CCLX. KETOGENESIS-ANTIKETOGENESIS. III. METABOLISM OF ALDEHYDES AND DICARBOXYLIC ACIDS.

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(*Received 1 September 1936.*)

ALIPHATIC ALDEHYDES.

BATTELLI & STERN [1910] and Parnas [1910] discovered independently an enzyme system which converted aldehydes into the corresponding alcohols and acids by dismutation (the Cannizzaro reaction). Parnas found that aliphatic aldehydes incubated with liver brei anaerobically in bicarbonate buffer were dismuted quantitatively. Reichel & Berczley [1931] prepared a purified "aldehydrase"; and the dismutation of propaldehyde was found by Reichel & Wetzel [1934] and by Reichel & Köhle [1935, 1] to occur equally well under aerobic and anaerobic conditions. Reichel & Köhle [1935, 2] believe that an alcohol dehydrogenase is associated with the "aldehydrase". It is possible that the two enzymes working together aerobically could completely convert aldehyde into acid. Although the mechanism of aldehyde oxidation is not yet clear, it is of interest to examine the behaviour of aldehydes in tissue slices taking into account both the anaerobic dismutation and the ketogenic properties under aerobic conditions. This is desirable because Friedmann [1908] has observed that acetaldehyde and aldol are ketogenic.

Methods.

The general methods employed have been described earlier [Edson, 1935]. Acetoacetic acids (or β-ketonic acids) was determined by the aniline citrate procedure. Since the higher aldehydes are almost insoluble in water, it was necessary in some cases to work with suspensions. The members of the series from valeraldehyde to octaldehyde inclusive were added to the Ringer solution as fine emulsions in quantity sufficient to provide about 2 mg.

The dismutation was investigated manometrically by measuring anaerobic acid production in bicarbonate-Ringer solution at *p*H 7·4 and 37·5°. For such experiments it was permissible to use 30–40 mg. (dry wt.) of liver slices. Respiration and ketone body formation were measured as usual in phosphate saline [Krebs, 1933].

EXPERIMENTAL.

Anaerobic dismutation of aldehydes in surviving liver tissue.

Since the chemistry of anaerobic dismutation is fully known, the rate of reaction can be determined by following the change in one of the reactants. Though it is not possible to estimate accurately the small amounts of alcohol which would be formed in a tissue slice experiment, a precise measurement of acid production can be made. Table I shows the rate of acid formation, as measured by CO_2 evolution from bicarbonate, in rat liver slices which were shaken in presence of aliphatic aldehydes (neutral).

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Table I. *Anaerobic acid formation by rat liver slices in presence of aliphatic aldehydes.*

3 ml. bicarbonate-Ringer solution [Krebs & Henseleit, 1932]. *p*H 7·4. Duration of exp. 1 hour. Gas: 5% CO_2; 95% N_2.

Substrate	Q_{Acid}	Substrate	Q_{Acid}
Nil	2·59	*n*-Valeraldehyde, saturated	3·26
Glucose, 0·01 *M*	2·60	*iso*Valeraldehyde, saturated	3·38
Acetaldehyde, 0·01 *M*	8·80	*n*-Hexaldehyde, saturated	2·26
Propaldehyde, 0·01 *M*	7·76	*n*-Heptaldehyde, saturated	2·09
Butaldehyde, 0·01 *M*	4·15	*n*-Octaldehyde, saturated	2·54

$$Q_{Acid} = \frac{\mu l\ CO_2}{\text{mg. dry wt. tissue} \times \text{hours}}.$$

It will be observed that acid production occurs to a marked extent with acetaldehyde and propaldehyde; to a smaller degree with butaldehyde, *n*- and *iso*-valeraldehydes; whilst the acid production in presence of hex-, hept- and oct-aldehydes is no greater than that of the control. Acid formation decreases during ascent of the homologous series, the quotients standing in the order of the water-solubilities of the aldehydes, but there is reason to believe that tissue penetration occurs with the higher homologues, since the slices are whitened throughout at the end of an experiment.

Working with ox and pig liver brei, Parnas [1910] found a quantitative dismutation in the aldehyde series from acetaldehyde to valeraldehyde and also with heptaldehyde; rat liver slices, however, form no additional acid in presence of heptaldehyde.

Ketogenesis from aldehydes under aerobic conditions.

Since certain aliphatic aldehydes undergo dismutation in liver, it would be expected that they would also give rise to ketone bodies by secondary oxidation of fatty acids. This prediction is borne out by the experiments recorded in Table II.

Table II. *Ketone body formation from aldehydes in rat liver slices.*

Phosphate saline, *p*H 7·4, in oxygen. Duration of exp. 2 hours.

Substrate	Q_{O_2}	$Q_{Acac.}$
Nil	−11·5	0·37
Acetaldehyde, 0·005 *M*	−10·2	0·76
Acetaldehyde, 0·01 *M*	− 8·3	0·67
Propaldehyde, 0·005 *M*	−10·3	0·27
Butaldehyde, 0·01 *M*	−12·6	3·11
n-Valeraldehyde, saturated	−13·2	2·11
*iso*Valeraldehyde, saturated	−11·7	2·18
n-Hexaldehyde, saturated	−12·8	4·33
n-Heptaldehyde, saturated	−13·0	2·43
n-Octaldehyde, saturated	−11·5	4·02

The figures of Table II show the following facts:

1. Acetaldehyde in a concentration of 0·005 *M* causes a small increase in the rate of acetoacetic acid production. Higher concentrations inhibit both respiration and ketone body formation.

2. Propaldehyde, like propionic acid, is not ketogenic.

3. Butaldehyde and its higher homologues are ketogenic and the rates of ketone body formation are approximately equal to those observed with the corresponding fatty acids, the odd-numbered members of the series giving smaller

quotients than the even-numbered members. The rate of disappearance of aldehyde is rapid, being practically complete after 2 hours. The aliphatic aldehydes react at a rate high enough to be consistent with the functions of intermediary metabolites.

It is difficult to reconcile the ketogenic actions of hex-, hept- and oct-aldehydes with their failure to exhibit dismutation anaerobically. It was thought that the ketogenesis was artificial in so far as aldehydes might be oxidized to fatty acids spontaneously. Experiments showed that no significant autoxidation occurred either in Ringer solution or in presence of boiled liver extracts during 2 hours at 37·5°. In liver slices aldehydes may be converted into ketone bodies by an alternative mechanism which does not involve dismutation.

DICARBOXYLIC ACIDS AND THEIR DERIVATIVES.

The theory of ω-oxidation has brought the dicarboxylic acids into prominence from the point of view of fat metabolism. It has long been known that some of the dicarboxylic acids such as succinic and glutaric acids are completely oxidized in the animal body [Dakin, 1921], but it was desirable to investigate their ketogenic and antiketogenic properties in liver slices of well-nourished and of starved animals. The experiments of Table III were performed in phosphate saline and β-ketonic acids were determined by the aniline citrate method. Acids were added as neutral sodium salts.

Table III. *Ketone body formation in presence of dicarboxylic acids and some of their derivatives.*

	Rat liver slices.			
	Well-nourished animal		Starved animal (24 hours)	
Substrate (M)	Q_{O_2}	$Q_{Acac.}$	Q_{O_2}	Q_{Acac}
	Saturated dicarboxylic acids.			
Oxalic, 0·01	−11·0	0·58	—	—
Oxalic, 0·005	−12·3	0·98	—	—
Oxalic, 0·0025	−12·1	1·07	—	—
Control (no substrate)	−12·0	0·48	—	—
Oxalic, 0·015	—	—	− 8·9	1·63
Oxalic, 0·003	—	—	−10·9	2·17
Control	—	—	−12·1	1·97
Malonic, 0·01	−13·1	2·12	− 8·5	2·80
Malonic, 0·02	−10·2	2·88	—	—
Control	−15·1	0·60	−10·0	1·66
Succinic, 0·01	−14·6	0·26	−16·1	1·60
Control	−11·5	0·27	−10·0	1·66
Glutaric, 0·01	−12·9	0·18	−10·7	1·43
Control	−11·5	0·27	−10·0	1·66
Adipic, 0·01	−12·1	0·16	−13·6	2·31
Control	−11·5	0·27	−10·2	2·04
Pimelic, 0·01	−13·4	0·30	−14·2	2·30
Control	−13·9	0·33	−10·2	2·04
Suberic, 0·01	−19·5	0·14	−15·1	1·96
Control	−13·9	0·33	−10·2	2·04
Azelaic, 0·01	−14·8	0·35	−13·4	1·62
Control	−13·9	0·33	−10·2	2·04
Sebacic, 0·01	−14·1	0·21	−14·1	1·43
Control	−11·5	0·27	−10·2	2·04
Thapsic, 0·1 ml. saturated solution	−10·1	0·48	−13·4	2·29
Control	−12·1	0·70	−13·7	2·55

Table III (*cont.*).

Substrate (*M*)	Well-nourished animal Q_{O_2}	Well-nourished animal $Q_{Acac.}$	Starved animal (24 hours) Q_{O_2}	Starved animal (24 hours) $Q_{Acac.}$
Unsaturated dicarboxylic acids.				
Fumaric, 0·01	−12·6	0·26	−16·4	2·81
Control	−12·1	0·27	−13·7	2·55
Maleic, 0·01	−11·9	1·07	−11·9	2·70
Control	−12·1	0·70	−13·7	2·55
Maleic, 0·01	−12·8	0·95	—	—
Maleic, 0·02	−12·4	1·09	—	—
Maleic, 0·04	−12·2	0·75	—	—
Control	−14·0	1·06	—	—
Hydroxy- and ketonic derivatives.				
Hydroxymalonic, 0·01	−13·9	3·35	−11·2	3·51
Hydroxymalonic, 0·02	−12·2	3·18	—	—
Hydroxymalonic, 0·03	−11·0	2·58	—	—
Hydroxymalonic, 0·04	− 9·5	2·07	—	—
Control	−15·1	0·60	−13·7	2·55
Malic, 0·01	−13·6	0·93	−10·5	1·63
Control	−12·1	0·70	−10·1	1·66
d-Tartaric, 0·01	−11·6	1·51	—	—
Control	−13·1	0·83	—	—
Mesoxalic, 0·01	−12·2	2·43	—	—
Control	−12·9	0·67	—	—
α-Ketoglutaric, 0·01	−11·5	0·14	−17·4	2·67
Control	−11·5	0·21	−13·7	2·55
The results with citric acid may be included with the above.				
Citric, 0·01	−13·2	0·43	−15·3	2·23
Control	−12·1	0·70	−13·7	2·55

NOTE. Concentrated solutions of aniline citrate cannot be used for the determination of acetoacetic acid in presence of mesoxalic acid since the latter decomposes slowly evolving CO_2. Instead the method of Ostern [1933] was employed, the decomposition of mesoxalic acid then being negligible. After removal of the slices 0·5 ml. 3 *M* acetate buffer was added to the fluid in the main compartment of the manometer vessel and to the side-bulb 0·2 ml. water and 0·05 ml. aniline. As usual the contents of the side-bulb were tipped into the main compartment after 20 min. equilibration at 25°. With quantities of acetoacetic acid corresponding to 100 μl. CO_2 pressure readings were constant in about 3·5 hours.

The following facts will be observed:

1. In general the dicarboxylic acids increase the oxygen uptake of liver slices, an effect which is most marked in starved animals. Succinic and suberic acids cause the greatest augmentation; smaller increases are observed with adipic, pimelic, azelaic and sebacic acids, and in the starved animal with α-ketoglutaric acid. Mazza [1936] has recently published similar results.

2. Certain dicarboxylic acids—malonic, hydroxymalonic, oxalic and tartaric —depress respiration when their concentrations are only 0·01 *M*.

3. The majority of the acids are not ketogenic in the well-nourished liver, nor are they antiketogenic in the liver of the starved animal.

4. Malonic, hydroxymalonic and mesoxalic acids are striking exceptions. Malonic acid and its hydroxy-derivative depress respiration and simultaneously produce large amounts of ketone bodies in the liver of the well-fed rat, and both are ketogenic in starved liver. Mesoxalic acid is ketogenic to a similar degree.

5. Maleic acid depresses respiration but is not ketogenic.

6. *d*-Tartaric acid is weakly ketogenic in agreement with the observations of Ohta [1912].

7. In presence of thapsic acid the respiration of well-nourished liver was diminished but ketone body formation was unaffected; larger amounts of thapsic acid strongly inhibited both respiration and ketogenesis.

8. Oxalic acid is ketogenic, particularly in low concentrations which do not inhibit respiration, but the effect is never large.

9. Citric acid is neither ketogenic nor antiketogenic under the conditions utilized.

The action of oxalate was examined in a Ca-free phosphate saline (Table IV). The results show that the ketogenic effect is not due to precipitation of Ca^{++} which occurs in ordinary Ringer-phosphate solution.

Table IV. *Ketogenic action of oxalate in rat liver slices.*

	Phosphate saline		Ca-free phosphate saline	
Substrate (*M*)	Q_{O_2}	$Q_{Acac.}$	Q_{O_2}	$Q_{Acac.}$
Nil	−12·8	0·36	−14·2	0·24
Oxalate, 0·01	−12·5	0·66	−11·5	0·46
Oxalate, 0·002	−13·1	0·99	−12·7	0·72
Oxalate, 0·001	−13·0	1·25	−15·5	0·73
Nil	−12·4	0·43	−13·6	0·16
Oxalate, 0·003	−13·5	1·43	−13·1	0·86
Oxalate, 0·0017	−13·6	1·56	−14·1	1·03
Oxalate, 0·0003	−13·6	0·96	−14·1	0·99

Table V. *Inhibition of malonate and hydroxymalonate ketogenesis.*

Rat liver slices.

Exp. 1.

Substrate (*M*)	Q_{O_2}	$Q_{Acac.}$
Control (no substrate)	−14·7	0·50
Malonate, 0·02	−12·4	2·77
Succinate, 0·01	−20·2	0·44
Fumarate, 0·01	−15·1	0·53
Pyruvate, 0·01	−19·1	0·98
Malonate, 0·02 + succinate, 0·01	−14·1	2·19
Malonate, 0·02 + fumarate, 0·01	−13·6	2·55
Malonate, 0·02 + pyruvate, 0·01	−17·4	2·29
Control	−12·7	0·33
Fumarate, 0·01	−14·4	0·61
Pyruvate, 0·01	−17·9	1·07
Malonate, 0·01	−12·4	1·71
Hydroxymalonate, 0·01	−11·5	2·15
Malonate, 0·01 + fumarate, 0·01	−13·0	1·36
Hydroxymalonate, 0·01 + pyruvate, 0·01	−14·6	3·38

Exp. 2. Malonate concentration constant, that of succinate varied.

	In absence of malonate		In presence of malonate, 0·007 *M*	
Substrate (*M*)	Q_{O_2}	$Q_{Acac.}$	Q_{O_2}	$Q_{Acac.}$
Succinate, 0·007	−15·9	0·80	−15·6	1·56
Succinate, 0·014	−18·5	0·58	−15·2	1·38
Succinate, 0·02	−19·1	0·70	−16·5	1·27
No succinate	−12·2	0·98	−14·4	2·01

Exp. 3.

Substrate (*M*)	Q_{O_2}	$Q_{Acac.}$
Control (no substrate)	−15·1	0·60
Malonate, 0·01	−11·8	2·12
Hydroxymalonate, 0·01	−10·2	2·88
Glycerol, 0·01	−15·8	0·53
Malonate, 0·01 + glycerol, 0·01	−15·8	1·17
Hydroxymalonate, 0·01 + glycerol, 0·01	−14·3	1·43

Inhibition of ketogenesis due to malonate and hydroxymalonate. It was found that the effects of malonic acid and hydroxymalonic acid were modified in the presence of certain added substrates (Table V).

Exps. 1 and 2 show that there is a small but distinct inhibition of malonate ketogenesis in presence of succinate, fumarate and pyruvate; the value of $Q_{\text{Acac.}}$ falls as the oxygen consumption rises, the effect being more marked with higher concentrations of succinate. Pyruvate has no significant influence on ketogenesis caused by hydroxymalonate. Exp. 3 shows a strong inhibition due to glycerol; oxygen uptake is increased and ketone body formation is reduced in both cases.

Discussion.

Consideration of all the facts now available leads to the conclusion that there are two classes of ketogenic substances. The first and larger class consists of substances which are converted into ketone bodies during metabolism: it includes fatty acids, some of their derivatives and the amino-acids leucine, tyrosine and phenylalanine. The existence of this group was established chiefly by the classical work of Embden and of Friedmann. The second class is composed of substances which are not converted into ketone bodies: they influence the metabolism anticatalytically, causing an accumulation of ketone bodies. Certain dicarboxylic acids, e.g. malonic acid, are representatives of this class, and ammonia should probably be placed in the same category. Jowett & Quastel [1935] have obtained evidence which caused them to reject the view that malonic acid could be transformed into acetoacetic acid via acetic acid. The conversion of the other ketogenic dicarboxylic acids (hydroxymalonic, mesoxalic, tartaric and oxalic) into ketone bodies also appears to be unlikely.

The ketogenic effect of malonic acid is now well established. It was first observed by Momose [1914] in liver perfusion experiments. Szent-Györgyi *et al.* [1935] showed that ketone bodies were excreted by a rabbit which had been injected intravenously with sodium malonate. In the absence of other substrates malonate also caused a production of acetoacetic acid in kidney slices, an effect which was inhibited by fumarate. Jowett & Quastel [1935] have studied the malonate effect in detail: they believe that malonic acid inhibits the normal breakdown of acetoacetic acid formed spontaneously from fatty acids already present in liver. Further, Quastel & Wheatley [1935] have shown that malonate prevents aerobic, but not anaerobic, disappearance of acetoacetic acid added to kidney and liver slices.

An alternative hypothesis dependent on substrate competition may be offered. Krebs [1936] has recently discovered a mechanism whereby succinic acid is formed from pyruvic acid in animal tissues. Moreover, malonate is known to be a specific inhibitor of succinic dehydrogenase [Thunberg, 1920; 1933; Quastel & Whetham, 1925; Quastel & Wheatley, 1931]. If the oxidation of carbohydrate be inhibited by malonate at the succinic acid stage, it is possible that fatty acids will be oxidized preferentially. This would account for the great increase in the spontaneous ketogenesis which is observed in presence of malonate. Similar causes may operate in other cases, e.g. (1) hydroxymalonate is known to be a strong inhibitor of lactic dehydrogenase [Quastel & Wooldridge, 1928]; (2) oxalate is a weak inhibitor of muscle succinic dehydrogenase [Thunberg 1920; 1933]; oxalic and tartaric acids inhibit the lactic dehydrogenase of toluene-treated *B. coli* [Quastel & Wooldridge, 1928]. Thus the actions of the ketogenic dicarboxylic acids may be ascribed to their specific anticatalytic

properties, which hinder carbohydrate oxidation and thereby favour fatty acids in the competition for available oxygen. Evidence relating to substrate competition in liver will be presented in the next paper of this series.

SUMMARY.

1. Anaerobically there is an acid production in rat liver slices in presence of acetaldehyde, propaldehyde, butaldehyde, *n*-valeraldehyde and *iso*valeraldehyde but not in presence of hex-, hept- and oct-aldehydes. The acid is probably formed by dismutation.

2. Under aerobic conditions the above aldehydes, with the exception of propaldehyde, are ketogenic in liver. The ketone body formation is of the same order as that found with the corresponding fatty acids.

3. The metabolism of dicarboxylic acids and their derivatives has been investigated in rat liver slices. In general there is increased oxygen consumption and the acids are neither ketogenic in well-nourished liver nor antiketogenic in the liver of the starved animal. Notable exceptions are malonic, hydroxymalonic, mesoxalic, tartaric and oxalic acids. The first three are strongly ketogenic; tartaric and oxalic acids weakly ketogenic.

It is a pleasure to thank Dr H. A. Krebs for valuable suggestions and advice. I am also indebted to Dr Krebs for specimens of sodium mesoxalate and α-ketoglutaric acid.

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