CCLXI. KETOGENESIS-ANTIKETOGENESIS.

IV. SUBSTRATE COMPETITION IN LIVER.

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KREBS [1935] has shown that the deamination of *l*-amino-acids in surviving kidney tissue is inhibited by readily oxidizable substrates such as lactate and pyruvate. Although less amino-acid is metabolized, the oxygen consumption remains constant, indicating that the second substrate is oxidized instead of the amino-acid. These experiments demonstrate clearly that when kidney tissue is offered several substrates there is competition for the oxygen available. Krebs also observed substrate competition in respiring yeast cells.

The experiments of Embden & Wirth [1910] suggest that the same phenomenon can take place in liver. Perfusing the liver with various combinations of substrates, they found that the usual ketone body formation due to *iso*-valeric acid was greatly inhibited by *n*-valeric acid. In a similar way *n*-valeric acid partially inhibited ketogenesis caused by *n*-hexanoic acid and by tyrosine; whilst α-amino-*n*-hexanoic acid, which itself is non-ketogenic, prevented acetoacetic acid production from leucine. When glutaric acid and glucose were separately opposed to ketogenic fatty acids, no antiketogenic effects were observed, and the position of glycerol was uncertain. Quastel & Wheatley [1933], using tissue slices, found that propionic acid, but not glucose or lactic acid, inhibited the formation of acetoacetic acid from butyric acid.

Macallum [1930] enunciated a general theory of antiketogenesis which in effect is a theory of substrate competition, although it was not formulated in those terms. The experiments of Krebs [1935] prove the reality of substrate competition, and so it became desirable to investigate this process in liver: first, by opposing different substrates to the spontaneous ketogenesis of starved liver, and secondly by allowing a wide range of substrates to compete with added fatty acids for the oxidizing enzymes of the liver of a well-nourished animal.

Methods.

Respiration and ketone body formation were measured by the methods already described [Edson, 1935]. β-Ketonic acids were determined by the aniline citrate method except in a few cases which were referred to the Van Slyke procedure. Rat tissue slices were used throughout, particular care being taken that the animals were in a good state of nutrition. Fresh food was offered to them 3 hours before they were killed; starved animals were deprived of food for 24 hours.

The competing substrates were generally present in equimolecular concentrations, 0·01 *M*, but sometimes the effect of unequal competition was investigated. The medium was Krebs's phosphate saline [1933].

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EXPERIMENTAL.

Antiketogenesis in starved liver.

Facts relating to the antiketogenic properties of amino-acids and dicarboxylic acids have been recorded in communications II and III of this series. Additional results obtained with other substrates are presented in the following tables.

Table I. *Carbohydrates and their derivatives.*

Liver slices of starved rat.

Substrate (*M*)	Q_{O_2}	Q_{Acac}
1. *Hexoses.*		
Nil	− 9·4	2·43
Glucose, 0·02	− 13·9	1·20
Fructose, 0·02	− 12·4	1·19
Mannose, 0·02	− 12·6	1·42
Galactose, 0·02	− 12·8	1·17
Nil	− 13·6	2·88
Glucose, 0·01	− 14·0	2·53
Glucose, 0·02	− 15·0	2·21
Glucose, 0·60	− 8·7	0·99
Nil	− 12·1	2·44
Hexosediphosphate, 0·01	− 13·8	1·53
2. *Pentoses.*		
Nil	− 12·9	2·41
Arabinose, 0·01	− 11·0	1·80
Xylose, 0·01	− 13·2	2·20
Nil	− 12·0	1·60
Rhamnose, 0·01	− 10·4	1·43
3. *Sucrose.*		
Nil	− 12·0	1·60
Sucrose, 0·01	− 12·2	1·62
Invert sugar (sucrose, 0·01 + 0·1 ml. 1% invertase in presence of liver slices)	− 15·2	0·86
0·1 ml. invertase (1% sol.)	− 12·4	1·70
4. *Glycogen.*		
Nil	− 12·7	2·43
Glycogen, 0·2 ml. 6% sol.	− 12·5	2·35
5. *Derivatives.*		
Nil	− 13·6	2·88
dl-Glyceraldehyde, 0·01	− 11·1	0·96
Nil	− 9·7	2·43
Dihydroxyacetone, 0·01	− 13·7	1·36
Nil	− 10·5	1·43
Lactate, 0·01 (*dl*)	− 16·2	0·76
Pyruvate, 0·01	− 15·3	0·97
Nil	− 12·1	2·21
α-Glycerophosphate, 0·01	− 13·3	2·26
Nil	− 12·1	2·44
β-Phosphoglycerate, 0·01	− 11·1	1·35

Table II. *Alcohols.*

Liver slices of starved rats.

Substrate (*M*)	Q_{O_2}	$Q_{Acac.}$
Nil	−12·0	1·60
Ethyl alcohol, 0·005	−11·0	1·46
Methyl alcohol, 0·005	−12·3	1·48
Methyl alcohol, 0·0025	−13·1	1·68
Ethylene glycol, 0·01	−12·6	1·86
Nil	− 9·7	2·43
Glycerol, 0·01	−11·7	0·63
Nil	−13·6	2·88
Glycerol, 0·01	−16·7	1·19
Glycerol, 0·02	−15·4	0·84
Nil	−12·9	2·41
Erythritol, 0·01	−11·6	1·88
Adonitol, 0·01	−13·2	1·90
Dulcitol, 0·01	−11·6	2·21
Mannitol, 0·01	−12·3	1·99
Sorbitol, 0·01	−15·8	0·59
Nil	−13·6	2·88
Sorbitol, 0·01	−17·0	0·85

Table III. *Some miscellaneous results.*

Liver slices of starved rats.

Substrate (*M*)	Q_{O_2}	$Q_{Acac.}$
Nil	−13·6	2·88
Ornithine, 0·01	−13·1	1·96
Arginine, 0·01	−12·6	1·77
Glutaric acid, 0·01	−12·9	2·26

NOTE. The experiments with the higher alcohols were performed on receipt of the information that Dr B. Mendel had discovered the antiketogenic effect of sorbitol (private communication to Dr H. A. Krebs). The action of glycerol had been found previously.

The data of Tables I, II and III show the following facts with regard to the ketogenesis of starvation.

1. Glycerol, sorbitol and glyceraldehyde are strongly antiketogenic, the inhibition being 70% or over. There is increased oxygen uptake in presence of sorbitol and glycerol.

2. The other alcohols have no effect or inhibit weakly.

3. Glucose is no more antiketogenic that the other hexoses, whose influence is seen to be much smaller than that of glycerol. The antiketogenic action of glucose increases with concentration, but in order to produce an effect as great as that characteristic of 0·01 *M* glycerol the glucose concentration must be raised to 0·6 *M*, which is sufficient to depress respiration.

4. Lactate and pyruvate reduce ketone body formation by nearly 50%.

5. Added glycogen is not antiketogenic.

It appears that almost any oxidizable substrate will inhibit the spontaneous ketogenesis of starved liver to a greater or less degree.

Substrate competition in the liver of the well-fed animal.

Many experiments were performed in which substrates were allowed to compete with added fatty acids in the normal glycogen-rich liver. The results of typical experiments are recorded below. Fatty acids were added as sodium salts.

Table IV.

A. *Glucose in competition with* n-*hexanoic acid.*

Substrate (M)	Q_{O_2}	Q_{Acac}
n-Hexanoic acid, 0·01	−14·3	4·91
,, ,, +glucose, 0·005	−13·8	4·97
,, ,, +glucose, 0·008	−14·1	5·21
,, ,, +glucose, 0·01	−13·9	4·42
,, ,, +glucose, 0·02	−14·3	4·63
,, ,, +glucose, 0·05	−13·7	3·86
,, ,, +glucose, 0·10	−12·4	3·20

B. *Sodium lactate in competition with even-numbered fatty acids.*

Nil	−12·8	0·22
dl-Lactate, 0·01	−15·4	0·29
n-Butyric acid, 0·01	−17·7	3·30
,, ,, +lactate, 0·01	−18·3	2·11
n-Hexanoic acid, 0·01	−14·7	5·18
,, ,, +lactate, 0·01	−18·1	3·11
,, ,, +lactate, 0·02	−19·0	2·92
n-Octanoic acid, 0·005	−12·9	4·10
,, ,, +lactate, 0·01	−15·8	4·40

C. *Sodium pyruvate in competition with even-numbered fatty acids.*

Nil	−12·9	0·28
Pyruvate, 0·01	−15·4	0·41
n-Butyric acid, 0·01	−16·5	2·61
,, ,, +pyruvate, 0·01	−19·5	1·59
n-Hexanoic acid, 0·01	−15·6	4·29
,, ,, +pyruvate, 0·01	−20·4	3·07
n-Octanoic acid, 0·005	−12·3	2·61
,, ,, +pyruvate, 0·01	−17·0	2·98

D. *Amino-acids in competition with even-numbered fatty acids.*

Nil	−12·3	0·42
dl-Alanine, 0·01	−12·3	0·29
Glycine, 0·01	−11·6	0·54
n-Butyric acid, 0·01	−13·9	2·16
,, ,, +alanine, 0·01	−14·0	2·78
,, ,, +glycine, 0·01	−13·5	2·15
n-Hexanoic acid, 0·01	−14·5	3·25
,, ,, +alanine, 0·01	−15·0	3·98
,, ,, +glycine, 0·01	−14·1	3·61
Nil	−12·7	0·82
l-Tyrosine, saturated	−13·0	1·32
l-Cysteine, 0·01	−16·6	0·89
n-Butyric acid, 0·01	−15·1	3·81
,, ,, +tyrosine, saturated	−20·2	5·27
,, ,, +cysteine, 0·01	−15·2	2·75
n-Hexanoic acid, 0·01	−17·4	6·90
,, ,, +tyrosine, saturated	−16·9	6·02
,, ,, +cysteine, 0·01	−22·5	6·20
Nil	−12·2	0·11
d-Arginine, 0·01	−11·5	0·19
d-Valine, 0·01	−11·7	0·21
n-Butyric acid, 0·01	−16·8	1·98
,, ,, +arginine, 0·01	−16·3	1·66
,, ,, +valine, 0·01	−17·4	2·33
n-Hexanoic acid, 0·01	−14·4	2·63
,, ,, +arginine, 0·01	−15·1	2·13
,, ,, +valine, 0·01	−14·4	2·79
Nil	−10·2	0·33
d-Ornithine, 0·01	−12·3	0·23
n-Butyric acid, 0·01	−13·4	2·76
,, ,, +ornithine, 0·01	−12·7	2·51
n-exanoic acid, 0·01	−15·4	5·19
,, ,, +ornithine, 0·01	−13·7	3·84

Table IV (*cont.*).

Substrate (*M*)		Q_{O_2}	$Q_{Acac.}$
E. *Dicarboxylic acids in competition with even-numbered fatty acids.*			
Nil		−10·2	0·33
Succinate, 0·01		−17·9	0·44
n-Butyric acid, 0·01	+	−13·4	2·76
,, ,,	+succinate, 0·01	−18·2	2·32
n-Hexanoic acid, 0·01		−15·4	5·19
,, ,,	+succinate, 0·01	−19·5	4·66
Nil		−11·9	0·52
Glutarate, 0·01		−14·2	0·61
Sebacate, 0·01		−14·5	0·21
n-Butyric acid, 0·01		−13·7	2·25
,, ,,	+glutarate, 0·01	−16·1	3·32
,, ,,	+sebacate, 0·01	−14·1	1·78
n-Hexanoic acid, 0·01		−13·3	3·76
,, ,,	+glutarate, 0·01	−15·3	5·10
,, ,,	+sebacate, 0·01	−14·5	4·51
F. *Glycerol, sorbitol and glyceraldehyde in competition with even-numbered fatty acids.*			
Nil		−10·2	0·41
n-Butyric acid, 0·01		−13·0	2·35
,, ,,	+glycerol, 0·01	−14·3	1·72
,, ,,	+glycerol, 0·02	−14·3	1·70
n-Hexanoic acid, 0·01		−15·0	3·92
,, ,,	+glycerol, 0·01	−15·0	2·80
n-Octanoic acid, 0·005		−15·0	3·93
,, ,,	+glycerol, 0·01	−14·3	2·97
Nil		−11·3	0·36
n-Butyric acid, 0·01		−17·0	2·96
,, ,,	+sorbitol, 0·01	−18·2	1·98
n-Hexanoic acid, 0·01		−17·2	4·48
,, ,,	+sorbitol, 0·01	−17·7	3·78
n-Octanoic acid, 0·005		−14·8	4·20
,, ,,	+sorbitol, 0·01	−15·0	4·88
Nil		−10·7	0·28
n-Butyric acid, 0·01		−14·6	2·48
,, ,,	+*dl*-glyceraldehyde, 0·01	−10·9	1·84
n-Hexanoic acid, 0·01		−16·6	4·91
,, ,,	+*dl*-glyceraldehyde, 0·01	−13·1	2·10
G. *Odd-numbered in competition with even-numbered fatty acids.*			
Nil		−11·6	0·26
n-Butyric acid, 0·01		−13·9	1·49
,, ,,	+formate, 0·01	−13·6	1·75
n-Hexanoic acid, 0·01		−13·5	2·78
,, ,,	+formate, 0·01	−14·9	2·83
n-Octanoic acid, 0·005		−13·7	2·92
,, ,,	+formate, 0·01	−12·7	2·59
Formate, 0·01		−11·3	0·25
Nil		− 9·6	0·21
n-Butyric acid, 0·01		−12·6	1·64
,, ,,	+propionate, 0·01	−12·8	0·91
,, ,,	+propionate, 0·02	−12·3	1·01
n-Hexanoic acid, 0·01		−15·7	3·63
,, ,,	+propionate, 0·01	−15·4	2·59
Propionate, 0·01		−11·1	0·30
Nil		−13·7	0·20
n-Butyric acid, 0·01		−15·6	2·03
,, ,,	+*n*-valerate, 0·01	−14·3	1·35
n-Hexanoic acid, 0·01		−16·9	3·30
,, ,,	+*n*-valerate, 0·01	−18·3	1·71
n-Octanoic acid, 0·005		−13·1	2·45
,, ,,	+*n*-valerate, 0·01	−16·6	2·33
n-Valerate, 0·01		−13·4	0·94

Table IV G (*cont.*).

Substrate (*M*)	Q_{O_2}	$Q_{Acac.}$
Nil	−12·6	0·40
n-Butyric acid, 0·01	−15·9	2·23
,, ,, +*n*-heptanoate, 0·005	−15·0	1·97
n-Hexanoic acid, 0·01	−17·4	4·20
,, ,, +*n*-heptanoate, 0·005	−21·4	2·59
n-Octanoic acid, 0·005	−12·0	2·80
,, ,, +*n*-heptanoate, 0·005	−14·3	2·70
n-Heptanoate, 0·005	−16·9	1·48
Nil	−12·7	0·23
n-Butyric acid, 0·01	−16·5	2·76
,, ,, +*n*-nonanoate, 0·0025	−16·1	2·15
n-Hexanoic acid, 0·01	−14·6	3·77
,, ,, +*n*-nonanoate, 0·0025	−17·6	2·84
n-Octanoic acid, 0·005	−13·1	3·65
,, ,, +*n*-nonanoate, 0·0025	−15·8	3·88
n-Nonanoate, 0·0025	−14·6	1·47

H. *Even-numbered fatty acids competing with one another.*

Nil	−12·9	0·85
n-Butyric acid, 0·01	−15·8	3·65
n-Hexanoic acid, 0·01	−14·5	5·20
n-Octanoic acid, 0·005	−13·5	3·87
Acetic acid, 0·01	−15·9	1·53
n-Butyric acid, 0·01 + *n*-hexanoic acid, 0·01	−15·7	5·76
n-Butyric acid, 0·01 + *n*-octanoic acid, 0·005	−13·1	4·56
n-Hexanoic acid, 0·01 + *n*-octanoic acid, 0·005	−13·4	4·77
n-Butyric acid, 0·01 + acetic acid, 0·01	−16·7	3·88
n-Hexanoic acid, 0·01 + acetic acid, 0·01	−15·3	5·40

The figures of the above table demonstrate the following facts.

1. Many oxidizable substances inhibit the formation of β-ketonic acids from fatty acids which have been added to liver slices. The inhibition is variable in degree, and it is usually produced without lowering of the oxygen uptake. Ketogenesis from *n*-octanoic acid appears to be difficult to inhibit.

2. Glucose, glycerol, sorbitol and glyceraldehyde behave as in the starved liver, but in presence of 0·01 *M* fatty acids the percentage inhibitions are smaller.

3. Lactate and pyruvate also inhibit, the oxygen consumption then being raised above that due to fatty acid alone.

4. The amino-acids cysteine, valine and alanine have little effect, but arginine and ornithine diminish ketogenesis.

5. The dicarboxylic acids—succinic, glutaric, sebacic—have no regular influence. Succinic acid inhibits slightly; glutaric acid appears to increase ketogenesis in presence of added fatty acid.

6. *Odd-numbered fatty acids.* Formic acid is indifferent towards the process of ketogenesis, but propionic acid inhibits it to a marked extent. Since these two substrates are non-ketogenic their behaviour is not surprising. Contrary to expectation, and in spite of their own capacity to yield ketone bodies, the higher homologues—*n*-valeric, *n*-heptanoic and *n*-nonanoic acids—inhibited the formation of β-ketonic acids from butyric and *n*-hexanoic acids; but they showed no inhibitory effect in the case of *n*-octanoic acid. The antiketogenic action was accompanied, not by diminished, but by increased oxygen uptake.

7. When two even-numbered fatty acids were present together there was not a summation of the separate ketogenic effects. Instead $Q_{Acac.}$ assumed a maximum value which was a little in excess of the quotient for the more strongly ketogenic member of the pair.

dl-*Glyceraldehyde*. The results obtained with glyceraldehyde must be interpreted with caution, since it was found that there is a considerable disappearance of acetoacetic acid when it is shaken with glyceraldehyde for 2 hours at 37·5° in the absence of tissue slices. Moreover, the effect of glyceraldehyde is not increased by the presence of slices (liver or kidney). The interaction of acetoacetic acid and glyceraldehyde resembles an effect described by Henze [1930; 1931; Henze & Müller, 1930] who found that acetoacetic acid reacted with methylglyoxal *in vitro* to give "ketol".

Discussion.

These results afford evidence in favour of the hypothesis that the rate of production of ketone bodies in liver is the outcome of competition between fatty acids and other oxidizable substrates, and since the liver is the chief site of ketone body formation, substrate competition becomes an important factor in regulating the general ketogenic-antiketogenic balance. If oxidizable substrates such as glucose, sorbitol and lactate were able to compete with fatty acids for available oxygen, and if this were dependent on local substrate concentration, then fatty acid oxidation might be inhibited in the way that has been observed. According to this view the antiketogenic power of carbohydrates and their metabolic derivatives depends on "sparing" of fatty acids.

Fatty acids are known to be strongly adsorbed. Should this occur in liver, it would help to explain the apparent selective oxidation of fatty acids. The oxidation of the higher homologue, *n*-octanoic acid, is scarcely affected by the presence of competitors, a fact which could be predicted if fatty acids are adsorbed by enzyme systems according to Traube's rule.

The effect of the odd-numbered fatty acids is noteworthy. If an even-numbered and an odd-numbered acid compete with each other on equal terms, inhibition may be expected, because the rates of ketone body formation are approximately in the ratio, odd : even :: 1 : 3. The observed $Q_{Acac.}$ of a competing pair is roughly the mean of the separate values obtained when only one acid is present.

A theory of substrate competition would require that the antiketogenic action should increase with the concentration of the antiketogenic substance, and that it should occur without diminution of oxygen uptake. In the case of glucose this is true, but it is not equally clear with other substrates. Regarding oxygen consumption, however, the requirements of the theory appear to be satisfied in all cases. The anticatalytic effects of malonate and other ketogenic dicarboxylic acids supply additional evidence in support of a competition theory.

Apart from substrate competition there may be other mechanisms controlling ketogenesis; for instance the powerful antiketogenic effects of glycerol and sorbitol may require some special explanation.

Summary.

1. Antiketogenesis has been studied in liver slices of starved rats and in slices of well-nourished livers in presence of added fatty acids.

2. The evidence leads to the conclusion that fatty acids compete with other oxidizable substrates for the oxidizing systems of the liver. Carbohydrates and their derivatives, e.g. lactate, pyruvate and dihydroxyacetone, alcohols and certain amino-acids are antiketogenic competitors. The fatty acids also compete amongst themselves.

3. Glycerol and sorbitol are the most powerful of the antiketogenic substances that have been examined.

4. Substrate competition is considered to be an important factor in the regulation of hepatic ketogenesis.

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