XCV. STUDIES ON BRAIN METABOLISM.

I. THE METABOLISM OF GLUTAMIC ACID IN BRAIN.

By HANS WEIL-MALHERBE.

From the Cancer Research Laboratory, North of England Council of the British Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle-upon-Tyne.

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It has long been realised that glutamic acid differs from most amino-acids, since it is oxidised in organs whose metabolism is supposed to be mainly concerned with carbohydrate and which are quite inert towards most of the other amino-acids. This suggests a connection between glutamic acid and carbohydrate metabolism.

Thunberg [1920] first showed that glutamic acid was the only amino-acid in presence of which washed frog muscle decolorised methylene blue. Harrison [1925] described aerobic oxidation of glutamic acid by washed frog muscle. Needham [1930] showed that glutamic acid is oxidised to succinic acid by the muscle of ox, rabbit, pigeon and frog. Holmberg [1934] prepared extracts from washed muscle which reduced methylene blue in presence of glutamic acid.

Thunberg [1923] demonstrated oxidation of glutamic acid by minced peripheral nerve and Quastel and Wheatley [1932] by brain. Krebs [1935, 1] observed increased respiration of brain and retina in the presence of glutamic acid.

The probable oxidation of glutamic acid by tumour tissue was indicated by the observation of Fleisch [1924] that a preparation of washed, minced Jensen rat sarcoma reduces methylene blue in presence of glutamic acid and by unpublished experiments of Dickens and Weil-Malherbe.

Methods.

Respiration was measured by the manometric method of Warburg [1926]. Unless otherwise stated, brain slices (grey matter only) of rats or guinea-pigs were used. The tissue (10–15 mg. dry wt.) was suspended either in phosphate saline [Krebs, 1933] or in bicarbonate saline [Krebs and Henseleit, 1932]. When phosphate saline was used, the manometer was filled with oxygen and the respiratory CO_2 was absorbed by 0·2 ml. of 10 % NaOH in the inner cup of the vessel. For the experiments with bicarbonate saline a gas mixture containing 5 % CO_2 was used. Any substrate added was neutralised to litmus paper. All experiments were done at 37·5°.

Ammonia and glutamine were determined according to Krebs [1935, 1, 2] with the apparatus of Parnas and Heller.

Units. The amount of metabolites formed or disappearing is expressed in μl. (1 millimol = 22400 μl.) or in Q-values $\left(\frac{\mu\text{l.}}{\text{mg. dry weight} \times \text{hours}}\right)$ with a corresponding index.

Nomenclature. The nomenclature of the amino-acids is that of Freudenberg and Karrer [*cf.* Krebs, 1935, 1].

Further experimental details will be given in the following sections.

I. l(+)-*Glutamic acid as a substrate of brain respiration.*

l(+) Glutamic acid maintains the respiration of brain slices which without the addition of a suitable substrate falls off rapidly. Glutamic acid is in this respect equal or even superior to glucose (Table I, see also Krebs, 1935, 1).

Table I. *Respiration of brain slices in presence of* l(+)*-glutamic acid. Phosphate saline.*

Species	Substrate added	Q_{O_2} 1st	2nd	3rd hr.
Rat	0	− 8·5	− 4·8	− 1·9
	0·2% glucose	− 9·5	− 9·5	−10·0
	M/100 *l*(+)-glutamic acid	−14·4	−12·0	−10·4
Guinea-pig	0	− 6·3	− 3·4	− 1·7
	0·2% glucose	− 8·8	− 8·7	− 9·4
	M/50 *l*(+)-glutamic acid	−12·8	−11·5	−10·6
Guinea-pig	*M*/100 *l*(+)-glutamic acid	−14·5	−11·2	− 9·1
	M/1000 *l*(+)-glutamic acid	−13·9	− 8·9	− 4·8
	M/4000 *l*(+)-glutamic acid	−13·4	− 6·3	− 3·7
	0	−10·3	− 5·7	− 3·6

If the concentration of *l*(+)-glutamic acid is diminished, respiration starts at a high rate and begins to fall after some time indicating exhaustion of the substrate (Table I, last exp.).

An increase of bicarbonate is observed when brain slices are incubated with *l*(+)-glutamic acid (Table II). This indicates disappearance of acid in the course of glutamic acid oxidation.

Table II. *Acid disappearance during the oxidation of* l(+)*-glutamic acid. Brain slices of guinea-pig in bicarbonate saline.*

Method	Conc. of *l*(+)-glutamic acid	Q_{Acid} 1st	2nd	3rd	Q_{O_2} 1st	2nd	3rd hr.
Warburg [1924], *cf.* Meyerhof and Lohmann [1926]	0	−2·0	−0·4	−0·1	− 5·7	−3·4	−2·2
	M/50	−4·9	−3·8	−3·6	−10·1	−9·2	−5·6
Dickens and Šimer [1931]	0		−0·86			− 3·5	
	M/50		−2·9			−14·3	

The R.Q. is little below unity (Table III). The theoretical value for complete oxidation is 1·1.

Table III. R.Q. *of brain slices in presence of* l(+)*-glutamic acid.*

Method	Species	Medium	Substrate added	Time of exp. hrs.	Q_{O_2}	R.Q.	Remarks
Dickens and Šimer [1930]	Rat	Phosphate saline	*M*/100 *l*(+)-glutamic acid	2	−10·7	0·91	
			0·2% glucose		−10·8	1·02	
			0		− 6·6	1·01	
Dickens and Šimer [1930]	Guinea-pig	Phosphate saline	*M*/100 *l*(+)-glutamic acid	2	−12·3	0·95	Duplicate
					−12·4	0·97	
			0·2% glucose		− 9·5	0·97	experiments
					− 9·9	0·98	
Dickens and Šimer [1931]	Guinea-pig	Bicarb. saline	*M*/100 *l*(+)-glutamic acid	3	−14·3	0·91	
			0		− 3·5	1·07	

In presence of other oxidisable substrates *l*(+)-glutamic acid raises the respiration still further (Table IV). This indicates a summation of oxidations. Brain thus differs from kidney or yeast, in which, in the presence of several substrates, there is competition for the available oxygen [Krebs, 1935, 1].

Table IV. *Summation of oxidations in brain. Phosphate saline.*

Species	Substrate added	Q_{O_2} 1st	2nd	3rd hr.
Guinea-pig	0·2% glucose	− 9·9	—	—
	M/50 *l*(+)-glutamic acid	− 11·3	—	—
	0·2% glucose + *M*/50 *l*(+)-glutamic acid	− 14·6	—	—
Rat	*M*/100 *l*(+)-glutamic acid	− 14·3	− 10·2	− 7·1
	M/100 lactic acid	− 17·1	− 15·3	− 12·9
	M/100 lactic acid + *M*/100 *l*(+)-glutamic acid	− 21·5	− 19·2	− 15·9
	M/100 pyruvic acid	− 21·1	− 18·2	− 16·6
	M/100 pyruvic acid + *M*/100 *l*(+)-glutamic acid	− 24·4	− 21·5	− 17·5
Guinea-pig	*M*/100 *l*(+)-glutamic acid	− 11·5	− 10·6	—
	M/100 succinic acid	− 10·9	− 4·6	—
	M/100 *l*(+)-glutamic acid + *M*/100 succinic acid	− 18·6	− 15·8	—

II. *Other amino-acids.*

l(+)-Glutamic acid is the only amino-acid oxidised in brain. The following 12 naturally occurring amino-acids have been tested and found ineffective in maintaining brain respiration: *l*(+)-alanine, *l*(+)-valine, *l*(−)-leucine, *l*(−)-methionine, *l*(−)-proline, *l*(−)-hydroxyproline, *dl*-serine, *l*(−)-aspartic acid, *l*(+)-ornithine, *l*(+)-arginine, *l*(−)-histidine and *l*(−)-tryptophan (concentration *M*/100 in all experiments).

III. *Glutamine and* d(−)*-glutamic acid.*[1]

Glutamine like *l*(+)-glutamic acid maintains brain respiration, although not quite so effectively (Table V). The oxidation of glutamine may proceed partially or totally *via* *l*(+)-glutamic acid, since Krebs [1935, 2] has shown the presence in brain of an enzyme capable of splitting glutamine to *l*(+)-glutamic acid and ammonia.

d(−)-Glutamic acid, the non-natural isomeride, is not oxidised by brain slices. There is even a slight depression of respiration in absence as well as in presence of glucose (Table V and Fig. 1).

The same specificity in the behaviour of brain slices towards the optical isomerides is observed in methylene blue experiments.

The experiments were done in ordinary Warburg manometers. The slices were previously shaken in oxygen long enough to exhaust their stores of preformed substrates and were transferred to the vessels containing bicarbonate saline in the main part and 0·2 ml. of a freshly prepared 2*N* solution of chromous chloride in the inner cup. The gas space was filled with $N_2 + 5\%$ CO_2. After 10 min. for absorption of the last traces of oxygen the methylene blue was tipped from the side bulb and the time necessary for complete decoloration was noted. After the experiment the slices were dried and weighed. In order to facilitate the comparison with the respiratory experiments the results are expressed in values of Q_{MB}, *i.e.* μl. of reduced methylene blue (373·5 mg. = 22400 μl.) per hour and mg. dry weight.

In view of the fact that 2 mols. of methylene blue correspond to 1 mol. of oxygen, it will be noted from Table VI that the anaerobic oxidation with

[1] I am indebted to Dr H. A. Krebs for samples of both substances.

methylene blue is about 15–20 times smaller than the oxidation in oxygen, a fact which has already been pointed out and discussed by Fleisch [1924]. The experiments however show clearly that *l*(+)-glutamic acid accelerates the decoloration of methylene blue whereas *d*(−)-glutamic acid is slightly inhibiting.

Table V. *Respiration of brain slices in presence of* l(+)*-glutamic acid,* d(−)*-glutamic acid and glutamine. Phosphate saline.*

Species	Substrate added	Q_{O_2} 1st	2nd	3rd hr.
Rat	0	− 8·5	− 4·8	− 1·9
	M/100 *l*(+)-glutamic acid	−14·4	−12·0	−10·4
	M/100 glutamine	−10·2	− 8·7	− 7·0
	M/100 *d*(−)-glutamic acid	− 4·6	− 2·5	− 1·6
Guinea-pig	0·2% glucose	− 9·5	− 9·3	—
	0·2% glucose + *M*/50 *d*(−)-glutamic acid	− 8·3	− 5·5	

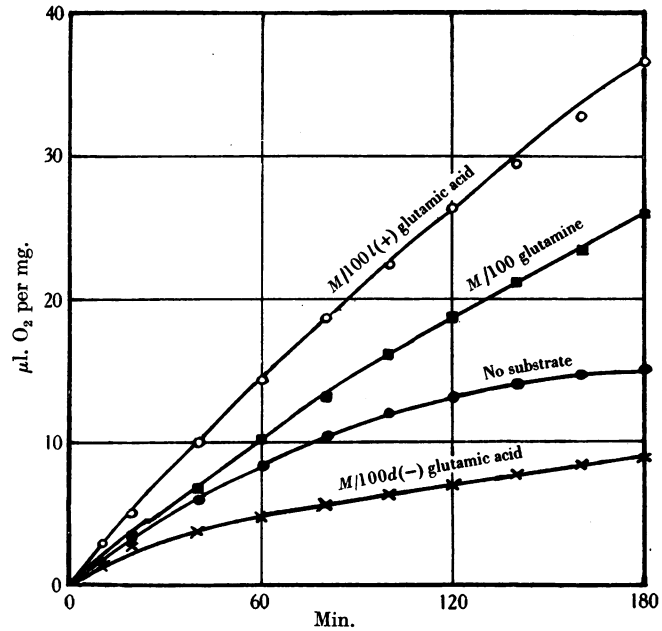

Fig. 1. Respiration of rat brain in presence of *l*(+)-glutamic acid, *d*(−)-glutamic acid and glutamine.

Table VI. *Reduction of methylene blue by slices of guinea-pig brain in presence of* d- *and* l-*glutamic acid. 0·2 mg. of methylene blue in each vessel.*

Substrate added	Dry weight of slices mg.	Decoloration time min.	Q_{MB}	Remarks
0	23·21	20	1·55	Slices previously shaken for 1 hour in O_2
M/50 *l*(+)-glutamic acid	26·06	12·5	2·21	
M/50 *d*(−)-glutamic acid	26·17	18	1·53	
0	19·36	49	0·76	Slices previously shaken for 2 hours in O_2
M/50 *l*(+)-glutamic acid	18·67	34	1·14	
M/50 *d*(−)-glutamic acid	21·62	48	0·69	

IV. *Glutamic acid deaminase*

Since brain slices only attack the natural isomeride it is the more surprising that extracts of brain not only attack the non-natural *d*(−)-glutamic acid, but even attack it preferentially. *l*(+)-Glutamic acid is also oxidised but more slowly and, relatively to the *d*-acid, the more slowly the more the extract is diluted. This behaviour is shown under aerobic (Table VII) as well as anaerobic conditions (Table VIII).

Table VII. *Oxygen uptake of an extract of fresh ox brain.*

50 g. of fresh ox brain (grey matter) are ground with 25 ml. water and centrifuged. The supernatant creamy emulsion is decanted and diluted with *M*/100 veronal buffer of p_H 8·2. Each vessel contains 2 ml. of the extract.

Parts of buffer added to 1 part of extract	Substrate added	μl. O_2 uptake (60 min.)
1	0	103
	M/50 *l*(+)-glutamic acid	114
	M/50 *d*(−)-glutamic acid	126
3	0	37
	M/50 *l*(+)-glutamic acid	46·5
	M/50 *d*(−)-glutamic acid	55
7	0	15·5
	M/50 *l*(+)-glutamic acid	22
	M/50 *d*(−)-glutamic acid	29·5

Table VIII. *Reduction of methylene blue by extracts of fresh brain. Thunberg technique.*

Extract 1 was identical with that used in the experiment of Table VII, diluted with 10 parts of veronal buffer p_H 8·2. Extract 2 was prepared by grinding a rat brain with 10 parts of *M*/50 phosphate buffer (p_H 7·4) and centrifuging. Each tube contained 2 ml. of extract and 0·2 ml. of methylene blue solution 1 : 5000.

Extract (no.)	Substrate added	Decoloration time (min.)
1	0	45
	M/50 *l*(+)-glutamic acid	33
	M/50 *d*(−)-glutamic acid	13
2	0	31
	M/50 *l*(+)-glutamic acid	20
	M/50 *d*(−)-glutamic acid	9

Unless it is assumed that an enzyme which was previously masked suddenly appears on extraction, one must conclude that on extraction the same enzyme undergoes a change of its specificity towards the two optical isomerides. Change of optical specificity is a well known fact in enzyme chemistry [*cf.* Rona and Ammon, 1933]. In this connection it is of special interest that steric selectivity may change with different methods of preparation [Bamann and Laeverenz, 1930] or with a different position of the catalytically active group on the colloidal carrier [Schwab *et al.*, 1933]. Bamann and Laeverenz [1930] conclude "that the difference of optical selectivity cannot be explained by differences of the active chemical group of the esterases, but is due to a changed association with specific carriers, brought about in the organism".

The behaviour of other organs towards optically isomeric amino-acids differs from that of brain. From the work of Krebs [1933; 1935, 1] it is known that kidney slices oxidise not only the natural, but also the non-natural amino-acids,

the latter even faster in many cases. As to the glutamic acids I find that the *d*-acid is oxidised by kidney slices little less rapidly than the *l*-acid in phosphate saline or in dialysed horse serum (Table IX).

Table IX. *Oxidation of* l(+)- *and* d(−)-*glutamic acid by kidney slices (guinea-pig), (1) in phosphate saline, (2) in dialysed horse serum.*

Preparation of the dialysed horse serum: 100 ml. of horse serum were saturated with CO_2 and dialysed in a collodion sac against 2 l. of distilled water saturated with CO_2 [Van Slyke *et al.*, 1923] for 2 days. The water was changed twice daily. The serum was then freed *in vacuo* from dissolved CO_2, neutralised with NaOH to p_H 7·4 (phenol red), made up with salts and phosphate buffer to correspond to the phosphate saline and finally inactivated at 56° for 2 hours.

		Phosphate saline			Dialysed horse serum		
Substrate added		Q_{O_2}	Q_{NH_3}	$Q_{\text{Amide-N}}$	Q_{O_2}	Q_{NH_3}	$Q_{\text{Amide-N}}$
0	1st hr.	−12·7	1·03	1·07	−13·4	0·86	0·94
	2nd hr.	− 9·7			−11·6		
M/50 *l*(+)-glutamic acid	1st hr.	−29·1	0·63	6·58	−29·8	0	5·00
	2nd hr.	−30·2			−31·6		
M/50 *d*(−)-glutamic acid	1st hr.	−23·1	1·34	1·22	−22·5	2·03	1·94
	2nd hr.	−16·4			−17·4		

Considerable amounts of the amino-acid deaminase pass into solution when kidney slices are incubated in saline [Krebs, 1933] and it is very probable that it is the dissolved part of the enzyme which accounts for the oxidation of the *d*-acids. When the cell has been killed with octyl alcohol oxidation of the *l*-acids is stopped, but that of the *d*-acids continues [Krebs, 1935, 1].

Dickens and Weil-Malherbe [1935] showed that tumour slices attack *l*- and *d*-glutamic acid with about the same velocity. Tumour in this respect behaves like kidney. On the other hand glutamic acid seems to be the only amino-acid oxidised by tumour.

Another difference between brain and kidney is important: whereas a fresh extract of brain still possesses some activity towards *l*(+)-glutamic acid, an extract of kidney is entirely inactive towards all *l*-amino-acids.

These discrepancies were thought possibly to be linked with the difference of the chemical composition of the two tissues, especially with the abundance of lipoids in brain. The assumption of a dissociable complex of the enzyme with some lipoid could explain why brain slices hold the enzyme more strongly than

Table X. *Extract of dry brain powder after acetone extraction. Thunberg technique.*

100 g. of fresh ox brain are treated with 500 ml. of acetone and dried *in vacuo* over P_2O_5. The finely ground dry powder is repeatedly treated at room temperature with 100 ml. of acetone.

Extract 1: 1 part of the powder extracted with 10 parts of *M*/100 veronal buffer (p_H 8·2) and centrifuged.

Extract 2: extract 1 diluted 5 times with veronal buffer.

Each tube contained 2 ml. of extract and 0·2 ml. of methylene blue solution 1 : 5000.

Extract (no.)	Substrate added	Decoloration time (min.)
1	0	33
	M/50 *l*(+)-glutamic acid	18
	M/50 *d*(−)-glutamic acid	22
2	0	78
	M/50 *l*(+)-glutamic acid	81
	M/50 *d*(−)-glutamic acid	51

kidney or tumour slices, which are comparatively poor in lipoids. The oxidation of the natural amino-acids might then be ascribed to the enzyme-lipoid complex as it exists within the cell and as it persists to a certain extent in the extract of brain, which is rich in lipoids.

The following facts support this view: a concentrated aqueous extract of an acetone-dried brain powder, rich in lipoids, attacks both glutamic acids with about the same velocity; the *l*-acid is even slightly ahead of the *d*-acid. When the same extract is diluted 5 times with water, the activity towards the *l*-acid is completely lost and the *d*-acid only is attacked (Table X).

When the same acetone-dried powder is extracted with ether, even a concentrated aqueous extract will only attack the *d*-acid, but not the *l*-acid. This behaviour is seen both in aerobic and anaerobic experiments. Table XI reproduces an oxygen uptake experiment.

Table XI. *Oxygen uptake with extract of dry brain powder after ether extraction.*

Two different preparations are used in these experiments. Powder 1 is the same as in the previous exp. (Table X) after repeated extraction with large quantities of ether at room temp. Powder 2 was prepared from another ox brain in the same way but was treated with chloroform after ether extraction. Both powders were extracted with 15 parts of $M/100$ veronal buffer (p_H 8·2) and centrifuged. Each vessel contained 2 ml. of extract.

Powder (no.)	Substrate added	Time (hours)	O_2-uptake (μl.)
1	0	6	54
	$M/50$ *l*(+)-glutamic acid		55
	$M/50$ *d*(−)-glutamic acid		80·5
2	0	4	20·5
	$M/50$ *l*(+)-glutamic acid		16
	$M/50$ *d*(−)-glutamic acid		44

Since the lipoid carrier responsible for the steric selectivity of the enzyme is very readily extractable with ether, it probably belongs to the monoamino-phosphatide fraction.

Attempts to restore the power of attacking *l*-amino-acids to kidney extracts by adding emulsions of brain lipoids failed. It therefore seems that the dissociation of the enzyme-lipoid complex is irreversible.

Enzyme-lipoid complexes have repeatedly been reported [*cf.* Przylecki, 1935]. Ro [1931] and Truszkowski [1934] found that uricase is bound to lipoids in the tissue.

The findings of Abderhalden and Tetzner [1935] that rats fed with *dl*-alanine excrete *d*(−)-alanine unchanged, agree well with the view that in the intact cell only the natural amino-acid is metabolised.

Some properties of the glutamic acid deaminase of brain.

1. *Oxygen uptake.* The oxygen uptake of an extract of the dry brain powder after exhaustive extraction with ether and alcohol or chloroform is small but constant for many hours.

2. *Ammonia production.* Ammonia estimations in the enzyme solution are impaired by the presence of a substance which causes a precipitation in the distillate on addition of Nessler's reagent. This difficulty could be overcome by repeated extraction of the brain powder with alcohol and by adding Nessler's reagent shortly before the colorimetric measurements were carried out. TableXII reproduces such an experiment. It will be noted that the ratio oxygen uptake:

ammonia formation is 1 : 1 and not, as would theoretically be expected, 1 : 2. Krebs [1935, 1], in certain cases, came across the same phenomenon which must be explained by the assumption of a coupled oxidation. Keilin and Hartree [1936] have recently shown that alcohols may serve as substrates for coupled oxidation in the presence of the deaminase-amino-acid system. Since our brain powder has been treated with alcohol, it might be suggested that the coupled oxidation observed was caused by retained alcohol. But the alcohol had been completely removed *in vacuo* over P_2O_5 and it is therefore probable that the powder contained other substances serving as substrates for coupled oxidation.

3. *Specificity of the glutamic acid deaminase.* Table XII shows that other amino-acids of the *d*-series are not oxidised by the enzyme.

Table XII. *Oxygen uptake and ammonia formation with extract of dry brain powder after ether and alcohol extraction.*

Powder 1 of Table XI repeatedly treated with abs. alcohol at room temp. Alcohol removed *in vacuo* over P_2O_5. Extracted with 15 parts of *M*/100 veronal buffer (p_H 8·2) and centrifuged. Each vessel contained 2 ml. of the extract. Duration of the experiment: 6 hours.

Substrate added	O_2 uptake μl.	Extra O_2 μl.	NH_3 formation μl.	Extra NH_3 μl.	Ratio $O_2 : NH_3$
0	33·5	—	48·9	—	—
M/50 *l*(+)-alanine	34	+ 0·5	46·6	− 2·3	—
M/50 *dl*-alanine	32	− 1·5	48·3	− 0·6	—
M/50 *dl*-valine	34	+ 0·5	50·6	+ 1·7	—
M/50 *l*(+)-glutamic acid	37	+ 3·5	45·4	− 3·5	—
M/50 *d*(−)-glutamic acid	58	+24·5	73·6	+24·7	1 : 1·01

V. *Isolation of α-ketoglutaric acid.*

The first product of the oxidation of *l*(+)-glutamic acid in brain is α-ketoglutaric acid. It was isolated as 2:4-dinitrophenylhydrazone. This was possible by checking its further oxidation with arsenic [Krebs, 1933]. α-Ketoglutaric acid could only be found in the presence of both *l*(+)-glutamic acid and arsenic. The controls with either arsenic or *l*(+)-glutamic acid alone were negative. Minced brain was used for these experiments instead of slices.

300 g. of fresh ox brain obtained from the slaughter house were minced, suspended in 300 ml. bicarbonate saline, containing *M*/25 *l*(+)-glutamic acid and *M*/1000 As_2O_3 and shaken in 3 wash-bottles with ground stoppers for 4 hours. The wash-bottles were filled with oxygen containing 5% CO_2. After incubation the contents of the bottles were centrifuged and the proteins in the supernatant fluids were removed by adding 1/10 vol. of 30% trichloroacetic acid. The filtrate was acidified with 1/5 vol. of conc. HCl and 100 ml. of 1% sol. of 2:4-dinitrophenylhydrazine in 2*N* HCl was added. After 12 hours in the ice chest the precipitate was filtered off, redissolved in 2*N* Na_2CO_3 and reprecipitated with 2*N* HCl. The precipitate was washed at the centrifuge with water, dried and recrystallised once from ethyl acetate—light petroleum and once from ethyl acetate. 123 mg. of a lemon-yellow crystalline product were obtained; M.P. 224° (uncorr.); M.P. of a sample prepared from pure α-ketoglutaric acid 224° (uncorr.); mixed M.P. 224° (uncorr.).

(Found: C, 40·26; H, 3·35; N, 17·12%. Calc.: C, 40·44; H, 3·09; N, 17·18%.)

The crude precipitate contained considerable amounts of the 2:4-dinitrophenylhydrazone of a second keto-acid which was encountered also in absence of either arsenic or glutamic acid. It was red, amorphous and easily soluble in Na_2CO_3 with a dark brown colour. With alcoholic KOH it gave a deep violet colour. Its properties agree in many respects with the bis-2:4-dinitrophenylhydrazone of glyoxylpropionic acid which has been described by Veibel [1931]

and Mayer [1931]. Yet the substance does not seem to be identical with this compound. Similar or identical 2:4-dinitrophenylhydrazones from brain have been described by Kraut and Nefflen [1935] and Johnson [1936].

Attempts to use semicarbazide for the fixation of α-ketoglutaric acid, following Hahn *et al.* [1929], were entirely unsuccessful. Semicarbazide in the concentrations used by Hahn did not even depress the oxygen uptake of brain slices in presence of α-ketoglutaric acid to any considerable extent. This experience makes the usefulness of this reagent for the accumulation of keto-acids under the experimental conditions described very doubtful.

VI. *The fate of the ammonia derived from deamination of* l(+)*-glutamic acid.*

Krebs [1935, 2] has shown that kidney and brain slices synthesise glutamine from *l*(+)-glutamic acid and ammonia. It is therefore not surprising that no free NH_3 is found when *l*(+)-glutamic acid is oxidised by brain slices, since all the NH_3 liberated reacts at once with excess glutamic acid to form glutamine. Krebs found however no increase of the total NH_3 (free NH_3 + amide-N) as compared with the control experiment, when glutamic acid was present. Only a shift in the relation: amide-N/free NH_3 was observed, the amide-N content being increased and the free NH_3 disappearing in the presence of glutamic acid. But the sum of both fractions was substantially the same as in the control. The same puzzling phenomenon was encountered by Needham [1930] in muscle where glutamic acid is oxidised to succinic acid without increased formation of either ammonia or amides.

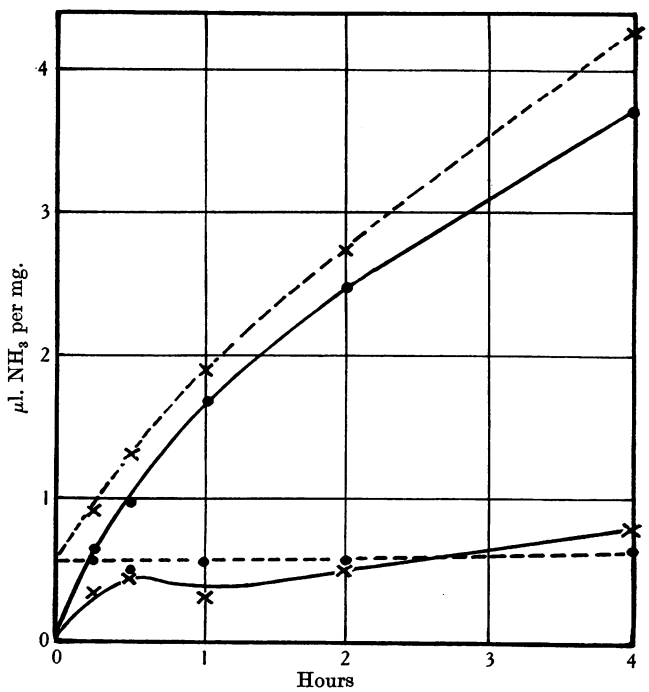

Fig. 2. Ammonia and glutamine formation by slices of guinea-pig brain.
Full line: free ammonia. Dotted line: amide-nitrogen.
× — × *M*/50 *l*(+)-glutamic acid. •—• without substrate.

Table XIII. *Ammonia and glutamine formation by slices of guinea-pig brain without added substrate and in presence of* M/50 l(+)*-glutamic acid. Phosphate saline.*

Time min.	No substrate			*l*(+)-Glutamic acid		
	μl. NH_3	μl Amide-N	μl. total NH_3*	μl. NH_3	μl. Amide-N	μl. total NH_3*
	per mg. tissue (dry weight)					
15	0·63	0·59	1·22	0·33	0·90	1·23
30	0·96	0·49	1·45	0·44	1·30	1·74
60	1·68	0·55	2·23	0·27	1·90	2·17
120	2·47	0·54	3·01	0·51	2·75	3·26
240	3·70	0·65	4·35	0·80	4·25	5·05

* Total NH_3 = sum of free NH_3 and amide-N.

Our own experiments have confirmed Krebs's findings. The increase of amide-N in the presence of *l*(+)-glutamic acid is accompanied in the control experiment by an increase of free NH_3 which is only slightly smaller, so that practically all the NH_3 derived from the deamination of glutamic acid disappears. Fig. 2 shows a time curve of the NH_3 and glutamine formation in presence and in absence of glutamic acid.

The fate of the NH_3 derived from deamination of glutamic acid will be discussed fully in a subsequent paper dealing with the problem of formation and metabolism of NH_3 in brain.

VII. *Reversibility of the reaction* l(+)*-glutamic acid* → α*-ketoglutaric acid.*

An increased amount of amide-N is found in the presence of glucose, pyruvic acid and α-ketoglutaric acid. Addition of NH_3 further enhances the formation of amide-N. Simultaneously NH_3 disappears from the solution, the largest dis-

Table XIV. *Ammonia-binding mechanism and glutamine formation. Guinea-pig brain.*

Substrate added	mg. tissue (dry weight)	Incubation (hours)	Medium	μl. NH_3 Initial	μl. NH_3 Final	Q_{NH_3}	$Q_{Amide-N}$
0	18·27	3	Phosphate saline	0	77·0	1·40	0·24
M/50 α-ketoglutaric acid	18·28			0	27·4	0·50	0·42
0	15·81	2	Bicarbonate saline	0	39·2	1·24	0·30
0·2% glucose	15·09			0	6·2	0·20	0·57
M/50 α-ketoglutaric acid	16·83			0	16·3	0·48	0·52
M/50 α-ketoglutaric acid + 0·2% glucose	16·17			0	0	0	0·46
0	15·64			85	102	0·54	0·31
0·2% glucose	18·26			85	41·3	−1·20	0·84
M/50 α-ketoglutaric acid	14·04			85	77·3	−0·27	0·55
M/50 α-ketoglutaric acid + 0·2% glucose	12·21			85	29·7	−2·26	1·22
0	17·67	2	Bicarbonate saline	0	47·2	1·34	0·41
0·2% glucose	18·85			0	10·6	0·28	0·52
M/50 pyruvic acid	14·50			0	8·7	0·30	0·45
M/50 pyruvic acid + 0·2% glucose	16·94			0	0	0	0·40
0	14·54			91·5	119	0·95	0·33
0·2% glucose	20·97			91·5	40·8	−1·21	0·82
M/50 pyruvic acid	17·64			91·5	80·5	−0·31	0·61
M/50 pyruvic acid + 0·2% glucose	10·09			91·5	57	−1·71	1·23
0	14·18	2	Bicarbonate saline	91	113	0·78	0·30
0·2% glucose	14·65			91	54	−1·26	0·78
M/50 pyruvic acid	22·89			91	75	−0·35	0·48
M/50 α-ketoglutaric acid	22·65			91	85	−0·13	0·55
0·2% glucose + *M*/50 pyruvic acid	13·62			91	48·5	−1·56	0·92
0·2% glucose + *M*/50 α-ketoglutaric acid	16·29			91	28	−1·94	1·35

appearance being observed in the presence of glucose and the smallest in the presence of α-ketoglutaric acid. But when glucose and pyruvate or glucose and α-ketoglutaric acid are added together, amounts of NH_3 disappear which exceed the sum of the disappearances in, presence of either substrate alone. The highest rate of NH_3 disappearance and at the same time the highest values of amide-N are observed when glucose + α-ketoglutaric acid are added (Table XIV).

To explain this, it is necessary to anticipate some results which will be fully dealt with in further publications of this series. It will be shown that α-ketoglutaric acid is an intermediate in the oxidation of pyruvic acid. We assume, following Toeniessen and Brinkmann [1930], a condensation of 2 mols. of pyruvic acid to form αα′-diketoadipic acid. The next step however is probably not, as these authors assumed, hydrolysis to succinic acid and 2 mols. of formic acid but decarboxylation and oxidation, the product being α-ketoglutaric acid.

$$\begin{array}{lllll} CH_3.CO.COOH & -H_2 & CH_2.CO.COOH & -CO_2 & CH_2.COOH \\ + & \longrightarrow & \cdot & \longrightarrow & \cdot \\ CH_3.CO.COOH & & CH_2.CO.COOH & +\tfrac{1}{2}O_2 & CH_2.CO.COOH \\ \text{Pyruvic acid} & & \alpha\alpha'\text{-Diketoadipic acid} & & \alpha\text{-Ketoglutaric acid} \end{array}$$

From the fact that the "NH_3-binding mechanism" described above is regularly accompanied by an increase of amide-N it is concluded that the same reactions which lead to the disappearance of NH_3 and formation of glutamine in the presence of *l*(+)-glutamic acid can be arrived at from α-ketoglutaric acid, pyruvic acid or glucose. Krebs [1935, 2] has already shown that the synthesis of glutamine from *l*(+)-glutamic acid and NH_3 in brain and retina is very small in absence of glucose. The synthesis of glutamine from α-ketoglutaric acid and NH_3 equally requires the presence of glucose. Therefore the NH_3-binding mechanism is smaller with α-ketoglutaric acid than with glucose, although the former is an intermediate in the process. The true effect of α-ketoglutaric acid is seen in the presence of glucose only. An analogy is the increasing effect of glucose and other substrates on urea formation in liver [Krebs and Henseleit, 1932].

Bicarbonate saline is a more favourable medium for the NH_3-binding mechanism than phosphate saline.

VIII. *The rôle of* l(+)*-glutamic acid in metabolism.*

It is very improbable that the amount of glutamic acid supplied to the brain is sufficient to be of importance as fuel comparable with carbohydrate. The peculiar position of glutamic acid among the amino-acids suggests that it fulfils a specific function in connection with carbohydrate metabolism. The view is held that *in vivo* the glutamic acid deaminase of brain is concerned rather with synthesis than with breakdown of glutamic acid. This synthesis is part of an NH_3-binding mechanism which leads from glucose *via* pyruvic acid and α-ketoglutaric acid to glutamic acid and glutamine. Glutamine is not the end product of the process, since it disappears also without liberating NH_3 [Krebs, 1935, 2]. Further work on these lines is in progress.

IX. Summary.

The only amino-acid oxidised by brain is *l*(+)-glutamic acid which is oxidised to α-ketoglutaric acid and NH_3 and further to H_2O and CO_2. The enzyme responsible for the oxidation of *l*(+)-glutamic acid to α-ketoglutaric acid and NH_3 does not attack *d*(−)-glutamic acid so long as it is bound in the cell or to some constituent of the cell, probably a lipoid. In solution however the specificity is changed and *d*(−)-glutamic acid alone is oxidised.

The NH_3 derived from the deamination of *l*(+)-glutamic acid disappears in secondary reactions leading to and beyond glutamine.

The reaction *l*(+)-glutamic acid → α-ketoglutaric acid is reversible. The existence of an NH_3-binding mechanism is shown leading from glucose *via* pyruvic acid and α-ketoglutaric acid to *l*(+)-glutamic acid, glutamine and further to an end product. The view is expressed that *in vivo* the glutamic acid deaminase is rather concerned with synthesis than with breakdown of glutamic acid.

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