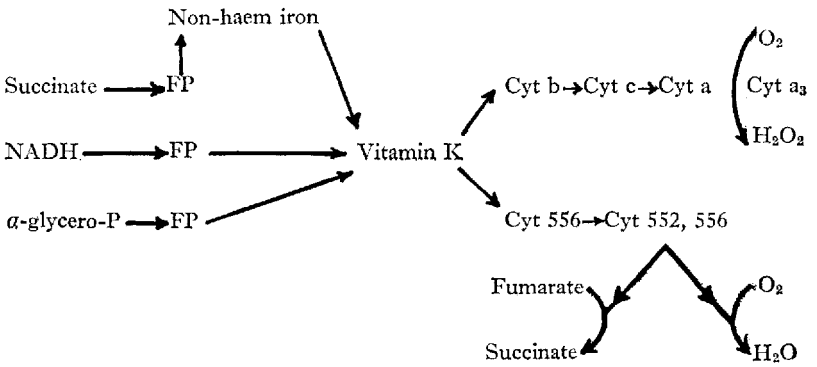


Fig. 4. Branched electron transport chain suggested to occur in *Moniezia expansa*. Oxidation of succinate and the formation of hydrogen peroxide or water only occurs at a significant rate under aerobic conditions. Under anaerobic conditions oxidative phosphorylation may still occur by the reduction of fumarate to succinate with NADH or α-glycerophosphate as the electron donors. By this means the TCA cycle enzymes can be coupled to ATP production under anaerobic conditions.

(produced by the reduction of dihydroxyacetone phosphate with NADH) and, under aerobic conditions, succinate, may feed electrons into a flavoprotein. Ubiquinone appears to be absent and vitamin K is suggested to be the link transporting electrons to the branched-chain system. The normal cytochrome chain is a minor pathway which occurs only in the presence of oxygen and produces hydrogen peroxide. The alternative route involves two new cytochromes, the second of which produces water under aerobic or, more usually, oxidizes fumarate to succinate under anaerobic conditions. Thus fumarate can replace oxygen as the terminal electron acceptor in an

energy-producing system. *Ascaris* appears to be somewhat different in that it contains no cytochrome oxidase or catalase (Laser, 1944; Magath, 1918) and a terminal flavin oxidase rather than a cytochrome is involved in the production of hydrogen peroxide under aerobic conditions (Saz & Bueding, 1966). Succinate can be decarboxylated to propionate and further metabolized to the branched-chain fatty acids, α-methylbutyrate and α-methylvalerate (von Brand, 1966; Saz & Weil, 1962). All three of these fatty acids are excreted by adult *Ascaris*.

The proportions of lactate and succinate produced by the glycolysis pathway of parasites depends on the ratio of pyruvate kinase to phosphoenolpyruvate carboxykinase (Bueding & Saz, 1968) although whether these two enzymes are part of a linked control system has still to be determined. Besides the Helminthes, succinate production has also been found among the Mollusca (Simpson & Awapara, 1964), although these animals also show a high pyruvate kinase activity suggesting that the normal glycolysis pathway might be preferred. Hammen & Lum (1966), have investigated the ratio of succinate dehydrogenase to fumarate reductase activities in a number of molluscs to find out whether the TCA cycle has its normal oxidative role or is being used anaerobically for succinate production. They found that animals living in aerobic environments have SD/FR ratios from 2 to 7, while those in anaerobic environments were less than 1. Hence succinate formation is a highly adaptive feature of the molluscs living in anaerobic environments.

Gluconeogenesis and glyconeogenesis

A high level of phosphoenolpyruvate carboxykinase was also found to be associated with a high level of glycogen in mollusc tissues (Simpson & Awapara, 1964). This is in accord with the suggestion that this enzyme is also associated with glucose synthesis to bypass the energetically unfavourable pyruvate kinase reaction (Lardy, 1966). Thus lipid metabolism via acetyl-CoA and amino acid metabolism via pyruvate, oxaloacetate and α-oxoglutarate can feed into the trichloroacetic acid cycle and form oxaloacetate. This can be converted into phosphoenolpyruvate which can go backwards up the glycolysis pathway as far as fructose 1,6-diphosphate. Phosphofructokinase, like pyruvate kinase, is energetically unfavourable for the reverse reaction and so breakdown of the diphosphate is carried out by a diphosphatase, the fructose 6-phosphate formed going via UDP glucose to glycogen (Fig. 5). In most animals aerobic conditions promote ATP synthesis which results in the inhibition of glycolysis—the Pasteur effect. A low oxygen level tends to result in a depleted ATP reserve and induction of glycolysis. The reverse tends to be true for glyconeogenesis (Fig. 5) but not for those animals such as helminths which can maintain their ATP level by an anaerobic process (Halton, 1967). For helminths, lipids (Passey & Fairbairn, 1957) and propionate (Saz & Lescure, 1966, 1968) may act as starting materials and seem particularly important in the early stages of development.

In mammals glycogen synthesis from oxaloacetate occurs in the liver and kidney and, in addition to control by enzyme feedback, is enhanced by glucagon, the adrenal hormones and by diabetes (Lardy, 1966). Skeletal muscle lacks phosphopyruvate carboxylase while smooth muscle and heart muscle lack phosphopyruvate

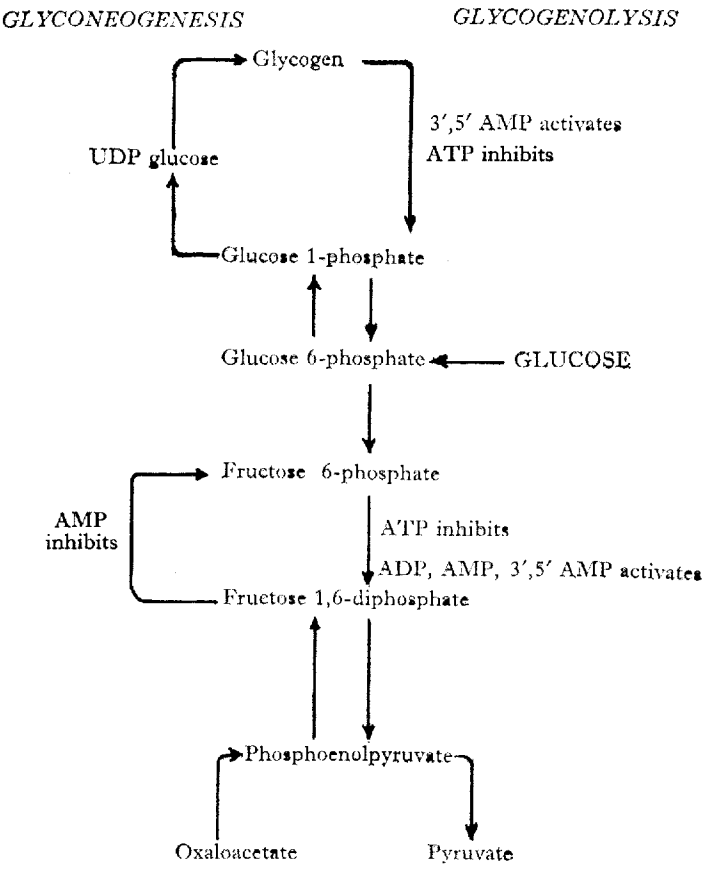


Fig. 5. Comparison of the pathways of glyconeogenesis and glycogenolysis.

carboxylase and fructose 1,6-diphosphatase and cannot carry out glyconeogenesis at all (Krebs & Woodford, 1965). Skeletal muscle can synthesize glycogen from triose phosphates, and the glycerol from lipid breakdown may be an important source. Krebs & Woodford (1965) point out that α-glycerophosphate may also be an important glycogen source. This compound can arise from the reduction of dihydroxyacetone phosphate by NADH in a reaction analogous to lactate production, from pyruvate. (Indeed, in insect flight muscle, which effectively lacks lactate dehydrogenase (Zebe & McShan, 1956–7), α-glycerophosphate formation fulfills exactly this role, as a result of which there is no net anaerobic synthesis of ATP.) A major difference between the two products is that α-glycerophosphate, unlike lactate, cannot escape from the muscle cell and must be further metabolized *in situ*: glycogen synthesis appears to be a major route.

In contrast to glycogen synthesis from ingested carbohydrate, glyconeogenesis is not affected by exercise, but it is markedly increased by starvation when the levels of phosphoenolpyruvate (Lardy, 1966) and fructose 1,6-diphosphatase (Krebs & Woodford, 1965) are increased, and stored lipid now becomes a major energy source.

At the same time the level of the malic enzyme (Fig. 3) goes down. The effect on the malic enzyme of refeeding the starved animal varies strikingly with the diet. With a carbohydrate-free diet there is no response, but with a high-carbohydrate diet free of fat a large (fortyfold) increase in enzyme activity occurs. Inclusion of a small amount of lipid in the diet (4% maize oil) significantly suppressed the magnitude of the response (Young, Shrago & Lardy, 1964). These observations suggest that the main role of the malic enzyme is to generate NADPH for fatty acid resynthesis. The NADPH produced by the pentose phosphate pathway alone is not sufficient to account for fat synthesis in adipose tissue (Flatt & Ball, 1964; Katz & Rognstad, 1966). The overall scheme is outlined in Fig. 6.

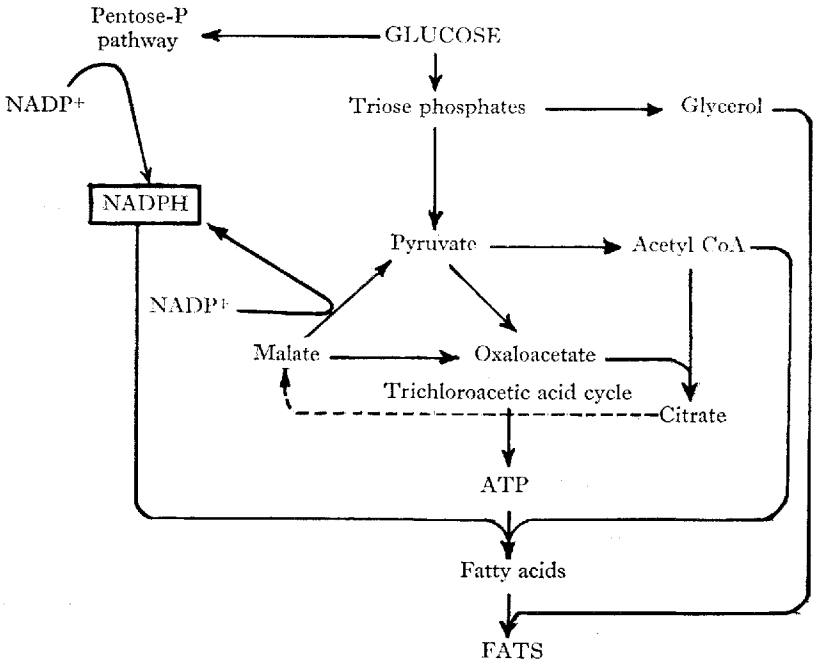


Fig. 6. Pathway of fat synthesis upon refeeding carbohydrate to a starved mammal.

Allied to starvation is hibernation, and during this period nervous tissue, even though relatively quiescent, still requires carbohydrate for energy (Solokoff, 1960). In the ground squirrel, *Citellus tridecemlineatus*, energy during hibernation is supplied by fat metabolism. A minimum blood sugar is maintained by glycogenolysis and gluconeogenesis in the liver (only liver contains the necessary glucose 6-phosphatase; Fig. 5), as a result of which the liver glycogen store is considerably depleted. At the same time glyconeogenesis in muscle builds up the glycogen store in muscle (Hannon & Vaughan, 1961). This apparently bizarre biochemical pattern is of vital importance for terminating hibernation. On arousal the respiratory quotient goes up from 0·7 to 1·0 and the animal switches from fat to an intensive carbohydrate metabolism which rapidly brings the body temperature and blood sugar level up to normal (Burlington & Klain, 1967). This is a striking example of the way in which

a basic metabolic pathway has become modified in different organs and yet has evolved in such a way as to provide complementary mechanisms in a complex physiological process. The use of gluconeogenesis to provide the glucose fuel for heat production is also a feature of cold adaptation but this process differs from hibernation in that a high liver glycogen will be maintained if a normal diet is available (Burlington, 1966). Finally, mention must be made of the use of gluconeogenesis in the developing chick embryo to utilize yolk lipids in contrast to the embryonic rat which has a good glucose supply from the mother and hence has a predominantly glycolytic energy pathway (Burch, Lowry, Kuhlman, Skerjance, Diamant, Lowry & Von Dippe, 1963).

This report has been concerned mainly with what might be called the natural variation in carbohydrate metabolism. Unexpected, 'artificially' induced effects may be observed with some compounds eaten with the normal diet. This is the case with the insecticide, DDT. DDT is detoxified by a NADPH-dependent microsomal oxidase (Agosin & Fine, 1965). Houseflies which show DDT resistance also have a greatly enhanced pentose phosphate pathway to ensure a plentiful supply of reduced $NADP^+$ (Agosin, Fine, Scaramelli, Ilivicky & Aravena, 1966). An increased turnover of glutathione also results from the increased pentose phosphate pathway and an enhanced protein synthesis, perhaps to synthesize detoxifying enzymes (Agosin & Fine, 1965). An enhanced metabolism will require an increased calorie intake for its maintenance. Hence ingestion of a single substance, perhaps inadvertently, may have profound metabolic effects. In a prolifically breeding species, such as the housefly, these effects may become constitutive if an appropriate mutation confers a selective advantage. However, one can only ponder on the possible long-term effects of such compounds on our breeding stocks of domestic animals or perhaps even upon ourselves.

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