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The Physiological Role of the Ketone Bodies

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THE THIRD HOPKINS MEMORIAL LECTURE

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A memorial lecturer may pay tribute to the man to be honoured by speaking about some of his outstanding qualities, and I will do so briefly towards the end of my lecture. Another way of honouring and commemorating a great man is to continue his endeavours and to pursue the aims for which he strove. Hopkins's aim was the furtherance of biochemistry, and so I think it is appropriate if I spend the greater part of this lecture on presenting an account of experiments with which I have been recently concerned.

Until 1953 it was generally believed that acetoacetate and D(-)- β -hydroxybutyrate were normal intermediates in the degradation of fatty acids. In that year two new discoveries made it clear that these two metabolites are not on the main route of fatty acid metabolism, but are formed by side reactions. The first was the discovery by Lynen & Ochoa (1953) that the normal intermediates of fatty acid degradation are the coenzyme A derivatives of the ketone bodies, rather than the free ketone bodies. The second was the discovery by Lehninger & Greville (1953) that the intermediate formed from long-chain fatty acids is L(+)- β -hydroxybutyryl-coenzyme A, whereas the free β -hydroxybutyrate appearing in the body fluids has the D-configuration.

If, then, the ketone bodies are not normal intermediates, we must ask why they are formed and what role they play in the animal body. The experiments which I am to discuss were initiated with the aim of finding answers to these questions. The plan was to study the metabolic behaviour of the ketone bodies, in the hope that this would throw light on their physiological significance.

When free acetoacetate is supplied to animal tissues, *in vitro* or *in vivo*, two main reactions take place in most tissues: a reduction to D(-)- β -hydroxybutyrate, and an oxidation leading to carbon dioxide and water, intermediates being acetoacetyl-coenzyme A, acetyl-coenzyme A and the tricarboxylic acid cycle. There are other reactions, but quantitatively they are of less importance: the non-enzymic decarboxylation to acetone and the conversion into cholesterol and fatty acids.

Liver differs from most other animal tissues in that no oxidation of acetoacetate takes place and that the acetoacetate which disappears can usually all be accounted for by the formation of β -hydroxybutyrate. This is illustrated by data given in Table 1. The removal of acetoacetate by the liver was accelerated by the addition of the various hydrogen donors such as α -oxoglutarate, L(+)-glutamate, pyruvate, citrate, malate and succinate. The yield of β -hydroxybutyrate was in all cases somewhat greater than the amount of acetoacetate removed, the difference being greatest in the case of pyruvate. This is due to the fact that pyruvate, via acetyl-coenzyme A, forms additional acetoacetate, and that all the other added hydrogen donors can form pyruvate on aerobic incubation. Thus some of the β -hydroxybutyrate formed was derived from the substrates shown in the first column of the Table.

In other metabolically active tissues the yield of β -hydroxybutyrate is usually lower than the loss of acetoacetate. This is shown in Tables 2 and 3. In sheep-heart homogenate about half the acetoacetate removed was converted into β -hydroxybutyrate. In homogenates of skeletal muscle and sheep-kidney cortex very little of the acetoacetate removed was recovered as β -hydroxybutyrate. Intermediates of the oxidation of acetoacetate (the acids of the tricarboxylic acid cycle) did not accumulate in measurable quantities, with the exception of citrate, which under some conditions appeared in very small amounts. There is no evidence suggesting that appreciable quantities of acetoacetate are used for a net synthesis of fat or other cell constituents under the test conditions. The decarboxylation to acetone was likewise negligible in relation to other reactions. This leads to the conclusion that the acetoacetate not accounted for by β -hydroxybutyrate underwent complete oxidation.

It has of course long been established that many tissues can oxidize ketone bodies and use them as fuel of respiration [for review see Peters & Van Slyke (1946) and Campbell & Best (1956)]. But virtually all the previous work was qualitative and did not deal with the question of how much the

Table 1. *Removal of acetoacetate and formation of β -hydroxybutyrate in rat-liver homogenate*

Data refer to 4 ml. of a 10% homogenate, incubated for 45 min. at 30° in O₂. The 4 ml. included 20 μ moles of acetoacetate and 80 μ moles of other substrates. The minus sign indicates disappearance, the plus sign formation.

Substrate added other than acetoacetate	Metabolic changes		
	O ₂ (μ moles)	Acetoacetate (μ moles)	β -Hydroxybutyrate (μ moles)
None	-27.6	-1.2	+2.2
α -Oxoglutarate	-31.1	-11.7	+14.3
L(+)-Glutamate	-38.5	-12.6	+15.9
Pyruvate	-34.5	-0.8	+5.9
Fumarate	-33.6	-5.4	+7.9
Citrate	-28.8	-8.5	+13.4
Succinate	-57.4	-8.9	+11.0

Table 2. *Metabolism of ketone bodies in various tissue homogenates in vitro*

The substrate was 5 mM-acetoacetate, incubated at 30° [for experimental details see Table 2 of Krebs *et al.* (1961)].

Tissue	Metabolic changes (μ moles/g. dry wt./hr.)				
	O ₂	Acetoacetate removed	β -Hydroxybutyrate formed	Acetoacetate oxidized	O ₂ equivalents Acetoacetate oxidized
Sheep heart	-476	-232	+112	-120	4.4
Sheep diaphragm	-100	-115	+88	-27	5.4
Rat skeletal muscle	-152	-13	0	-13	11.3
Pigeon skeletal muscle	-553	-62	0	-62	9.1
Sheep-kidney cortex	-445	-108	9	-99	4.7

Table 3. *Metabolism of ketone bodies in animal tissues (sliced or intact)*

The substrate was 5 mM-acetoacetate, incubated at 40°.

Tissue	Metabolic changes (μ moles/g. dry wt./hr.)				
	O ₂	Acetoacetate removed	β -Hydroxybutyrate formed	Acetoacetate oxidized	O ₂ equivalents Acetoacetate oxidized
Rat-kidney cortex (sliced)	-814	-235	+69	-166	5.1
Rat spleen (sliced)	-398	-135	+24	-111	3.7
Rat-brain cortex (sliced)	-651	-167	+45	-122	5.5
Rat testis (teased out)	-454	-84	+16	-68	6.8
Guinea-pig smooth muscle (taenia of colon, intact)	-206	-67	+20	-47	4.6
Pigeon pancreas (sliced)	-331	-99	+27	-72	4.8

ketone bodies can contribute to the respiration of individual tissues. Information on this point can be obtained by relating the amounts of acetoacetate oxidized to the oxygen consumption. The complete oxidation of acetoacetate requires 4 molecules of O₂ per molecule of acetoacetate. As shown in Table 2, the ratio (O₂ equivalents used)/(acetoacetate oxidized) was a little over 4 in sheep heart and kidney cortex and somewhat higher in the other tissues.* If acetoacetate is the only sub-

strate of respiration the value of this ratio is 4. If the value of the ratio is n , the percentage contribution of acetoacetate to the fuel of respiration is $(4/n) \times 100$. It can be calculated from this formula that acetoacetate contributed over 90% to the fuel of respiration in the heart, 85% in the sheep-kidney cortex and 74% in the diaphragm. Data given in Table 3, which refer to tissue slices or intact isolated tissues, show that the contribution of acetoacetate is of a similar magnitude in rat-kidney cortex, spleen, brain, testis, pancreas and smooth muscle.

* When acetoacetate is aerobically reduced to β -hydroxybutyrate there are two major electron acceptors, namely O₂ and acetoacetate. This must be borne in mind when calculating the percentage contribution to respiration of acetoacetate. In Tables 2 and 3 the sum of β -hydroxybutyrate and O₂ has been expressed as 'O₂ equivalents', taking 2 molecules of β -hydroxybutyrate as equivalent to 1 molecule of O₂.

Experiments on isolated tissue may be frowned upon as being unphysiological, especially as far as quantitative aspects are concerned. Together with J. R. Williamson (Williamson & Krebs, 1961) I have therefore repeated the experiments on the intact rat heart perfused with saline according to

Table 4. *Metabolism of acetoacetate in perfused rat heart*

Concentrations of substrate were 5 mM and of insulin 2 milliunits/ml.; for full experimental details see William-son & Krebs (1961).

	Substances added to perfusate			
	Acetoacetate	Acetoacetate, insulin	Acetoacetate, glucose	Acetoacetate, glucose, insulin
Acetoacetate (μ moles/g./hr.)	-503	-375	-515	-361
β -Hydroxybutyrate (μ moles/g./hr.)	+211	+172	+205	+147
O ₂ (μ moles/g./hr.)	-1455	-1455	-1607	-1607
O ₂ equivalents	5.33	7.54	5.52	7.86
Acetoacetate oxidized				
Contribution of acetoacetate to fuel of respiration (%)	75	55	73	51

Table 5. *Effect of crude anterior pituitary extract on acetoacetate from pyruvate in rat-liver homogenate*

Data refer to 4 ml. of a 10% homogenate with 20 mM-pyruvate, incubated for 60 min. at 30°. The values shown are means, standard error of the means and, in parentheses, the number of observations.

	Acetoacetate formed (μ moles/hr.)
Control	2.48 $\pm$ 0.267 (24)
With 5 mg. of dried anterior pituitary	3.80 $\pm$ 0.245 (24)

Bleeheh & Fisher (1954). A summary of some results is given in Table 4. As in the homogenates, some acetoacetate was reduced and some oxidized. When acetoacetate was the only added substrate it contributed 75% to the fuel of respiration. The addition of glucose and even insulin [which in this preparation causes a rapid uptake of glucose by the tissue (Bleeheh & Fisher, 1954)] did not depress the utilization of acetoacetate very much. Thus in the intact rat heart, as in the sheep-heart homogenate, acetoacetate is the preferred fuel of respiration. It might be argued that the tissues of ruminants are expected to utilize ketone bodies readily because these arise in large amounts in the rumen, but this does not apply to rat tissues.

The finding that ketone bodies are ready substrates of respiration suggests that their presence in the circulating blood serves to supply tissues with a fuel of respiration; that their function in this respect is analogous to that of glucose and the non-esterified fatty acids. This concept would make it plausible why the ketone body level is raised when the supply of utilizable glucose is diminished, as is the case in starvation (when the glucose concentration is lowered) or in diabetes (when glucose is present but not utilizable). The relatively low glucose level in the blood of ruminants is also in accordance with this idea.

If this concept is correct it may be expected that control mechanisms exist which adjust the blood ketone body level to requirements so that the lack of utilizable glucose is compensated by a raised

ketone body level. A hormonal control mechanism is in fact indicated by the observation that anterior pituitary extracts are ketogenic in the intact animal (Burn & Ling, 1930). Experiments on liver homogenates show that the liver is the site (or one of the sites) of action of the hormone. Crude extracts of the anterior pituitary, when added to liver homogenates in the presence of pyruvate, raise the ketone body production on average by 50% [Table 5; L. Adler & H. A. Krebs, unpublished work; see also Shipley, (1944)]. The active principle of the anterior pituitary is not identical with growth hormone, adrenocorticotrophic hormone or corticotrophin A. It is not precipitated by trichloroacetic acid and is heat-stable. The problem of its chemical nature and mechanism of action is at present under investigation.

Acetoacetate is not the only substrate which competes with glucose. In many tissues fatty acids are a ready fuel of respiration. According to Wertheimer & Ben-Tor (1952) rat diaphragm can oxidize fatty acids *in vitro* but prefers glucose if available. Cardiac muscle also oxidizes fatty acids (Bing, 1954-55; Ballard, Danforth, Neagle & Bing, 1960; Shipp, Opie & Challoner, 1961). In the experiments on rat heart (Table 4) the fraction of respiration not accounted for by the oxidation of acetoacetate was largely due to the oxidation of endogenous material, presumably fat, as indicated by the non-disappearance of sufficient glucose or glycogen. That fat can serve as a major fuel of respiration in muscular exercise has been especially brought home by the observations of Pugh *et al.* (1960) on channel swimmers who spend about 10 000 kcal. during the swim and in the absence of sufficient carbohydrate cannot defray the greater part of their energy expenditure except by the combustion of fat. Migratory insects such as certain leaf-hoppers or the Monarch butterfly (see Weis-Fogh, 1952) and birds of passage (Odum & Connell, 1956; Odum, 1960) are also known to rely mainly on fat as a source of energy during flight.

When Gemmill (1942) reviewed the question of the fuel of muscular exercise 20 years ago he came

to the conclusion that carbohydrate was *the* fuel, and that fat was not used directly by muscle. These views are no longer tenable. Ketone bodies and fatty acids can in many circumstances replace carbohydrate as an aerobic fuel of muscular exercise. Carbohydrate, however, has the advantage of being a source of energy also under anaerobic conditions.

I now wish to turn to another aspect of ketone body metabolism, the ready reversibility of the reaction:

catalysed by D(-)- β -hydroxybutyric dehydrogenase. The oxidation of β -hydroxybutyrate to acetoacetate occurs in many tissues when conditions are aerobic and D(-)- β -hydroxybutyrate is available (Jowett & Quastel, 1935). The reduction of acetoacetate is particularly rapid under anaerobic conditions and is accelerated in tissue homogenates when these are supplemented with hydrogen donors such as citrate, α -oxoglutarate, glutamate, pyruvate or malate. But as already shown in Table 1, the reduction also occurs aerobically at great speed. As a rule the reduction of acetoacetate is the result of a coupled oxido-reduction mediated by diphosphopyridine nucleotide, according to the general equation:

This mechanism accounts for the effects of all substrates except succinate. As the redox potential of the succinate-fumarate couple is about 300 mv more positive than that of the β -hydroxybutyrate-acetoacetate couple, succinate cannot donate appreciable quantities of hydrogen atoms to acetoacetate. Yet (see Table 1) succinate can substantially accelerate the reduction of acetoacetate. What, then, is the mechanism of the succinate effect?

Since reduced diphosphopyridine nucleotide is certain to be the immediate hydrogen donor for acetoacetate, succinate must increase the rate of supply of reduced pyridine nucleotide. That it increases its concentration is in fact already well established. It was first demonstrated by Chance & Williams (1955), and thermodynamic considerations postulate, as in the case of β -hydroxybutyrate, that this cannot be a direct transfer of hydrogen atoms from succinate to pyridine nucleotide. The effects of succinate on the reduction of diphosphopyridine nucleotide and of acetoacetate

have thus a common basis, namely the reduction of diphosphopyridine nucleotide, which precedes the reduction of acetoacetate. Owing to catalytic action of diphosphopyridine nucleotide, the accumulation of β -hydroxybutyrate magnifies the primary reduction of diphosphopyridine nucleotide, and, whereas the reduction of diphosphopyridine nucleotide is a reaction which owing to the low concentration of pyridine nucleotide usually comes to an end within a few minutes, if not seconds, the reduction of acetoacetate may continue over long periods, an advantage from the point of view of experimentation.

The question of the mechanism of diphosphopyridine nucleotide and acetoacetate reduction in the presence of succinate is the subject of a current controversy between Chance and his pupils (Chance & Hollunger, 1957, 1960; Chance & Hagihara, 1960; Klingenberg, Slenczka & Ritt, 1957; Azzone, Ernster & Klingenberg, 1960; Klingenberg & Schollmeyer, 1960) on the one hand, and the Oxford team on the other (Birt & Bartley, 1960; Kulka, Krebs & Eggleston, 1961; Krebs, Eggleston & d'Alessandro, 1961). Chance assumes that the oxidation of succinate causes an increased formation of adenosine triphosphate, and that the accumulating adenosine triphosphate causes a reversal of oxidative phosphorylation, as illustrated by the following scheme of the electron transport between reduced diphosphopyridine nucleotide and flavoprotein (FP):

If this system is in equilibrium, addition of adenosine triphosphate would cause the reaction to proceed from right to left, which means a reduction of diphosphopyridine nucleotide. That some stages of oxidative phosphorylation are reversible is shown by the ATP-P and ATP-ADP exchange reactions; the hypothesis is therefore feasible.

An alternative explanation is based on kinetic considerations and is illustrated by Fig. 1, which shows a diagram of the relevant aspects of electron transport. The main feature is the joint terminal pathway of the electrons from succinate and from reduced diphosphopyridine nucleotide, the separate pathways joining at the cytochrome level (see also Wu & Tsou, 1955; Ringler & Singer, 1959; Kimura, Singer & Lusty, 1960). At this stage a competition occurs between the electrons from succinate and those from reduced diphosphopyridine nucleotide. Under many conditions those from succinate win in this competition as shown by the measurement of the succinate disappearance, the formation of fumarate and malate and the oxygen consumption. Most of the respiration can be accounted for by the reaction:

Fig. 1. Diagram of electron transport. For details see text.

It is further of importance that the oxidation of succinate yields a product, malate, which readily donates electrons to diphosphopyridine nucleotide by undergoing the reactions of the tricarboxylic acid cycle. This causes a rise of the reduced diphosphopyridine nucleotide concentration because the transfer of electrons from reduced diphosphopyridine nucleotide to cytochrome is blocked by the flow of electrons from succinate to cytochrome.

If acetoacetate is present it is reduced whenever the transfer of electrons to diphosphopyridine nucleotide [reaction (1)] is more rapid than the transfer of electrons from diphosphopyridine nucleotide to oxygen [reactions (2) and (3)]. According to this concept succinate competitively inhibits reaction (2) and therefore favours the accumulation of reduced diphosphopyridine nucleotide formed by reaction (1) and hence promotes reaction (5).

There are two observations which Chance and his followers cite as evidence in favour of the concept of a reversal of oxidative phosphorylation. First, under some conditions, 2:4-dinitrophenol and other uncouplers prevent the reduction of diphosphopyridine nucleotide on addition of succinate. If the uncoupling of oxidative phosphorylation were the only effect of dinitrophenol this would indeed be powerful evidence in support of Chance's hypothesis. But it is not the only effect; dinitrophenol also accelerates the oxygen consumption and in particular the electron transport from reduced diphosphopyridine nucleotide to oxygen, and (as I shall show later) it inhibits the oxidation of succinate. According to the kinetic concept circumstances which cause a lowering of the rate of succinate oxidation and a rise in the rate of electron transport from reduced diphosphopyridine nucleotide to oxygen are bound to lower the reduced diphosphopyridine nucleotide concentration and the formation of β -hydroxybutyrate. The dinitrophenol experiments are therefore inconclusive.

The second observation which Chance (1961) and Klingenberg & Schollmeyer (1960) consider as evidence for a reversal of oxidative phosphorylation is the reduction of diphosphopyridine nucleotide observed in mitochondrial suspensions on addition of adenosine triphosphate. Again if this were the only effect of adenosine triphosphate it would support Chance's hypothesis, but this is not the case. In the experiments which are quoted as evidence adenosine triphosphate also inhibited the oxygen uptake (see Fig. 2 of Klingenberg & Schollmeyer, 1960). The reason for this is not certain, but whatever the mechanism a decreased oxygen consumption [i.e. a decreased rate of (2) and (3)] would account for a raised reduced diphosphopyridine nucleotide level. Thus the effects of adenosine triphosphate are likewise inconclusive, as they can be explained by both hypotheses. There are two further findings which are not compatible with the idea of the reversal of oxidative phosphorylation but agree with the kinetic concept. First, 1 mM-amytal accelerates the reduction of acetoacetate and this occurs in the presence as well as in the absence of succinate (see Table 6). In the absence of succinate 1 mM-amytal is a slight inhibitor of oxidative phosphorylation and of respiration, but in spite of this it increases the rate of reduction of acetoacetate. This increase cannot be explained by a reversal of oxidative phosphorylation, but is accounted for by the kinetic concept. Amytal is known to inhibit reaction (2) of Fig. 1 without blocking it completely. It still permits the oxidative formation of reducing agents which convert diphosphopyridine nucleotide into reduced diphosphopyridine nucleotide.

Still more convincing are experiments with high concentrations of dinitrophenol (0.5 mM) plus amytal. In the presence of these two agents oxidative phosphorylation is strongly inhibited, yet the reduction of acetoacetate in the presence of succinate is very rapid (Table 7). In this experiment dinitrophenol alone abolished the reduction

of acetoacetate, but this is explained by a specific effect of dinitrophenol on the oxidation of succinate not hitherto observed. As seen from Table 7, 0.5 mM-dinitrophenol inhibited the oxidation of succinate about 40% whilst the total oxygen consumption fell by 15% only. The differences in the inhibition of succinate removal and oxygen consumption mean that dinitrophenol affects the competition for the common electron pathway in favour of reduced diphosphopyridine nucleotide and against succinate. Amytal, by inhibiting electron transport from reduced diphosphopyridine nucleotide to cytochrome, affects the competition in the opposite direction and restores the oxidation of succinate. Why dinitrophenol inhibits the oxidation of succinate and favours the oxidation of reduced diphosphopyridine nucleotide, and further why amytal counteracts the slight inhibition of the oxygen uptake by dinitrophenol in the presence of succinate cannot yet be explained. The effects are observed only when the mitochondrial structure is essentially intact and not with purified succinic dehydrogenase. Dr W. N. Aldridge (personal communication) suggests that they may be connected with mitochondrial swelling. This is being investigated.

The observations on the reduction of diphosphopyridine nucleotide or acetoacetate are all

accounted for by the kinetic concept whilst the occurrence of the reduction of acetoacetate in the presence of dinitrophenol plus amytal (Table 7) when oxidative phosphorylation is virtually abolished is incompatible with the hypothesis of Chance.

The occurrence of a reversal of oxidative phosphorylation, as postulated by Chance, is of course not denied, and it is likely that under some experimental conditions the reversal can cause a reduction of diphosphopyridine nucleotide and even acetoacetate. But it is very improbable that it plays a major role in respiring tissue preparations. The view is firmly supported that a reduction of acetoacetate occurs whenever the rate of hydrogen transport from available substrates to diphosphopyridine nucleotide is greater than the rate of hydrogen transport from reduced diphosphopyridine nucleotide to cytochrome. Under these circumstances more reduced diphosphopyridine nucleotide is available than is required as substrate of oxidative phosphorylation, and if acetoacetate is present it is reduced by the surplus of reduced diphosphopyridine nucleotide.

The ready physiological, as opposed to experimental, interconversion of the two ketone bodies is a remarkable phenomenon. Although many oxidoreductions are reversible in animal tissues, the

Table 6. *Effect of amytal on acetoacetate metabolism in sheep heart*

Data refer to 4 ml. of a 10% homogenate, incubated for 45 min. at 30°. The gas space was filled with O₂, and 20 μ moles of acetoacetate and 80 μ moles of succinate were added [for experimental details see Krebs *et al.* (1961)]

Substrate added	Amytal (mM)	Metabolic changes		
		O ₂ uptake (μ moles)	Acetoacetate (μ moles)	β -Hydroxy- butyrate (μ moles)
Acetoacetate	0	-27.2	-10.9	+ 4.3
Acetoacetate, succinate	0	-56.0	-15.1	+11.4
Acetoacetate	1	-21.0	-13.4	+ 8.3
Acetoacetate, succinate	1	-43.0	-16.8	+17.3

Table 7. *Effects of 2:4-dinitrophenol and amytal on the metabolism of rat-liver homogenates in the presence of succinate and acetoacetate*

Data refer to 4 ml. of a 10% homogenate, incubated for 30 min. at 30°. All vessels contained 20 μ moles of acetoacetate and 80 μ moles of succinate. [General technique was as described by Krebs *et al.* (1961).]

Vessel no.	...	...	...	1	2	3	4
Further additions (final concn.)					Amytal (1.25 mM)	Dinitrophenol (0.5 mM)	Amytal (1.25 mM) Dinitrophenol (0.5 mM)
Metabolic changes (μ moles):							
O ₂				-38.3	-38.8	-32.6	-41.2
Acetoacetate				-10.2	- 9.4	- 0.2	- 7.3
β -Hydroxybutyrate				+ 9.5	+ 9.3	+ 1.7	+ 9.2
Succinate				-71.8	-70.6	-41.2	-72.5
Fumarate + malate				+57.5	+54.3	+23.7	+51.3

reductions do not as a rule occur readily because under physiological conditions, i.e. in the presence of oxygen, the equilibria are in favour of the oxidation. The exceptional reversibility of the acetoacetate- β -hydroxybutyrate system raises the question whether a special physiological significance attaches to it. A relevant observation was recently reported by Devlin & Bedell (1960) who showed that reduced diphosphopyridine nucleotide, which is not attacked when added to liver mitochondria, undergoes oxidation in the presence of acetoacetate. They suggest that the acetoacetate- β -hydroxybutyrate system may act as a hydrogen carrier which transfers electrons from extramitochondrial reduced diphosphopyridine nucleotide to an intramitochondrial site where the oxidation of reduced diphosphopyridine nucleotide, coupled with phosphorylation, takes place (see Fig. 2). It is assumed that oxidative phosphorylation can only occur at special sites and that β -hydroxybutyrate acts as a 'shuttle', transporting electrons from other sites to the effective ones. If this is correct and if a substantial proportion of the hydrogen atoms of organic substrates pass through this 'shuttle' then the potential rates of the reduction of acetoacetate should be comparable with those of respiration. Measurements of the maximum rates which are obtained with mixtures of substrates in rat-liver homogenates show that the rates may be sufficient to permit the assumption that about half of the respiration passes through the 'shuttle'. In other tissues it is rather less.

As yet it is not possible to decide to what extent the acetoacetate- β -hydroxybutyrate system acts as a hydrogen carrier in the manner suggested by Devlin & Bedell (1960). Another significance of the ready reduction of acetoacetate is suggested by the fact that the ketone bodies serve as fuels of respiration in many tissues. Acetoacetate is relatively

unstable, especially in blood, where it is decarboxylated to acetone (Rossi, 1938). β -Hydroxybutyrate is stable and is therefore a more suitable form of nutrient in the circulating blood. The idea that β -hydroxybutyrate is a nutrient in the blood plasma recalls the fact that β -hydroxybutyrate, in the form of a polymer, is a reserve material in many micro-organisms such as *Bacillus megaterium*, *Bacillus cereus*, *Azotobacter*, *Bacillus anthracis*, *Rhizobium*, *Pseudomonas*, *Chromatium*, *Rhodospseudomonas* and *Spirillum* (see Hayward, Forsyth, & Roberts, 1959).

I will not attempt to summarize my sketchy attempts to throw light on the physiological role of the ketone bodies. Instead I prefer to use the rest of my time to make a few references to Hopkins in whose honour we are assembled today. Appreciations of Hopkins's contributions to biochemistry and of the qualities of his personality have been eloquently recorded not long ago by many who were closely associated with him (see Needham & Needham, 1949; Stephenson, 1948, 1949; Dale, 1947, 1948; Mellanby, 1948; Peters, 1959; Sherrington, 1947; Dixon, 1947; Harris, 1947). In view of this I thought it appropriate if I confine myself to a few words about my personal encounters with Hopkins, and I hope you will be lenient with me if in so doing I speak as much about myself as about Hopkins. The circumstances under which I joined the Cambridge laboratory in 1933 were somewhat unusual. In his annual Presidential Address to the Royal Society, on 30 November 1932, Hopkins (1932) had discussed, in complimentary terms, my investigations on the synthesis of urea, then just published, quoting them as an example of current research on the chemistry of living tissues. I was working at that time in the Department of Medicine of the University of Freiburg im Breisgau. A few

Fig. 2. The β -hydroxybutyrate shuttle. Scheme suggested by Devlin & Bedell (1960) to account for the effect of acetoacetate on the oxidizability of extramitochondrial DPNH_2 . 'Extra-mitochondrial' DPNH_2 , which has no access to the 'intramitochondrial' site of oxidative phosphorylation reduces acetoacetate to β -hydroxybutyrate. The latter enters the mitochondrion and interacts with DPN situated at the site of the electron-transfer chain. The acetoacetate formed diffuses into the extramitochondrial area.

weeks after Hopkins's address, on 30 January 1933, the Nazis assumed power, and their policy of persecution reached the German universities soon afterwards. At this critical moment when my future was very uncertain, on 22 April 1933, I received a letter from Albert Szent-Györgyi who had visited Cambridge some days earlier, telling me that people in the Cambridge biochemical laboratory (whom I had never met personally) had expressed concern about my fate and would be willing to help me should I get into difficulties, and he suggested that I should write to Hopkins. I responded without delay and received a prompt reply from Hopkins beginning with the words 'I admire your work so much that I am very anxious to help you'. This was indeed a cheering message. He promised to look for financial support and explained that this might take a few weeks. Soon afterwards Dr Robert Lambert of the Rockefeller Foundation, which at that time supported my research—and has done so ever since—told me that the Foundation might possibly provide me with a one-year maintenance grant so that I could continue my work in England. The warmth of Hopkins's letters, the goodwill of the Rockefeller Foundation, and the unpleasantness of the German scene, encouraged me to set off for Cambridge although there were no definite prospects. I arrived there at the end of June. Hopkins confirmed that with the help of the Rockefeller Foundation I could work in his laboratory and characteristically he apologized for the inadequacy of the maintenance grant. In the circumstances, having just escaped the horrors of Nazi Germany, the grant did not seem to me at all inadequate. On the contrary I thought it was princely and I accepted at once.

The contrast between the world from which I had come away and the world which I entered was indeed enormous. Nazi philosophy had infiltrated all strata of German life and had created a dreadful atmosphere. The teaching of *Mein Kampf* had whipped up the worst of primitive instincts. There were savage brutalities in day-to-day life in the form of beatings, shootings and murder, personal vituperation, bad faith, incredible arrogance. Before the Nazis assumed power the worst features of their creed had been somewhat restrained, in particular in the universities, but after 30 January they swept into the open everywhere. It was a great shock to see how some of one's immediate colleagues, either because of conviction or because of blackmail and political pressure, adopted the official Nazi doctrines and cold-shouldered the political opponents of the Nazis. After this experience the warmth which greeted me at Cambridge from the minute of my arrival was indeed touching and heartening. There was a

spirit of friendliness, of humanity, of tolerance and of fairness which had vanished in Germany. It was in Hopkins's laboratory where I saw for the first time at close quarters some of the characteristics of what is sometimes referred to as 'the British way of life'. The Cambridge laboratory included people of many different dispositions, convictions and abilities. I saw them argue without quarrelling, quarrel without suspecting, suspect without abusing, criticize without vilifying or ridiculing, and praise without flattering. Hopkins was the central figure, beloved and respected as a natural leader, exercising leadership from within and not from above, utterly humble, modest, gentle, but by no means weak. For he had sufficient confidence in himself, as a leader must, to make decisions and to fight with tenacity for the aims in which he believed. Although President of the Royal Society, and 72 years old, he was accessible to all and always ready to help. I never heard him plead that he was too busy and had no time. His concern ranged far beyond biochemistry, Cambridge University and the Royal Society. What struck me, in particular contrast with the German scene, was the strong 'social conscience' of Hopkins and his school, their deep concern for affairs of the world at large. One of its many manifestations was the practical help given, with substantial personal sacrifices, to refugees. Between 1933 and 1935 the laboratory sheltered six refugees from central Europe: Friedmann, Lemberg, Chain, Weil-Malherbe, Bach and myself.

To me, as to many others, life in Hopkins's laboratory was a great education. When I arrived in Cambridge I had already some years of research experience, mainly under the guidance of Otto Warburg. Warburg had taught me the alphabet of scientific research; he set an example of asking the right question, of forging new tools for tackling the chosen problem, of being ruthless in self-criticism, of taking pains in verifying facts, of expressing results clearly and concisely, of focusing life on true values. Hopkins's laboratory became to me a model of an academic community, harmoniously working for a common purpose, in an atmosphere of mutual trust, encouragement and help, inspired by the outstanding qualities of character and intellect of its central figure.

Hopkins's laboratory was a model also of how true and lasting strength can be achieved in a team of scientists. Biological principles teach us that the strength of any population derives from the diversity of its individual members. A population is more likely to survive hazards the greater its diversity (see Medawar, 1957). Hopkins's school never concentrated on a few limited topics as many other schools did. His own research group was small. He encouraged the team to be

versatile; everyone competent and enthusiastic was given the chance to venture ahead with his own ideas. This was probably a major cause of the far-reaching influence which the Cambridge school exerted on the development of the subject. It safeguarded the school against one of the worst hazards which can befall a team of scientists, the danger of losing the spirit of adventure and becoming obsolete in outlook and method.

You will appreciate that to me Hopkins is not only the great biochemist but also a man to whom I became deeply attached for very personal reasons. At a critical stage of my life, when the country of my birth proscribed me, he helped me, though a stranger, and welcomed me to the community of his laboratory, and together with his collaborators he initiated me into a new society. Sooner than I thought possible I was transformed from the stage of the visitor to that of a member. A token of this transformation was a touching set of incidents at the annual Sir William Dunn dinners, where, with Hopkins presiding, the memory of the benefactor of the Cambridge School of Biochemistry was honoured. When I attended this for the first time, after almost a year's stay, I was asked to reply to the Toast of the Visitors from Overseas, and a year later I was invited to propose, on behalf of the School, the Toast of the Visitors from Overseas. I cannot help feeling that the invitation of your Committee to stand in this place today, on the special occasion of the 100th Anniversary of Hopkins's birth and the 50th Anniversary of The Biochemical Society, is in the tradition of the very same spirit, demonstrating the acceptance of the one-time stranger into the family of British biochemists. For this I shall always be truly grateful.

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