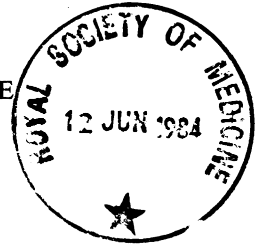
Section of Experimental Medicine and Therapeutics

President—Professor W. D. M. PATON, D.M., F.R.S.

Meeting
October 22, 1959



WALTER ERNEST DIXON MEMORIAL LECTURE
[Number 9]

Biochemical Aspects of Ketosis

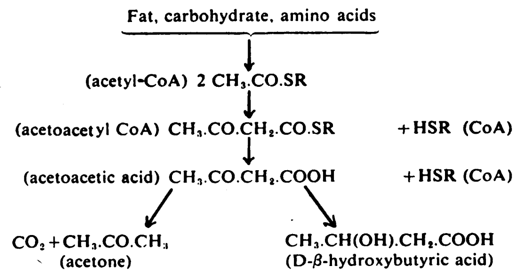
By Sir HANS KREBS, M.D., F.R.S.
Oxford

I PROPOSE to discuss the concept that the accumulation of ketone bodies arises from an inability of the liver to regulate the relative rates of certain metabolic processes. According to this concept, ketosis is a disorder of the regulation of metabolic processes rather than an insufficiency of certain enzymes or of key metabolites.

The Origin of Ketone Bodies

The bulk of the ketone bodies arises by condensation of acetate which reacts in the form of acetyl coenzyme A (CoA) (*see* Scheme 1). Two

SCHEME 1.*—PATHWAY OF FORMATION OF KETONE BODIES



*Another pathway leading from acetoacetyl CoA to free acetoacetate has been found by Lynen *et al.* (1958). This involves a reaction between acetoacetyl CoA and acetyl CoA leading to β-hydroxy-β-methylglutaryl CoA which is subsequently hydrolysed to free acetoacetic acid and acetyl CoA. It is not yet possible to assess the relative quantitative importance of the two pathways.

molecules of acetyl coenzyme A first form acetoacetyl coenzyme A and this is hydrolysed by a specific enzyme—acetoacetyl-coenzyme A deacylase. This enzyme is present in liver but absent from most other tissues (Mahler, 1953) which accounts for the fact that liver is the main site of ketone body formation.

All foodstuffs—fats as well as carbohydrates and proteins—yield acetyl coenzyme A in the course of their combustion and are therefore potential sources of ketone bodies. However, in the intact organism, the fate of acetyl coenzyme A depends partly on its origin. Only the acetyl coenzyme A derived from fatty acids with even carbon numbers and the three "ketogenic" amino acids—leucine, phenylalanine and tyrosine—produce major quantities of ketone bodies, and long-chain fatty acids rarely form more than one molecule of ketone body per molecule (Magnus-Levy, 1899, 1901, 1925). Whilst the acetyl coenzyme A arising from most sources undergoes complete oxidation through the tricarboxylic acid cycle (except for a relatively small fraction which is utilized for various synthetic processes), some of the acetyl coenzyme A arising from fatty acids and from the ketogenic amino acids can yield ketone bodies.

Site of Formation and Utilization of Ketone Bodies

The liver is by far the most important site of ketone body formation though small quantities can be formed in other tissues, e.g. kidney (Weinhouse and Millington, 1951; Jowett and Quastel, 1935). In ruminants, the wall of the rumen is an additional major source (Pennington, 1952; Hird and Symons, 1959) and, in the lactating animals, ketone bodies can also be formed by the mammary gland (Terner, 1958).

Many tissues are capable of utilizing ketone bodies as a substrate of respiration and of oxidizing them completely to carbon dioxide and water (Stadie *et al.*, 1940; Shipley, 1944; Krebs and Eggleston, 1948). This applies in particular to the mammalian heart which appears to use

acetoacetate in preference to most other substrates of oxidation (Kulka, 1958; Williamson, 1959).

Accumulation of Ketone Bodies

It is relevant to the problem of the nature of ketosis that there are many physiological or semiphysiological conditions which can be associated with an accumulation of ketone bodies above the normal level in blood and urine. These include starvation, diets low in carbohydrate (fat-protein diets, Deuel *et al.*, 1932), severe muscular exertion —"post-exercise ketosis" (Courtice and Douglas, 1936; Courtice *et al.*, 1939; Passmore and Johnson, 1958; Drury *et al.*, 1941; Gemmill, 1940), exposure to a cold environment (Sargent *et al.*, 1958), alkalosis due to hyperventilation (Davies *et al.*, 1920) or to a dose of sodium bicarbonate (Deuel *et al.*, 1935), and anæsthesia (F. L. Engel and M. G. Engel, 1958).

Some of the pathological conditions which can lead to ketosis of varying degrees have a common basis: the failure to utilize carbohydrate at the normal rate. This applies to all forms of diabetes—diabetes mellitus, diabetes due to depancreatization or to alloxan poisoning (Lukens, 1948), or to phlorizin poisoning (Goldfarb *et al.*, 1934)—to glycogen storage disease, and probably also to ketosis following vomiting (hyperemesis gravidarum and periodic vomiting of children), and to the ketosis accompanying pregnancy toxæmia of sheep.

The ketosis of lactating cows, first described in Holland in 1923 by Sjollema and Van Der Zande, though not fatal, may seriously reduce milk production and is a disease of major economic importance.

Experimentally, ketosis can be produced both in the intact body and in isolated liver preparations by substances which lower the oxaloacetate level in tissues. Examples are malonate (Recknagel and Potter, 1951), fluoroacetate (Cole *et al.*, 1955), fluorocitrate (Gal *et al.*, 1954), mesoxalate and tartronate (Edson, 1936; Krebs and Stickland, 1958. One of the most interesting forms of experimental ketosis is that produced by anterior pituitary extracts (Bennett *et al.*, 1948). The ketogenic effect of the anterior pituitary is suppressed by cortisone, hydrocortisone and related substances (Engel, 1957; M. G. Engel and F. L. Engel, 1958). These effects indicate that the metabolism of ketone bodies is under hormonal control.

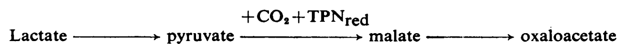
Relations Between Carbohydrate and Fat Metabolism

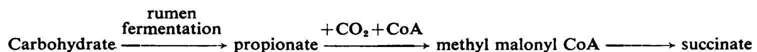
The elucidation of intermediary stages of the oxidation of fat has made it possible to re-examine the meaning of the old remark (Rosenfeld, 1885, 1906) that "fats burn in the fire of carbohydrate", a statement circumscribing the fact that ketone bodies are mainly derived from fat, and do not accumulate when carbohydrate is burned.

Fatty acids are known to be oxidized by β-oxidation to acetyl coenzyme A; this condenses with oxaloacetate to enter the tricarboxylic acid cycle, one turn of which represents the complete oxidation of one acetate equivalent. Oxaloacetate is thus necessary for the complete oxidation of fatty acids. It cannot be formed from fatty acids but it can be synthesized from carbohydrate. Thus the need for oxaloacetate represents a link between fat and carbohydrate metabolism, even though carbohydrate is not the only source of oxaloacetate: propionate and certain amino acids—especially those which have long been recognized as glucogenic amino acids (aspartate, glutamate, proline, arginine)—can also serve as precursors of oxaloacetate. The various reactions which can yield oxaloacetate in the animal body are listed in Table I.

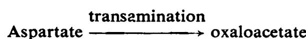
TABLE I.—MAIN SOURCES OF OXALOACETATE IN THE ANIMAL BODY

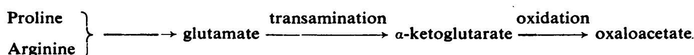
(1) *Carbohydrate*





(2) *Amino acids*





The Role of Oxaloacetate in Ketosis

It has been suggested that ketosis arises from lack of oxaloacetate. However, the experimental tests show that this is not true for most forms of ketosis. The oxaloacetate levels in the livers of well-fed and starved rats were found by Kalnitsky and Tapley (1958) to be identical after twenty-four hours although the acetoacetate level had increased over tenfold (*see* Table II). These findings indicate that the ketosis of starvation is not due to lack of oxaloacetate. Similarly, the oxaloacetate levels of the livers of normal and alloxan-diabetic rats were equal whilst the acetoacetate levels were seven times higher in the diabetic rat. Thus the ketosis of alloxan diabetes cannot be due to lack of oxaloacetate either.

TABLE II.—OXALOACETATE AND ACETOACETATE LEVELS IN RAT LIVER

	Average values	
	Oxaloacetate (µmole/100 g fresh weight)	Acetoacetate (µmole/100 g fresh weight)
Series 1 (Kalnitsky and Tapley, 1958)		
Liver, well-fed	1·0	1·5
Liver, fasting 24 hours ..	1·1	15·2
Series 2 (Shaw and Tapley, 1958)		
Liver, normal..	0·9	0·4
Liver, alloxan diabetes ..	1·0	2·8

Another way of testing the role of oxaloacetate in ketogenesis is the administration of oxaloacetate or its precursors to the ketotic organism. Experiments on various animals show that the dietary ketonuria produced by feeding butyrate is greatly reduced by the administration of oxaloacetate or its precursors (Beatty and West, 1951; Fasella *et al.*, 1958). The ketosis of alloxan-diabetic animals (Beatty and West, 1955) or of human diabetics (MacKay *et al.*, 1939; Dunlop and Arnott, 1937; Lawrence, 1937; Dibold *et al.*, 1937) or of cows suffering from bovine ketosis on the other hand, is not appreciably influenced by oxaloacetate and its precursors. Some authors (Fasella *et al.*, 1958) have drawn the conclusion from these findings that the mechanisms responsible for diabetic and dietary ketosis are "fundamentally different". I prefer to express this difference by the statement that different factors limit the formation and the utilization of acetyl coenzyme A in dietary ketosis on the one hand, and diabetic ketosis on the other.

That lack of oxaloacetate can be a cause of an accumulation of ketone bodies can be shown *in vitro* with liver preparations. As Lehninger (1946) has shown, liver mitochondria convert octanoic acid almost quantitatively to acetoacetate when added as the sole substrate, but when oxaloacetate is available some of the octanoate is converted to the acids of the tricarboxylic acid cycle or completely oxidized. The importance of oxaloacetate in ketone body accumulation can also be demonstrated in a preparation of washed liver particles suspended in saline. On addition of pyruvate, varying amounts of acetoacetate, tricarboxylic acid cycle intermediates and CO_2 are formed. The yield of acetoacetate from pyruvate is substantially increased by a variety of agents, which in different ways all reduce the supply of oxaloacetate. Malonate, oxalate, tartronate, mesoxalate, fluoromalate, fluorocitrate, and ammonium chloride are examples. Their ketogenic action is shown in Table III. Malonate prevents the formation of oxaloacetate mainly by inhibiting succinic dehydrogenase. Ammonium chloride interferes with the tricarboxylic acid cycle at the stage of α-oxoglutarate by causing its conversion to glutamate and glutamine. Most of the other agents inhibit the synthesis of C_4-dicarboxylic acids from pyruvate and CO_2.

The determination of the level of oxaloacetate in the presence of various inhibitors directly shows the reduced steady state level of oxaloacetate on addition of the ketogenic agents (Table IV). Fumarate, on the other hand, raises the

TABLE III.—EFFECT OF VARIOUS INHIBITORS ON ACETOACETATE FORMATION BY LIVER PARTICLES IN THE PRESENCE OF PYRUVATE

(0·02 M pyruvate; 30°C; 60 min; 4 ml suspension)

	Pigeon liver (22·1 mg dry weight)		Rat liver (35·0 mg dry weight)	
Additions (other than pyruvate)	O_2 (µmoles)	acetoacetate (µmoles)	O_2 (µmoles)	acetoacetate (µmoles)
None	−29·1	+ 6·5	−26·2	+ 2·7
Malonate (5 × 10^{-3}M) ..	−19·8	+ 9·4	−19·8	+ 8·8
Tartronate (10^{-3}M) ..	−14·6	+12·0	−21·5	+10·8
Mesoxalate (5 × 10^{-3}M)..	−12·7	+13·9	− 7·3	+ 7·5
Oxalate (10^{-3}M)	−20·0	+12·2	−16·3	+10·4
Fluoromalate (5 × 10^{-4}M)	−13·6	+12·4	−11·8	+ 8·0
Fluorocitrate (10^{-4}M) ..	−31·6	+ 7·9	−16·8	+10·4
NH_4Cl (5 × 10^{-3}M) ..	−25·4	+ 8·2	−27·1	+ 6·5

TABLE IV.—OXALOACETATE LEVEL IN PIGEON LIVER PARTICLE SUSPENSIONS IN THE PRESENCE OF KETOGENIC SUBSTANCES

The data refer to µmoles in 8 ml suspension; 0·02 M pyruvate; 30°C; 60 min; 32·6 mg dry weight.

Additions (other than pyruvate)	None	Fluoromalate (5×10^{-4}M)	Oxalate (10^{-3}M)	Fluorocitrate (10^{-4}M)
O_2 used	−39·4	−23·0	−23·2	−21·0
Acetoacetate formed	+11·6	+21·4	+20·1	+21·0
Oxaloacetate present at end of experiment	0·12	0·06	0·07	0·05

oxaloacetate level and substantially reduces the accumulation of acetoacetate in the presence of pyruvate (Table V).

TABLE V.—EFFECT OF FUMARATE ON THE FORMATION OF ACETOACETATE AND ON THE LEVEL OF OXALOACETATE IN PIGEON LIVER PARTICLES INCUBATED WITH PYRUVATE

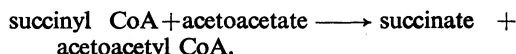
The data refer to µmoles in 8 ml suspension; 30°C; 60 min.

Substrates added	Pyruvate (0·02 M)	Fumarate (0·02 M)	Pyruvate (0·02 M) fumarate (0·02 M)
O_2 used	−42·7	−25·7	−47·1
Acetoacetate formed ..	+17·6	+ 0·5	+ 7·6
Oxaloacetate present at end of experiment	0·15	0·41	0·21

Thus the supply of oxaloacetate can be a factor controlling ketone body formation, i.e. the fate of acetyl coenzyme A. But from what has already been said about the failure of oxaloacetate to relieve some forms of ketosis, it is not the only limiting factor in ketone body accumulation.

The Role of Coenzyme A Transferase in Ketosis

It might be thought that apart from oxaloacetate derived from carbohydrate, there is also a link between fat and carbohydrate metabolism at the level of ketone body utilization. The most important reaction in animal tissues which initiates the disposal of free ketone bodies is probably that catalysed by coenzyme A transferase (Stern *et al.*, 1956). This enzyme converts acetoacetate to its reactive form, e.g. acetoacetyl CoA:


succinyl CoA+acetoacetate ⟶ succinate + acetoacetyl CoA.

However, the supply of succinyl CoA is not dependent on carbohydrate; it arises as an intermediate in the tricarboxylic acid cycle at the rate of 2 molecules per molecule of acetoacetate. It is self-generating and therefore cannot be expected to be a factor limiting the oxidative disposal of acetoacetate.

Control of Substrate Utilization as a Factor in Ketosis

There is, then, no evidence of a simple stoichiometric link between fatty acid and carbohydrate metabolism, in the form of an enzymic reaction involving one reactant derived from carbohydrate and another one derived from fat, such as earlier authors (Shaffer, 1921; Henze, 1930; Stöhr and Henze, 1932) had visualized. Yet that there is a further link between the metabolism of carbohydrate and fat is evident from the fact that when both are available they are not oxidized independently of one another. The body as a whole, and many individual tissues, burn carbohydrate in preference to fat, and fat takes the place of carbohydrate as a fuel when the supply of carbohydrate is exhausted. The total energy supply remains approximately constant, irrespective of the nature of the fuel burned. The "sparing" effect of carbohydrate on the oxidation of fat may also be expressed by the statement that carbohydrate prevents the oxidation of fat.

This competition between different substrates as fuels of respiration is shown by the following experiment in which various substrates were added to pigeon liver homogenate and the oxygen uptake, as well as some of the substrate changes, was measured (Table VI). Addition of sub-

TABLE VI.—OXIDATIVE METABOLISM OF PIGEON LIVER HOMOGENATES IN THE PRESENCE OF DIFFERENT SUBSTRATES

(The data refer to 8 ml liver homogenate containing 91·8 mg dry weight tissue. 30°C. 60 min O_2; substrates 0·01 M. The homogenates were prepared in the apparatus of Potter and Elvehjem from a mixture of 4 g of minced liver in 20 ml 0·9% KCl at 0°C. Each cup contained 2 ml of this homogenate, 1 ml 0·1 M sodium phosphate buffer of pH 7·4, 0·4 ml 0·155 M $KHCO_3$, 0·4 ml 0·02 M $MgCl_2$, 0·4 ml 0·02 M sodium ATP, 0·4 ml 0·2 M substrate solution (sodium salt) and sufficient 1·15% KCl to make up 8 ml)

Substrates added: Metabolic changes (µmoles)	None	Citrate	α-Oxoglutarate	Succinate	Fumarate
O_2	−54·5	−48·0	−54·8	−73·8	−54·8
Citrate	0	−34·0	+ 0·4	+ 3·0	+ 5·3
Malate + fumarate	0	+ 5·1	+26·6	+39·0	−52·5
α-Oxoglutarate ..	0	+29·0	−41·1	+ 5·9	+14·4
Succinate	0	+ 1·4	+ 6·0	−61·6	0
Pyruvate	0	+ 4·2	+10·3	+ 9·0	+ 9·8

TABLE VII.—OXIDATIVE METABOLISM OF WASHED PIGEON LIVER PARTICLES IN THE PRESENCE OF DIFFERENT SUBSTRATES

Washed pigeon liver particles, 8 ml suspension in saline medium (47·0 mg dry weight), 30°C. 60 min O_2. Medium: 1 ml 0·1 M Na-phosphate buffer pH 7·4; 0·4 ml 0·02 M $MgCl_2$; 0·4 ml 0·155 M $KHCO_3$; 0·4 ml 0·02 M ATP; 2 ml washed particles in 1·15% KCl; substrate solution 0·2 M (0·8 or 0·4 ml); 1·15% KCl to 8 ml.

Substrates added:	None	Pyruvate (0·02 M)	Citrate (0·01 M)	α-Oxoglutarate (0·01 M)	Fumarate (0·01 M)
Metabolic changes (μmoles)					
O_2	−6·7	− 71·3	−43·0	−48·1	−56·0
Pyruvate	0	−117	+ 2·4	+ 4·4	+ 2·7
Acetoacetate ..	+2·7	+ 12·5	+ 1·4	+ 0·7	+ 1·3
Citrate	0	+ 11·2	−37·0	+ 1·6	+ 2·8
α-Oxoglutarate ..	+0·8	+ 11·9	+16·0	−20·6	+ 7·5
Malate + fumarate	0	+ 17·6	+ 1·8	+11·6	−54·2

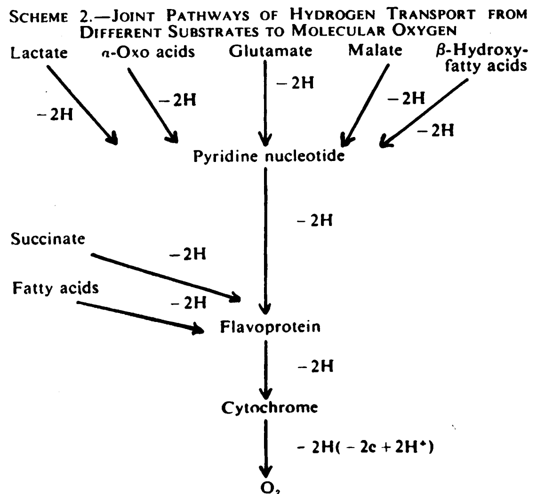
strates did not affect the rate of oxygen consumption very much except in the case of succinate, which caused a substantial increase. In contrast, the substrate changes were greatly affected by the additions. When no substrate was added, endogenous material served as a fuel and no intermediates accumulated. The data for the removal of the added substrates indicate that the added material largely replaced the endogenous material as a fuel. Thus, in the presence of citrate, about 30% of the oxygen consumption was due to the conversion of citrate to α-oxoglutarate, about 3% to the formation of succinate, about 16% to the formation of malate and fumarate, and about 17% to the formation of pyruvate. When α-oxoglutarate was added, the main oxidative reactions were the conversion of the substrate to succinate (5·5%), to malate and fumarate (48·5%) and to pyruvate (28%). In the presence of succinate, the conversion of succinate to fumarate and malate accounted for 26·5% of the oxygen consumption. With fumarate as a substrate, most of the oxygen consumption was due to the stages of the tricarboxylic acid cycle between fumarate and α-oxoglutarate.

Similar data were obtained with washed liver particles (Table VII). Again, the main metabolic reactions were those stages of the cycle nearest to the added substrate. In the case of pyruvate, this was the conversion of pyruvate to acetyl coenzyme A.

In these experiments, the high concentration of the added substrate was no doubt an important factor in displacing other oxidizable materials as fuels of respiration. How the mechanisms operate which regulate the choice of substrate is by no means fully known, but it can be explained in general terms why the available nutrients are not simultaneously burned.

A major factor is the circumstance that all oxidations involve the same co-factors and are therefore not entirely independent processes. The transport of hydrogen from a substrate to molecular oxygen generally involves three types of catalysts which are arranged in series. They are the pyridine nucleotides, flavoproteins and iron-porphyrins and only the first step of the transport chain varies from substrate to substrate. This is shown diagrammatically in Scheme 2.

SCHEME 2.—JOINT PATHWAYS OF HYDROGEN TRANSPORT FROM DIFFERENT SUBSTRATES TO MOLECULAR OXYGEN



Choice of substrate thus means competition of several substances for the same catalyst.

Over-production or Under-utilization of Acetyl Coenzyme A

The concept of substrate competition suggests that ketosis may be a condition where the balance of substrate utilization is disturbed. Ever since it has been appreciated that ketone bodies can be normal intermediates of metabolism it has been realized that the accumulation of ketone bodies could be due either to over-production or to under-utilization. In the light of present knowledge, this means over-production or under-utilization of acetyl coenzyme A. If the

utilization of acetyl coenzyme A through the tricarboxylic acid cycle is impeded, then any excess, instead of undergoing oxidation, would be expected to be shunted in the liver to the synthesis of ketone bodies. On the other hand, an overproduction would mean that more acetyl coenzyme A is formed than is needed for energy release and this excess would again be expected to be shunted to the synthesis of ketone bodies.

There is no evidence of under-utilization of acetyl coenzyme A in the ketotic organism, in the sense of the reduced rate of the tricarboxylic acid cycle. The capacity of the body as a whole to burn acetate is never fully used in a resting organism. On exercise, the total energy supply may be trebled and of this increase about two-thirds is due to the oxidation of acetate. It is true that the differences in respiration between the resting and maximally active tissue are small in liver (where most of the ketone bodies are produced) but numerous measurements of the respiration of liver preparations *in vitro* have shown that the rate of respiration, in terms of Q_{O_2}, is not depressed in starvation, although the rate of ketone body accumulation may rise fourfold (e.g. Edson, 1935, *see* Table VIII). It is thus

TABLE VIII.—COMPARISON OF RATES OF RESPIRATION AND OF ACETOACETATE FORMATION IN THE LIVER OF STARVED AND WELL-FED RATS

Average values (Edson, 1935)

	Q_{O_2}	$Q_{Acetoacetate}$
Well-fed liver ..	−11·5	+0·33
Starved (24 h) liver..	−10·6	+1·34

clear that ketosis can occur whilst the total oxidative capacity of the liver and the rate of utilization of acetyl coenzyme A through the tricarboxylic acid cycle are normal.

This indicates that ketone body accumulation is not due to under-utilization, but to over-production of acetyl coenzyme A, and raises the question of why over-production occurs.

Accumulation of Intermediary Metabolites

An over-production of intermediary metabolites and their accumulation is a most exceptional event in animal tissues. The non-accumulation indicates that intermediates when available react in preference to the starting material. A substrate molecule of respiration, once its oxidation has been initiated, burns to completion before a new molecule is attacked. This can be directly demonstrated by adding intermediates of the tricarboxylic acid cycle to respiring tissues. As already discussed (*see* Table VI) the result of the addition is a suppression of the oxidation of the endogenous substrates, such as glucose or fat.

Whilst this inhibition by intermediates is the rule, it does not apply to the ketotic state where the presence of acetyl coenzyme A or ketone bodies does not suppress the attack of new substrate molecules. The ketotic disorder might thus be looked upon as a failure of acetyl coenzyme A or of ketone bodies to suppress the breakdown of new substrate molecules which yield more acetyl coenzyme A. The reasons for such a failure are still a matter of speculation. There are many factors on which the rate of degradation of a particular foodstuff depends—the activity of the enzymes, the availability of coenzymes and the presence of other foodstuffs. The fact that ketosis can be caused by extracts of the anterior pituitary suggests that pituitary hormones also play a role in co-ordinating acetyl coenzyme A utilization and production. The ketogenic activity of the anterior pituitary appears to be inseparable from its fat-utilizing ("adipokinetic") activity. This would support the assumption that the anterior pituitary plays a part in the control of the rate at which fatty acids are oxidized to acetyl coenzyme A.

How such a control is exerted is unknown. It is likely that the hormone regulates the activity of a key enzyme. Several cases are known where the activity of enzyme is under hormonal control. An example is adrenaline which promotes the conversion of inactive phosphorylase to the active enzyme and thereby accelerates the conversion of glycogen to hexose phosphates (Sutherland and Cori, 1951; Sutherland and Wosilait, 1956; Rall *et al.*, 1957).

Prevention of Ketosis by Dietary Measures

Even without full knowledge of the reasons of ketosis and of the factors controlling the formation of acetyl coenzyme A, ketosis can to some extent be controlled experimentally and therapeutically by dietary measures, i.e. by the administration of antiketogenic instead of ketogenic food. Whether a food is ketogenic or antiketogenic has long been established empirically for the main foods (*see* Magnus-Levy, 1925). The knowledge of intermediary metabolism makes it now possible to predict the ketogenic and antiketogenic properties of food constituents on the following basis.

The energy supply from all foodstuffs comprises two major stages (Scheme 3). The first consists of the reactions leading from the foodstuffs to acetyl coenzymes A or to other intermediates of the tricarboxylic acid cycle. The second is represented by this cycle itself. On ordinary diets, about two-thirds of the energy is set free by the tricarboxylic acid cycle and one-

SCHEME 3.—ENERGY-YIELDING REACTIONS LEADING TO THE TRICARBOXYLIC ACID CYCLE

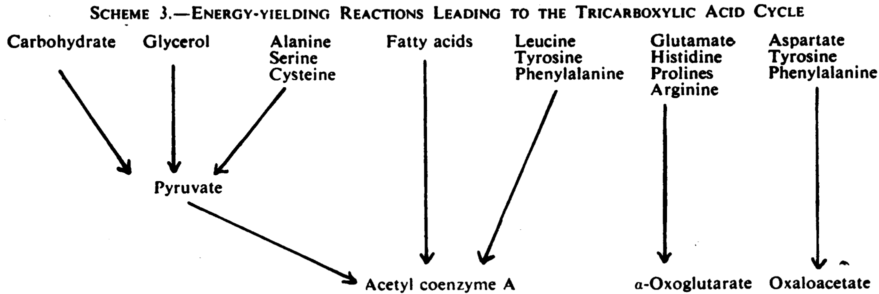


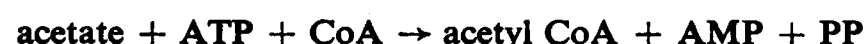
TABLE IX.—RELATIVE AMOUNTS OF ENERGY OBTAINED FROM VARIOUS SUBSTRATES (1) BY OXIDATION OF ACETATE (I.E. TRICARBOXYLIC ACID CYCLE) (2) BY STEPS LEADING TO ACETATE

(The amounts of energy are expressed by amounts of O_2 required and of ATP formed. ATP formation is calculated on the assumption that all oxidative steps yield 3 ATP per one oxygen atom except the reactions α-ketoglutarate → succinate (4 ATP) and $-H_2C-CH_2-$ → $-HC{=}{=}CH-$(2 ATP). The latter type of reaction occurs in the oxidation of succinate and of fatty acids)

Substrate	Mole O_2 required for: Steps leading to tricarboxylic acid cycle	Mole O_2 required for: Tricarboxylic acid cycle	ATP formed: Steps leading to tricarboxylic acid cycle	ATP formed: Tricarboxylic acid cycle	% of ATP formed through tricarboxylic acid cycle
Carbohydrate (per triose equivalent)	1	2	7*	12	63
Fatty acids (long chains, per each C_2 fraction)	1	2	5	12	71
Fatty acids (C_4-residue or butyrate)	1	4	5	24	83
Glycerol	1½	2	9	12	57
Lactic acid	1	2	6	12	67
Propionic acid	1½	2	6‡	12	67
Succinic acid	1½	2	8	12	60
Citric acid	2½	2	15	12	44
Glutamic acid	2½	2	15	12	44
Propanol	2½	2	15	12	44
1, 2-Propylene glycol	2	2	12	12	50
Acetic acid	0	2	−2†	12	100
Leucine	1½	6	8	36	82
Tyrosine	3½	6	9§	36	80
Phenylalanine	4	6	12§	36	75

*The ATP formed from carbohydrate includes that formed anaerobically. This amounts to one ATP per triose equivalent when glucose is the starting material.

†Acetic acid requires the expenditure of two pyrophosphate bonds before it can enter the tricarboxylic acid cycle, and yield energy, the reactions by which acetate is activated being:



acetate + ATP + CoA → acetyl CoA + AMP + PP

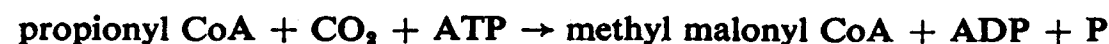
As far as is known the pyrophosphate is subsequently hydrolysed, and the AMP is phosphorylated by a second molecule of ATP to form 2 ADP:

AMP + ATP → 2 ADP

The balance of the reaction leading to the entry of acetate into the cycle is therefore

acetate + 2 ATP + CoA → acetyl CoA + 2 ADP + 2 phosphate

‡Like acetate, propionate requires the expenditure of two pyrophosphate bonds, to be converted into its reactive form, i.e. propionyl-CoA. Propionyl CoA is converted to succinyl CoA before it can yield energy and a further molecule of ATP is used for this conversion:



propionyl CoA + CO_2 + ATP → methyl malonyl CoA + ADP + P

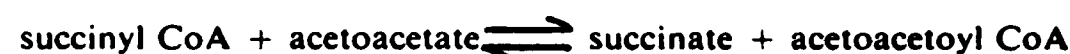
methyl malonyl CoA ⇌ succinyl CoA

It is likely that the energy-rich bond of succinyl CoA can be utilized for the generation of ATP through the reactions:

succinyl CoA + GDP + P ⇌ coenzyme A + GTP + succinate

GTP + ADP ⇌ GDP + ATP

which are intermediary stages in the oxidation of α-ketoglutarate and are taken to occur in all cells which perform the tricarboxylic acid cycle. It is also known that succinyl-CoA can be used for "activating" acetoacetate through the reaction:



succinyl CoA + acetoacetate ⇌ succinate + acetoacetoyl CoA

and it can thus save ATP required for the "activation" of acetoacetate. Thus, allowing for the net effect of these reactions, 2 ATP molecules are required for the "activation" of propionate.

§Several steps of the oxidation of tyrosine (p-hydroxyphenyl pyruvate → maleyl-acetoacetate, equivalent to 2 O_2) are probably not utilized for the synthesis of ATP (*see* text).

third by the reactions leading to it. A diet can be so modified that the proportion of the total energy supplied derived from the cycle, i.e. from the utilization of acetyl coenzyme A, varies. If ketosis is due to an excess of acetyl coenzyme A, irrespective of whether the excess arises from under-utilization or over-production, it should be relieved by a diet in which less of the energy supply depends on the utilization of acetate and more on the reactions leading to the formation of acetate (it being understood that the total oxygen consumption stays at a given level).

Table IX lists the distribution of the energy-supplying stages between these two phases of metabolism. The list does not aim at completeness but includes substances which may be of therapeutic interest, especially with reference to bovine ketosis. The relative contributions to the energy supply are expressed by two different standards, viz. the amounts of O_2 required and of ATP formed. In calculating the formation of ATP, the assumption has been made that all oxidative steps yield 3 ATP molecules per oxygen atom except the reaction α-oxoglutarate ⟶ succinate which yields 4 ATP and the reaction $-CH_2.CH_2-$ ⟶ $-CH:CH-$ which yields 2 ATP. The latter reaction occurs in the dehydrogenation of succinic and of fatty acids, including propionic acid and the fatty acid derived from leucine, i.e. isovaleric acid. There are uncertainties in the calculation of the ATP yield from tyrosine and phenylalanine because the degradation of these two amino acids includes oxidative steps which probably cannot be coupled with phosphorylation. They are the steps between hydroxy-phenylpyruvate, via homogentisic acid, to maleyl-acetoacetate (Hager *et al.*, 1957; Schepartz, 1953; Knox and Edwards, 1955; Dalgliesh, 1955). The enzyme systems concerned with these stages do not seem to contain pyridine nucleotides, flavoproteins and iron-porphyrins and as a rule only oxidations which involve these catalysts can be coupled to phosphorylation.

The two standards used for measuring the energy supply—O_2 consumption and ATP formation—give in general parallel values, but there are some differences, and the ATP values are of more immediate interest because they are a measure of the *utilizable* energy.

There are three substances in the list which release more than 80% of the energy through the tricarboxylic acid cycle. They are acetate (100%), the C_4-fatty acid (83%), and leucine (82%). These substances are known to be the main ketogenic materials of a normal diet.

Antiketogenic are those substances which relieve the pressure on the tricarboxylic acid cycle by providing energy through reactions outside the cycle. The more energy that can be obtained from reactions other than those of the cycle, the greater this antiketogenic effect can be expected to be. This is in fact the case. According to Table IX, the most effective antiketogenic substances are expected to be propanol, citrate and glutamate. These substances yield more ATP by reactions outside the cycle than is yielded by the cycle itself. The next best substance is 1,2-propylene glycol, followed by glycerol, succinate, propionate, glucose and lactate.

A precise quantitative measurement of the antiketogenic activity in the intact animal is difficult if not impossible, but qualitatively the experience gained from experiments on the ketosis of starvation, of pancreas diabetes and of cows suffering from bovine ketosis is as expected. The results of earlier experiments on the antiketogenic effects of various substances have been summarized by Magnus-Levy (1925). The antiketogenic effect of glucose has long been known. Those of lactate, glycerol, citrate, glutamate and propionate have been observed on humans and on dogs by Baer and Blum (1907), Satta (1906), and Borchardt and Lange (1907). More recently their antiketogenic effects, as well as those of 1,2-propylene glycol, have also been noted in cases of bovine ketosis (Schultz and Smith, 1951; Schultz, 1952, 1954; Johnson, 1951, 1954; Mills, 1954; Maplesden, 1954). In testing the antiketogenic activity, complications may arise when the substance under test is acidic and administered as an alkali salt. Neutral substances have probably the advantage of greater palatability and of rendering unnecessary an intake of a major quantity of alkali.

There is thus sufficient information to supplement a diet so as to make it less ketogenic.

Hormonal Therapy

Since, however, the type of energy-supplying reaction—whether energy is derived from carbohydrate, fat or protein—is not solely controlled by the diet but also by hormones, methods of treatment may be based on either dietary changes or hormonal supplements. An example of the effectiveness of a hormonal supplement is the insulin therapy of diabetes, and insulin is certainly also the most effective remedy for those types of ketosis where carbohydrate cannot be utilized because of lack of insulin, i.e. the common forms of diabetic ketosis. It is of course of no avail when it is not a limiting factor in carbohydrate utilization, as is the case in bovine ketosis. Numerous reports have appeared in recent years on the successful treatment of this type of ketosis

with cortisone, hydrocortisone and related substances (Shaw, 1955, 1956; Paterson, 1957; Gessert *et al.*, 1955; Shaw *et al.*, 1955; Link *et al.*, 1957; Vigue, 1955). The glucocorticoids are antiketogenic because they inhibit the degradation of fat and favour the utilization of carbohydrate. The mechanism of action of these hormones is not yet known in detail, but its study is a field now ready for further experimental work. The immediate problem is to pin-point the enzymes of fat and/or carbohydrate metabolism on which these hormones act.

Whilst, thanks to insulin, the problem of treating diabetic ketosis is now well in hand, this cannot be said with equal confidence of bovine ketosis in which, however, the difficulty is in practical management rather than ignorance of basic principles. A pattern for the treatment of ketosis is provided by the experience of diabetes mellitus in man. Diabetes, like bovine ketosis, is primarily an upset of endocrine balance and in this type of condition the two therapeutic approaches, dietary and hormonal, aim at restoring to normality a dis-equilibrium: to replace the deficient hormone by supplements and to modify the diet in order to assist the body's diminished capacity for maintaining the metabolic balance. In some cases, depending on the type and severity of the disease, one method of treatment, either dietary or hormonal, may be adequate. In other cases, a combined dietary and hormonal treatment is called for.

Conclusion

The development of biochemical knowledge has substantially deepened our understanding of ketosis but the final answer to the question of why ketone bodies accumulate under certain conditions still cannot be given. The newer knowledge allows us to formulate the problem more precisely: in ketosis, acetyl coenzyme A or acetoacetate fail to prevent the oxidation of substrates which provide further acetyl coenzyme A. This is in contrast to the general rule that intermediates do not accumulate because their presence suppresses the degradation of the material from which they arise. Full understanding of the ketosis problem will have to await more information on how the degradation of foodstuffs is controlled, but, on the practical side, enough information is available to treat ketosis effectively in man and in farm animals.

REFERENCES

BAER, J., and BLUM, L. (1907) *Beitr. chem. Physiol. Path.*, **10**, 80.

BEATTY, C. H., and WEST, E. S. (1951) *J. biol. Chem.*, **190**, 603.

——, —— (1955) *J. biol. Chem.*, **215**, 661.

BENNETT, L. L., KREISS, R. E., LI, C. H., and EVANS, H. M. (1948) *Amer. J. Physiol.*, **152**, 210.

BORCHARDT, L., and LANGE, F. (1907) *Beitr. chem. Physiol. Path.*, **9**, 116.

COLE, B. T., ENGEL, F. L., and FREDERICKS, J. (1955) *Endocrinology*, **56**, 675.

COURTICE, F. C., and DOUGLAS, C. G. (1936) *Proc. Roy. Soc.*, Ser. B., **119**, 381.

——, ——, and PRIESTLEY, J. G. (1939) *Proc. Roy. Soc.*, Ser. B., **127**, 41.

DALGLIESH, C. E. (1955) *Advanc. Protein Chem.*, **10**, 31.

DAVIES, H. W., HALDANE, J. B. S., and KENNAWAY, E. L. (1920) *J. Physiol.*, **54**, 32.

DEUEL, H. J., Jr., BUTTS, J. S., and HALLMAN, L. (1935) *Proc. Soc. exp. Biol., N.Y.*, **32**, 897.

——, GULICK, M., and BUTTS, J. S. (1932) *J. biol. Chem.*, **98**, 333.

DIBOLD, H., FREY, L., and LAPP, F. W. (1937) *Dtsch. med. Wschr.*, **63**, 1505.

DRURY, D. R., WICK, A. N., and MACKAY, E. M. (1941) *Amer. J. Physiol.*, **134**, 761.

DUNLOP, D. M., and ARNOTT, W. M. (1937) *Lancet*, ii, 738.

EDSON, N. L. (1935) *Biochem. J.*, **29**, 2082.

—— (1936) *Biochem. J.*, **30**, 1855.

ENGEL, F. L. (1957) *Yale J. Biol. Med.*, **30**, 201.

——, and ENGEL, M. G. (1958) *Endocrinology*, **62**, 150.

ENGEL, M. G., and ENGEL, F. L. (1958) *Endocrinology*, **62**, 75.

FASELLA, P., BAGLIONI, C., TURANO, C., and SILIPRANDI, N. (1958) *Lancet*, i, 1097.

GAL, E. M., PETERS, R. A., and WAKELIN, R. W. (1954) *Biochem. J.*, **58**, xlii.

GEMMILL, C. L. (1940) *Bull. Johns Hopk. Hosp.*, **66**, 71.

GESSERT, R. A., SHAW, J. C., and CHUNG, A. C. (1955) *J. Amer. vet. med. Ass.*, **127**, 215.

GOLDFARB, W., BARKER, S. B., and HIMWICH, H. E. (1934) *J. biol. Chem.*, **105**, 283.

HAGER, S. E., GREGERMAN, R. I., and KNOX, W. E. (1957) *J. biol. Chem.*, **225**, 935.

HENZE, M. (1930) *Hoppe-Seyl. Z.*, **189**, 121.

HIRD, F. J. R., and SYMONS, R. H. (1959) *Biochim. biophys. Acta*, **35**, 422.

JOHNSON, R. B. (1951) *N. Amer. Vet.*, **32**, 327.

—— (1954) *Cornell Vet.*, **44**, 6.

JOWETT, M., and QUASTEL, J. H. (1935) *Biochem. J.*, **29**, 2181.

KALNITSKY, G., and TAPLEY, D. F. (1958) *Biochem. J.*, **70**, 28.

KNOX, W. E., and EDWARDS, S. W. (1955) *J. biol. Chem.*, **216**, 479.

KREBS, H. A., and EGGLESTON, L. V. (1948) *Biochem. J.*, **42**, 294.

——, and STICKLAND, R. (1958) Unpublished.

KULKA, R. (1958) Unpublished.

LAWRENCE, R. D. (1937) *Lancet*, ii, 286.

LEHNINGER, A. L. (1946) *J. biol. Chem.*, **164**, 291.

LINK, R. P., NEWTON, D. I., and HUBER, W. G. (1957) *J. Amer. vet. med. Ass.*, **130**, 137.

LUKENS, F. D. W. (1948) *Physiol. Rev.*, **28**, 304.

LYNEN, F., HENNING, U., BUBLITZ, C., SÖRBO, B., and KRÖPLIN-RUEFF, L. (1958) *Biochem. Z.*, **330**, 269.

MACKAY, E. M., SHERRILL, J. W., and BARNES, R. H. (1939) *J. clin. Invest.*, **18**, 301.

MAGNUS-LEVY, A. (1899) *Arch. exp. Path. Pharmak.*, **42**, 149.

—— (1901) *Arch. exp. Path. Pharmak.*, **45**, 389.

—— (1925) In: Oppenheimer's Handbuch der Biochemie. 2nd ed. Jena; **8**, 461.

MAHLER, H. R. (1953) *Fed. Proc.*, **12**, 694.

MAPLESDEN, D. C. (1954) *Canad. J. comp. Med.*, **18**, 287.

MILLS, A. M. (1954) *Georgia Vet.*, **6**, 9.

PASSMORE, R., and JOHNSON, R. E. (1958) *Quart. J. exp. Physiol.*, **43**, 352.

PATERSON, R. A. (1957) *Vet. Rec.*, **69**, 1097.

PENNINGTON, R. J. (1952) *Biochem. J.*, **51**, 251.

RALL, T. W., SUTHERLAND, E. W., and BERTHET, J. (1957) *J. biol. Chem.*, **224**, 463.

RECKNAGEL, R. O., and POTTER, V. R. (1951) *J. biol. Chem.*, **191**, 263.

ROSENFELD, G. (1885) *Dtsch. med. Wschr.*, **11**, 683.

—— (1906) *Berl. klin. Wschr.*, **43**, 978.

SARGENT, F., II, JOHNSON, R. E., ROBBINS, E., and SAWYER, L. (1958) *Quart. J. exp. Physiol.*, **43**, 345.

SATTA, G. (1906) *Beitr. chem. physiol. Path.*, **6**, 376.

SCHEPARTZ, B. (1953) *J. biol. Chem.*, **205**, 185.

SCHULTZ, L. H. (1952) *Cornell Vet.*, **42**, 148.

—— (1954) Proceedings of the Cornell Nutrition Conference for Feed Manufacturers. p. 76.

——, and SMITH, V. R. (1951) *J. Dairy Sci.*, **34**, 1191.

SHAFFER, P. A. (1921) *J. biol. Chem.*, **47**, 433.

SHAW, J. C. (1955) Advances in Veterinary Science. New York; **2**, 262.

—— (1956) Proceedings of the University of Maryland Nutrition Conference for Feed Manufacturers, p. 76.

——, GESSERT, R. A., and CHUNG, A. C. (1955) *N. Amer. Vet.*, **36**, 918.

SHAW, W. V., and TAPLEY, D. F. (1958) *Biochim. biophys. Acta*, **30**, 426.

SHIPLEY, R. A. (1944) *Amer. J. Physiol.*, **141**, 662.

SJOLLEMA, B., and VAN DER ZANDE, J. E. (1923) *J. metab. Res.*, **4**, 525.

STADIE, W. C., ZAPP, J. A., Jr., and LUKENS, F. D. W. (1940) *J. biol. Chem.*, **132**, 423.

STERN, J. R., COON, M. J., and DEL CAMPILLO, A. (1956) *J. biol. Chem.*, **221**, 1.

STÖHR, R., and HENZE, M. (1932) *Hoppe-Seyl. Z.*, **206**, 1.

SUTHERLAND, E. W., and CORI, C. F. (1951) *J. biol. Chem.*, **188**, 531.

——, and WOSILAIT, W. D. (1956) *J. biol. Chem.*, **218**, 459.

TERNER, C. (1958) *Biochem. J.*, **70**, 402.

VIGUE, F. R. (1955) *N. Amer. Vet.*, **36**, 356.

WEINHOUSE, S., and MILLINGTON, R. H. (1951) *J. biol. Chem.*, **193**, 1.

WILLIAMSON, J. R. (1959) Unpublished.