CCXCIX. KETOGENESIS-ANTIKETOGENESIS.
II. KETOGENESIS FROM AMINO-ACIDS.

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THE fundamental facts of ketone-body formation from the amino-acids were established by Gustav Embden. The possible existence of a new factor arises from the work of Annau [1934], who discovered that ammonium chloride is a ketogenic agent in chopped liver. Recently the ammonia effect was examined in further detail with rat liver slices [Edson, 1935].

As a result it became important to decide if the ammonia which is liberated during deamination plays a ketogenic rôle in the intermediary metabolism of the amino-acids. This question was investigated by determining ketone-body formation in liver slices which were permitted to survive in phosphate-Ringer solution containing amino-acids. Since it is known that the naturally occurring stereoisomerides of the amino-acids are deaminated more slowly than the non-natural ones [Krebs, 1935], it was of interest to examine both series.

Methods.

The methods employed, including those used for the estimation of ketone-bodies, have been described in earlier work [Edson, 1935]. As before the animals were young male rats (3–6 months) of a uniform laboratory strain. The liver slices (20–30 mg. dry weight) were immersed in 2 ml. phosphate-Ringer solution, p_H 7·4, and shaken under an oxygen atmosphere for 2 hours in a thermostat at 37·5°. Respiration and acetoacetic (β-ketonic) acid production were measured as a routine.

Amino-acid solutions, 0·2 M, were added to the Ringer solution in such quantities as to make a final concentration of 0·01 or 0·02 M, except in those cases—tyrosine and cystine—where the solubility was very low, and then the Ringer solution was saturated with amino-acid at the temperature of the thermostat. Tyrosine and cystine were added as solids in amounts sufficient to give 0·01 M solutions, but owing to the low solubility the saline remained saturated with excess substrate after 2 hours' contact with tissue. Slices which had been immersed in saturated solutions required thorough washing to remove solid particles. Histidine was used in a concentration of 0·005 M because stronger solutions depressed respiratory activity. The dicarboxylic amino-acids were neutralised with sodium bicarbonate and the diamino-acids with HCl.

The values for β-ketonic acid production which are reported in this paper were determined manometrically by the aniline citrate method; but wherever possible they were checked by means of a modified Van Slyke procedure, which however is not applicable to the special cases of tryptophan, tyrosine, cystine and histidine.

Units. The tissue metabolism is expressed by the following quotients:

Q_{O_2} = μl. oxygen consumption per mg. dry weight of tissue per hour.

$Q_{Acac.}$ = μl. CO_2 (acetoacetic (β-ketonic) acid) formed per mg. dry weight of tissue per hour. 1 millimol. β-ketonic acid = 1 millimol. CO_2.

Experimental.

Ketogenesis from the amino-acids.

It was desirable to examine the antiketogenic properties of amino-acids in the liver of a starved animal as well as to investigate any ketogenic tendencies which they might have in the well-nourished organ. Accordingly the experiments were conducted in two separate series, one with rats which had been given full liberty to feed and the other with rats that had been starved for 24 hours.

(*a*) *The well-nourished animal.* In every case two controls were provided for comparison with the effect of the amino-acid: to one no substrate was added, and the other contained ammonium chloride in an initial concentration of 0·04 *M*. The results of typical experiments are collected in Table I.

When the data of Table I are examined the following points will be noticed:

1. The absolute values for acetoacetic (or β-ketonic) acid production from amino-acids which yield ketone-bodies are small and in no way comparable with those obtained when the substrates are fatty acids.

2. In the presence of tryptophan, proline, cystine, ornithine, lysine, aspartic acid and *l*(+)-*iso*leucine the values of $Q_{\text{Acac.}}$ are no higher than those of the controls.

3. In accordance with the traditional view tyrosine, phenylalanine and leucine prove to be ketogenic.

4. Hydroxyproline, the ultimate fate of which is not recorded in the literature, is ketogenic to about the same degree. The remaining amino-acids—glycine, alanine, serine, α-aminobutyric acid, valine, norleucine, *d*(−)-*iso*leucine, methionine, glutamic acid, histidine and arginine—present low values of $Q_{\text{Acac.}}$, which nevertheless are higher than those of the controls.

5. If the ketogenic effect of amino-acids be compared with that of 0·04 *M* ammonium chloride, it is observed that tyrosine, phenylalanine, *dl*-leucine and hydroxyproline give values of $Q_{\text{Acac.}}$ of the same order; $Q_{\text{Acac.}}$ for *l*(−)-leucine is somewhat lower but in the case of all other amino-acids the quotients for ketone-body formation are lower than those for ammonium chloride.

In this work the term "ketogenic" has been used to denote an agent which increases the yield of ketone-bodies in living tissues. This does not necessarily mean that the substance itself is converted into ketone-bodies, *e.g.* certain amino-acids are ketogenic although the carbon skeleton does not form ketone-bodies.

(*b*) *The starved animal.* The series was repeated on rats which had been starved for 24 hours. Representative results are shown in Table II. Ammonium chloride controls were omitted, because ammonia has no ketogenic action on starved liver in the absence of substrate.

With regard to the starved animal the following points are noteworthy:

1. The only amino-acid which is markedly ketogenic is *dl*-leucine.

2. Certain amino-acids appear to be neither ketogenic nor ketolytic. They are *l*(+)-valine, *l*(−)-methionine, cystine, hydroxyproline and tyrosine.

3. The rest present antiketogenic properties which vary in degree but are best exemplified by arginine and ornithine.

Table I. *Ketogenesis from amino-acids in liver slices of well-nourished rats.*

Amino-acid	Concentration *M*	Control No substrate Q_{O_2}	Control No substrate $Q_{Acac.}$	In presence of amino-acid Q_{O_2}	In presence of amino-acid $Q_{Acac.}$	Ammonium chloride, 0·04 *M* Q_{O_2}	Ammonium chloride, 0·04 *M* $Q_{Acac.}$	Source
Glycine	0·02	−11·3	0·39	−11·4	0·49	−10·7	0·86	B.D.H.
dl-Alanine	0·02	−11·3	0·39	−12·0	0·57	−10·7	0·86	B.D.H.
l(+)-Alanine	0·02	− 9·5	0·30	−10·0	0·79	− 9·1	0·87	H.L.R.
dl-Serine	0·01	− 9·3	0·22	−13·1	0·37	−10·7	0·87	H.L.R.
	0·02	− 9·3	0·22	−11·9	0·32	−10·7	0·87	
dl-α-Aminobutyric acid	0·02	−11·3	0·39	−12·4	0·57	−10·7	0·86	F.L.
l(+)-Valine	0·02	− 9·3	0·23	− 9·5	0·48	− 9·2	0·65	H.L.R.
d(−)-Valine	0·02	−10·7	0·35	−11·3	0·49	− 9·7	0·71	H.L.R.
dl-Leucine	0·01	−11·5	0·27	−12·9	0·89	− 9·7	0·79	F.L.
	0·02	−12·0	0·27	−10·9	1·02	− 8·6	0·85	
l(−)-Leucine	0·01	−11·5	0·27	−12·1	0·37	− 9·7	0·79	H.L.R.
	0·02	−12·0	0·29	− 9·3	0·44	− 8·6	0·85	
dl-Norleucine	0·01	−11·5	0·27	−13·3	0·37	− 9·7	0·79	F.L.
	0·02	−11·3	0·39	−12·2	0·67	−10·7	0·86	
l(+)-*iso*Leucine	0·01	−11·5	0·27	−12·7	0·27	− 9·7	0·79	H.L.R.
d(−)-*iso*Leucine	0·01	−11·5	0·27	−11·8	0·67	− 9·7	0·79	H.L.R.
dl-Methionine	0·01	−11·5	0·27	−15·0	0·38	− 9·7	0·79	Prepared by Dr N. W. Pirie
l(−)-Methionine	0·01	−11·5	0·27	−14·3	0·34	− 9·7	0·79	Prepared by Dr N. W. Pirie
dl-Cystine	Saturated	− 8·2	0·32	− 9·8	0·25	− 9·5	0·72	Prepared by Dr N. W. Pirie
l(−)-Cystine	Saturated	−10·5	0·28	−11·8	0·32	− 9·4	0·96	Prepared by Dr N. W. Pirie
l(−)-Tryptophan	0·01	−11·5	0·27	−11·9	0·30	− 9·7	0·79	Prepared by Mr D. D. Woods
dl-Tyrosine	Saturated	−10·7	0·35	−13·5	0·86	− 9·7	0·71	Prepared by Dr N. W. Pirie
l(−)-Tyrosine	Saturated	−12·0	0·29	−12·0	0·91	− 8·6	0·85	H.L.R.
	—	−10·7	0·35	−11·4	0·80	− 9·7	0·71	
l(−)-Phenylalanine	0·01	−11·4	0·40	−11·3	0·72	− 9·2	0·71	H.L.R.
d(+)-Phenylalanine	0·01	−11·4	0·40	−13·3	0·75	− 9·2	0·71	H.L.R.
l(−)-Proline	0·01	−10·7	0·35	−12·5	0·22	− 9·7	0·71	H.L.R.
	0·02	−10·5	0·28	−11·6	0·23	− 9·4	0·96	
l(−)-Hydroxyproline	0·01	−10·5	0·28	−11·0	0·83	− 9·4	0·96	H.L.R.
	0·02	−10·7	0·35	−10·3	0·74	− 9·7	0·71	
l(−)-Aspartic acid (neutral salt)	0·01	− 8·2	0·32	− 8·9	0·31	− 9·5	0·72	B.D.H.
	0·02	−10·5	0·24	−11·5	0·24	− 9·4	0·96	
l(+)-Glutamic acid (neutral salt)	0·01	− 8·2	0·32	− 8·1	0·14	− 9·5	0·72	Prepared from Ajinomoto
	0·02	−10·5	0·24	−12·1	0·46	− 9·4	0·96	
l(−)-Histidine (neutral salt)	0·005	− 8·2	0·32	− 9·1	0·44	− 9·5	0·72	H.L.R.
l(+)-Arginine (neutral salt)	0·02	−10·0	0·36	−10·0	0·43	−10·2	0·72	Prepared according to Kossel and Gross [1924]
l(+)-Lysine (neutral salt)	0·02	−10·0	0·36	−11·9	0·36	−10·2	0·72	H.L.R.
l(+)-Ornithine (neutral salt)	0·02]	−11·2	0·25	−11·8	0·38	−11·7	0·70	H.L.R.

Note: B.D.H. = British Drug Houses; H.L.R. = Hoffmann-La Roche; F.L. = Fränkel and Landau.

Table II. *Ketogenesis from amino-acids in liver slices of starved rats.*

Amino-acid	Concentration *M*	Control No substrate Q_{O_2}	Control No substrate $Q_{Acac.}$	In presence of amino-acid Q_{O_2}	In presence of amino-acid $Q_{Acac.}$
Glycine	0·04	−10·0	1·82	−12·1	1·30
dl-Alanine	0·02	−10·0	1·82	−13·0	1·20
l(+)-Alanine	0·02	−10·5	1·13	−13·4	0·65
	0·02	− 9·3	1·98	−11·7	1·42
dl-Serine	0·02	− 8·6	1·65	−12·8	1·22
l(+)-Valine	0·02	− 9·0	1·60	−10·0	1·60
	0·02	−10·5	1·13	−12·4	1·12
d(−)-Valine	0·02	− 9·0	1·60	−10·9	1·14
	0·02	−10·5	1·13	−11·2	0·79
dl-Leucine	0·02	−10·6	3·27	−10·6	4·20
l(−)-Leucine	0·02	−10·6	3·27	−10·6	2·84
	0·02	− 9·3	1·98	− 9·8	1·38
dl-Norleucine	0·01	−12·1	1·94	−13·5	1·60
	0·02	− 9·3	1·98	− 9·7	1·64
l(+)-*iso*Leucine	0·01	−12·1	1·94	− 9·6	1·29
	0·04	− 9·3	1·98	− 8·0	1·34
d(−)-*iso*Leucine	0·01	−12·1	1·94	−12·4	2·21
	0·04	− 9·3	1·98	− 9·4	1·68
dl-Methionine	0·02	− 8·6	1·65	−11·7	1·01
l(−)-Methionine	0·02	− 8·6	1·65	−10·7	1·64
dl-Cystine	Saturated	− 8·6	1·65	−10·5	1·50
l(−)-Cystine	Saturated	− 9·0	1·60	−10·7	1·73
l(−)-Tryptophan	0·01	−10·5	1·13	−13·1	0·67
	0·02	−10·0	1·82	−10·6	0·83
dl-Tyrosine	Saturated	− 9·0	1·60	−12·8	1·74
l(−)-Tyrosine	Saturated	− 9·0	1·60	− 9·2	1·33
l(−)-Phenylalanine	0·01	− 9·3	1·98	− 9·4	0·62
d(+)-Phenylalanine	0·01	− 9·3	1·98	− 9·2	1·34
l(−)-Proline	0·01	−10·5	1·33	−11·2	0·79
	0·02	− 9·0	1·60	−14·0	1·71
l(−)-Hydroxyproline	0·01	−10·5	1·33	−12·0	1·66
	0·02	− 9·0	1·60	−11·3	1·34
l(−)-Aspartic acid (neutral salt)	0·01	−10·5	1·41	− 9·8	1·27
l(+)-Glutamic acid (neutral salt)	0·01	−10·5	1·41	−13·1	1·32
l(−)-Histidine (neutral salt)	0·005	−10·0	1·82	−10·1	1·32
l(+)-Arginine (neutral salt)	0·02	−10·0	1·82	− 8·9	0·68
l(+)-Lysine (neutral salt)	0·02	−10·0	1·82	− 8·3	1·51
l(+)-Ornithine (neutral salt)	0·01	−11·6	2·17	−11·9	0·97

Some special problems.

(*a*) *The catabolism of phenylalanine in liver.*

Embden and Baldes [1913] have shown that phenylpyruvic acid does not yield ketone-bodies in the perfused liver, whereas phenylalanine does. They concluded that phenylpyruvic acid is not an intermediate in the breakdown of phenylalanine. On the other hand Embden *et al.* [1906] found that β-phenyllactic acid gave acetoacetic acid, and it was assumed by them to be the more probable intermediate.

Both these substances have been added to the liver slices of a well-nourished rat, and the effects examined in presence and in absence of ammonium chloride. The results are given in Table III. Phenylpyruvic acid was prepared by the method of Erlenmeyer [1892]; M.P. 153°. β-Phenyllactic acid was the product of Fränkel and Landau; M.P. 98°.

Table III. *Formation of ketone-bodies from phenylpyruvic and phenyllactic acids in the liver of the well-nourished rat.*

Substrate	Respiration Q_{O_2}	Ketone-body formation $Q_{Acac.}$
Nil	−12·3	0·45
Phenylpyruvic acid, 0·01 *M* (sodium salt)	−11·7	0·48
Phenylpyruvic acid, 0·01 *M* (sodium salt) + NH_4Cl, 0·04 *M*	− 8·0	0·88
NH_4Cl, 0·04 *M*	−11·0	1·02
Nil	−10·0	0·33
NH_4Cl, 0·04 *M*	− 9·8	1·10
β-Phenyllactic acid, 0·01 *M* (sodium salt)	−10·7	0·35
β-Phenyllactic acid, 0·01 *M* (sodium salt) + NH_4Cl, 0·04 *M*	− 8·6	1·14

The figures show that neither phenylpyruvic acid nor phenyllactic acid can be intermediates in the catabolism of phenylalanine, since they fail to give acetoacetic acid under conditions in which phenylalanine does so readily. The increased yields in the presence of ammonia are merely due to ammonia ketogenesis which always occurs even in the absence of added substrate. Embden's results with phenyllactic acid may be explained by the fact that he perfused with the ammonium salt of the acid.

It will be shown in a succeeding section that the path of phenylalanine catabolism in liver lies more probably through tyrosine and *p*-hydroxyphenylpyruvic acid.

Table IV. *Formation of ketone-bodies from* p-*hydroxyphenylpyruvic, homogentisic and muconic acids in rat liver slices.*

Animals well fed.

Substrate	Respiration Q_{O_2}	Ketone-body formation $Q_{Acac.}$
Nil	−13·9	0·45
NH_4Cl, 0·04 *M*	−11·0	1·02
p-Hydroxyphenylpyruvic acid, 0·01 *M* (sodium salt)	−14·6	0·97
Ditto + NH_4Cl, 0·04 *M*	−15·0	2·76
Nil	−11·7	0·26
NH_4Cl, 0·04 *M*	− 9·2	0·77
Homogentisic acid, 0·01 *M* (sodium salt)	−15·1	1·11
Ditto + NH_4Cl, 0·04 *M*	−11·2	1·98
Nil	−13·6	0·21
NH_4Cl, 0·04 *M*	− 9·0	0·55
Muconic acid, 0·01 *M* (sodium salt)	−11·8	0·33
Ditto + NH_4Cl, 0·04 *M*	− 8·9	0·72

Note: The apparent Q_{O_2} for homogentisic acid is a little too high, because slow autoxidation occurs in phosphate-Ringer solution at p_H 7·4. The autoxidation accounts for about 6% of the total oxygen uptake.

(*b*) *The catabolism of tyrosine in liver.*

(1) *Ketone-body formation.* Certain substances which have been suggested as intermediates of tyrosine metabolism were examined in a similar way. *p*-Hydroxyphenylpyruvic acid and homogentisic acid formed large amounts of acetoacetic acid whether ammonium chloride was present or not, which is in agreement with previous perfusion experiments. Muconic acid gave little or none, and therefore it is not a possible intermediate (Table IV). Hensel and Riesser [1913] obtained acetoacetic acid on perfusing the liver with muconic acid, but, since they employed the ammonium salt, their conclusions are not valid.

p-Hydroxybenzamidocinnamic acid was prepared by the method of Erlenmeyer and Halsey [1899], and *p*-hydroxyphenylpyruvic acid was obtained from it by the method of Neubauer and Fromherz [1910]. M.P. 213°. Homogentisic acid was separated from alcaptonuric urine according to Garrod [1899] and Orton and Garrod [1901]; M.P. 152°. Muconic acid was synthesised by the method of Behrend and Koolman [1912]; M.P. 293°.

(2) *The oxidation of l(−)-tyrosine.* Bernheim and Bernheim [1934] state that *l*(−)-tyrosine is oxidised by liver "brei" in such a way that one molecule of tyrosine takes up four atoms of oxygen. Experiments were performed with rat and rabbit liver "brei" under the conditions stipulated by Bernheim and Bernheim. It was found however that there was no constancy in the additional oxygen uptake, which never exceeded two atoms of oxygen for one molecule of tyrosine; and the additional oxygen consumption was such a small fraction of the blank that the measurements possessed no great accuracy.

(*c*) *Relationship of phenylalanine to tyrosine.*

The following facts are relevant to a discussion of phenylalanine and tyrosine metabolism:

1. Both phenylpyruvic acid and phenyllactic acid, like phenylalanine, cause an increased elimination of homogentisic acid in the alcaptonuric patient [Neubauer and Falta, 1904]. In one particular individual phenylpyruvic acid was converted quantitatively into homogentisic acid, but of the administered phenyllactic acid only 41·5 % was recovered as homogentisic acid.

2. Phenylpyruvic acid and phenyllactic acid, unlike phenylalanine, do not give rise to acetoacetic acid in liver.

3. *p*-Hydroxyphenylpyruvic acid and homogentisic acid form large amounts of acetoacetic acid both in perfused liver and in slices.

4. Embden and Baldes [1913] have demonstrated that a certain amount of *l*(−)-tyrosine is formed along with acetoacetic acid when the liver is perfused with *dl*-phenylalanine.

5. Following the oral administration of phenylalanine to rabbits the urine has been found to contain *p*-hydroxyphenylpyruvic acid (as well as phenylpyruvic acid) [Kotake *et al.*, 1922]. Shambaugh *et al.* [1931] and Chandler and Lewis [1932] have reported the results of injecting rabbits subcutaneously with phenylalanine and tyrosine. After phenylalanine injection significant amounts of phenylpyruvic acid were excreted but no *p*-hydroxyphenylpyruvic acid. The latter was not obtained even after administration of tyrosine.

6. Medes [1932] described a rare metabolic anomaly, tyrosinosis, in which it was discovered (amongst other phenomena) that the ingestion of phenylalanine was followed by an increased excretion of *p*-hydroxyphenylpyruvic acid and of tyrosine.

7. In the condition of imbecillitas phenylpyrouvica, recently described by Fölling [1934], phenylpyruvic acid is excreted continuously.

From these facts certain conclusions may be drawn:

(1) Phenylpyruvic acid can arise from phenylalanine. This may be the preferential path of breakdown in kidney [Krebs, 1933].

(2) Since phenylpyruvic acid fails to yield acetoacetic acid in liver, there must be another catabolic path for phenylalanine.

(3) The evidence leads to the assumption that tyrosine is the intermediate. This is not merely in agreement with all experimental observations, but it is proved by the perfusion work of Embden and Baldes and by the observations of Medes on tyrosinosis. It is possible that the metabolic error of imbecillitas phenylpyrouvica is a failure of the conversion of phenylalanine into tyrosine, but it may be a block of an alternative path.

The rôle of ammonia in ketogenesis from amino-acids.

A number of amino-acids caused a small but distinct increase in ketogenesis in cases where the carbon skeleton could not of itself give rise to β-ketonic acid, *e.g.* valine, glycine, serine. In these instances the ketogenic effect of ammonia liberated during deamination can explain the results. Where ammonium chloride was added a much greater value was obtained due to the high initial concentration ($0{\cdot}04\,M$). Since the liberation and removal of ammonia are concurrent processes of normal metabolism, the intracellular concentration cannot approach $0{\cdot}04\,M$, but it seems reasonable to argue that ammonia could cause effects of the observed order of magnitude.

The antiketogenic influence of arginine and ornithine is interesting in this connection. It may be assumed that these substances lower the effective concentration of ammonia by promoting urea synthesis.

Comparison of the more rapidly deaminated amino-acids of the *d*-series with those of the *l*-series did not lead to any positive general conclusions. In cases where the carbon skeleton was non-ketogenic the *d*-amino-acids as a group were no more ketogenic than the members of the *l*-series.

Summary.

1. The formation of ketone-bodies from amino-acids has been studied with rat liver slices, and in general the results confirm the perfusion work of Embden. The most strongly ketogenic amino-acids are leucine, tyrosine and phenylalanine.

2. The only other amino-acid which is considerably ketogenic is hydroxyproline.

3. Of the remaining amino-acids some are non-ketogenic, but others show a small ketone-body formation, which seems to be due to ammonia liberated in their metabolism.

4. A difference was observed between the fed and the starved animal. With the exception of leucine, tyrosine, phenylalanine and hydroxyproline the amino-acids show no marked ketogenesis in the fed rat; *dl*-leucine alone is ketogenic in the starved animal, whilst many others are antiketogenic.

5. The evidence relevant to the breakdown of phenylalanine has been discussed. Two pathways of phenylalanine catabolism are possible:

(i) Conversion into tyrosine and breakdown through *p*-hydroxyphenylpyruvic acid and homogentisic acid, which takes place in the liver, and

(ii) Primary deamination and formation of phenylpyruvic acid.

6. Muconic acid, contrary to statements in the literature, is not ketogenic Previous results are due to use of the ammonium salt.

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