CCXXX. METABOLISM OF AMINO-ACIDS.

IV. THE SYNTHESIS OF GLUTAMINE FROM GLUTAMIC ACID AND AMMONIA, AND THE ENZYMIC HYDROLYSIS OF GLUTAMINE IN ANIMAL TISSUES.

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1. *Oxidation of* l(+)*-glutamic acid without formation of ammonia.*

THE starting-point of this investigation was the observation that glutamic acid behaves differently from all the other α-amino-acids in guinea-pig and rabbit kidneys, in that, although it increases the oxygen uptake even more than any other amino-acid of the *l*-series, in most cases it actually diminishes ammonia formation. In guinea-pig kidney, the oxygen uptake is increased about 100–150% (Table I) whilst in most experiments no ammonia at all is detectable in the presence of *l*(+)-glutamic acid [Krebs, 1933, 2][1].

Table I. *Oxygen uptake and ammonia formation in the presence of* l(+)*-glutamic acid.*

	Without substrate		With *l*(+)-glutamic acid (*M*/50)	
Tissue	Q_{O_2}	Q_{NH_3}	Q_{O_2}	Q_{NH_3}
Kidney, guinea-pig	16·8	1·20	25·7	~0
	14·6	0·82	35·8	~0
	14·1	0·90	30·9	0·18
Kidney, rabbit	14·8	0·92	35·5	0·57
	13·8	0·37	23·4	0·40

However, if guinea-pig kidney is poisoned with arsenious oxide, ammonia is formed from glutamic acid (Table II). This could be explained by assuming that ammonia is formed in a primary reaction but disappears in a secondary reaction, the latter being inhibited by arsenious oxide. Experiments were therefore set up to see whether ammonia reacts in the expected way if it is added to kidney.

Table II. *Influence of* As_2O_3 *on ammonia formation in guinea-pig kidney.*

	Without substrate		With *l*(+)-glutamic acid (*M*/50)	
Concentration of As_2O_3	Q_{O_2}	Q_{NH_3}	Q_{O_2}	Q_{NH_3}
0	16·8	1·20	25·7	~0
M/5000	8·5	1·18	10·2	2·30
0	14·6	0·82	35·8	~0
M/1000	5·1	1·41	6·4	2·00

In muscle, too, glutamic acid causes an increase in oxygen uptake without influencing ammonia formation (D. M. Needham). This however is an effect different from that in kidney.

2. *Disappearance of ammonia from kidney in the presence of glutamic acid.*

If ammonium salts are added to guinea-pig or rabbit kidney in the presence of *l*(+)-glutamic acid, the ammonia disappears from the solution (Table III, Fig. 1). 11·79 mg. kidney, for instance, removed 95 μl. of ammonia in 30 mins., $Q_{NH_3}\left[\frac{\mu l.\ NH_3\ used}{mg. \times hours}\right]$ being −16·1. Similar figures were obtained with rabbit kidney (Table V). The rate of disappearance of ammonia is even higher than the rate of synthesis of urea in liver under average conditions.

Table III. *Disappearance of ammonia from guinea-pig kidney.*

11·79 mg. kidney in 2 ml. phosphate saline containing *M*/50 *l*(+)-glutamic acid and 166 μl. NH_3; 37·5°; O_2.

Time mins.	Ammonia present in 2 ml. μl.	Ammonia used μl.
0	166	—
30	71	95
60	14	152
90	0	166

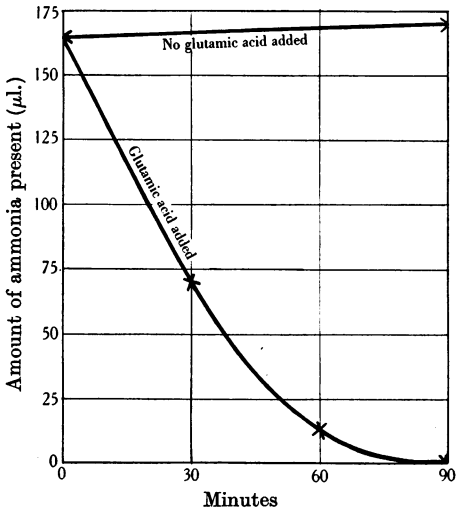

Fig. 1. Disappearance of ammonia in the presence of *l*(+)-glutamic acid (guinea-pig kidney).

No ammonia disappears from kidney in the absence of *l*(+)-glutamic acid; in the presence of amino-acids such as *d*(−)-glutamic acid, aspartic acid, β-hydroxyglutamic acid and numerous others the concentration of added ammonia is either unchanged or increased on account of deamination. The only two nitrogenous substances which behave similarly to glutamic acid are proline and hydroxyproline. The metabolism of these two substances will be dealt with in the next paper of this series.

In the presence of lactate or pyruvate, small quantities of ammonia can be utilised by kidney as shown in Table IV; the amount however is only 5–8% of that which disappears in the presence of glutamic acid.

Table IV. *Disappearance of ammonia from guinea-pig kidney in the presence of lactate and pyruvate.*

Substrate added	mg. tissue	NH_3 per flask (μl.) At start	NH_3 per flask (μl.) After 60 min.	NH_3 used μl.	Q_{NH_3}
dl-Lactate (*M*/50)	10·64	86	74·0	12·0	−1·13
Pyruvate (*M*/50)	10·32	86	73·9	12·1	−1·17

3. *Formation of amide-nitrogen in kidney.*

If the solution from which ammonia has disappeared in the presence of glutamic acid is heated for 5 min. with 5 % sulphuric acid, the ammonia appears again in the solution (Table V). Amino-acids or amino-purines do not split

Table V. *Disappearance of ammonia and formation of amide-nitrogen in kidney.*

Phosphate saline; *M*/50 *l*(+)-glutamic acid.

Tissue	Dry wt. of tissue mg.	NH_3 added μl.	Duration of exp. min.	NH_3 at end μl.	Change NH_3 μl.	Q_{NH_3}	Amide-N found μl.	$Q_{Amide\text{-}N}$
Guinea-pig kidney	11·79	166	30	71	− 95	−16·1	124	+21·0
	18·82	202	20	21	−181	−28·8	191	+30·4
	7·66	232	60	38	−194	−25·3	222	+29·0
Rabbit kidney	15·00	232	60	53	−179	−12·0	187	+13·9
	9·33	224	80	70	−154	−12·4	186	+14·9
	9·87	218	60	25	−193	−19·5	236	+24·0

off ammonia when heated for 5 min. in dilute acid, whereas acid amides are hydrolysed by hot dilute acid [Sachsse, 1873]. Asparagine requires heating for one hour, whereas glutamine is completely hydrolysed within 5 min. by 5 % sulphuric acid at 100° (Table VI). We may therefore conclude that the kidney tissue has converted ammonium glutamate into glutamine.

The amount of glutamine formed is usually somewhat larger than the amount of ammonia which disappears (Table V). The source of the excess of amide-nitrogen is ammonia formed by deamination of the glutamic acid. Whilst one part of the added glutamic acid is deaminated, another part unites with the ammonia in the synthesis of glutamine.

Table VI. *Acid hydrolysis of acid amides.*

0·005 *M* amide heated with 5 % (final concentration) sulphuric acid at 100°.
Calculated for complete splitting 112 μl. NH_3 per ml.

Amide	Time of heating min.	NH_3 formed (μl.) per 1 ml.	% splitting
Glutamine	2	91·5	82
	4	111·5	99·6
	8	113·0	100·9
Asparagine	5	26·0	23·2
Urea	5	0	0

4. *Formation of glutamine in various tissues.*

In the kidneys of guinea-pig and rabbit synthesis of glutamine proceeds more rapidly than deamination of glutamic acid; thus no free ammonia but only glutamine accumulates in these kidneys after addition of glutamic acid

(Table VII). Kidneys of rat and sheep deaminate more quickly than they form glutamine, and ammonia and glutamine appear in the solution simultaneously. Added ammonia does not disappear in these cases, the tissue being already saturated with ammonia from deamination. On the other hand no amide-nitrogen is formed in the kidneys of pig, dog or cat if glutamic acid and ammonia are present.

Table VII. *Formation of glutamine in kidney cortex of various mammals.*

No ammonia added.

Tissue (kidney)	No glutamic acid added			*M*/50 *l*(+)-glutamic acid		
	Q_{O_2}	Q_{NH_3}	$Q_{Amide\text{-}N}$	Q_{O_2}	Q_{NH_3}	$Q_{Amide\text{-}N}$
Guinea-pig	14·5	0·98	0·20	38·0	0	6·36
Rabbit	13·8	0·37	1·38	24·6	0·40	2·92
Sheep	13·9	1·77	0·68	26·6	3·11	7·50
Rat	21·0	2·90	1·40	43·2	6·00	2·83
Pig	16·9	1·80	~0	21·7	2·23	~0
Cat	16·6	1·57	~0	24·4	2·74	~0
Dog	21·3	1·36	~0	27·2	2·69	~0
Pigeon	18·2	2·21	~0	26·4	4·40	~0

Thus the system which synthesises glutamine is not found in all kidneys, but it is found in the retina and central nervous system of all the vertebrates which have been investigated (various mammals, birds, tortoise, frog, trout (see

Table VIII. *Consumption of ammonia and formation of glutamine in brain cortex and retina.*

Solution: 3 ml. phosphate saline; *M*/50 *l*(−)-glutamic acid; *M*/30 glucose; 37·5°, unless otherwise stated.

Tissue	Dry weight mg.	Time min.	Total amount of NH_3 in experimental solution (μl.) At beginning	At the end	Q_{NH_3}	NH_3 found after acid hydrol. μl.	$Q_{Amide\text{-}N}$
Brain, guinea-pig	8·59	60	67·2	26·4	− 4·77	39·4	4·60
	13·08	60	80·0	49·5	− 2·34	13·4	1·03
Retina, guinea-pig	3·57	60	67·2	31·0	−10·1	32·8	9·20
	4·00	60	33·6	1·6	− 8·0	29·0	7·25
Brain, pig	18·82	80	33·6	0	—	38·1	—
Retina, pig	20·00	40	33·6	0	—	41·0	—
Brain, rat	9·54	120	85·0	28·6	− 2·96	44·4	2·34
	7·50	60	85·0	62·6	− 2·99	20·6	2·74
Retina, rat	2·58	90	66·0	36·0	− 7·75	24·4	6·30
Retina, sheep	30·98	120	431·0	48·0	− 6·20	227·0	3·67
	21·45	120	448·0	204·0	− 5·70	138·0	3·22
Brain, pigeon	11·12	80	67·2	20·6	− 3·10	61·5	4·11
	6·43	80	67·2	37·2	− 3·49	33·6	3·93
Retina, pigeon	19·10	80	67·2	5·0	− 2·42	61·0	2·40
	7·82	60	165·0	81·0	−10·7	88·0	11·2
	9·00	40	89·6	52·2	− 6·23	33·0	5·50
	10·87	40	134·0	68·2	− 9·12	46·2	6·35
Retina, domestic fowl	14·70	40	131·0	79·0	− 5·33	49·0	5·00
Brain, frog (27°)	9·16	180	39·0	9·2	− 1·08	41·5	1·51
Retina, frog (27°)	6·09	180	39·0	19·5	− 1·07	32·5	1·78
Brain, tortoise (30°) (*Testudo graeca*)	17·34	240	68·5	30·0	− 0·55	34·0	0·49
Retina, tortoise (30°) (*Testudo graeca*)	1·21	240	68·5	24·5	− 9·00	42·5	8·70
Brain, trout (30°)	22·4	60	127·0	95·0	− 1·44	33·0	1·47
Retina, trout (30°)	8·71	60	127·0	25·0	−11·7	88·0	10·1

Table VIII)). The retina of warm-blooded animals forms about 6–10 μl. of amide-nitrogen per mg. or 5–7 % of its own dry weight of glutamine per hour. In brain cortex the rate is about a third of that in retina.

Whilst in kidney, as mentioned earlier, the amount of amide-nitrogen formed is usually slightly higher than the amount of ammonia consumed, in brain and in retina the amount of ammonia which disappears is often greater than the amount of amide-nitrogen found. This will be further studied in section 10.

No formation of amide-nitrogen from ammonium glutamate was detectable in the following tissues of the guinea-pig: kidney medulla, liver, spleen, testis, placenta, chorion, muscle, heart, salivary glands, pancreas, white matter of brain, red blood cells, small intestine; or in Jensen rat sarcoma, mice Crocker tumour, fowl tumour and pigeon blood.

5. *Some properties of the glutamine-synthesising system.*

A. *Influence of* p_H. The optimum p_H for the synthesis of glutamine in guinea-pig kidney is 7·2–7·4, as shown in Table IX and Fig. 2.

Table IX. *Influence of* p_H *on the synthesis of glutamine.*

Guinea-pig kidney; 37·5°. p_H varied by varying the concentration of bicarbonate in the saline and the concentration of CO_2 in the gas mixture. Various concentrations of bicarbonate were obtained by mixing 1·3% bicarbonate with bicarbonate-free saline. $M/50$ $l(+)$-glutamic acid. Ammonium chloride: $2{\cdot}72 \times 10^{-3} M$; 3 ml. solution for each flask.

Conc. of bicarbonate (M)	0·155	0·125	0·0950	0·0339	0·0136	0·00358	0·00358
Percentage of CO_2 in the gas mixture	2·5	5·0	5·0	5·0	5·0	5·0	20·0
p_H	8·58	8·19	8·07	7·62	7·22	6·64	6·04
mg. of kidney cortex	7·23	7·71	9·20	8·24	5·57	12·83	10·21
NH_3 at the beginning (μl. per flask)	201·0	201·0	201·0	201·0	201·0	201·0	201·0
NH_3 found after 40 min. (μl.)	204·0	148·5	114·0	105·5	132·0	100·0	172·0
NH_3 used (μl.)	3·0	−53·5	−87·0	−95·5	−69·0	−101·0	−29·0
Q_{NH_3}	~0·0	−10·4	−14·2	−17·4	−18·6	−11·8	−4·25
Amide-N found after 40 min. (μl.)	38·0	56·5	83·6	123·0	84·2	114·5	42·4
$Q_{\text{Amide-N}}$	7·9	11·0	13·6	22·4	22·6	13·4	6·2

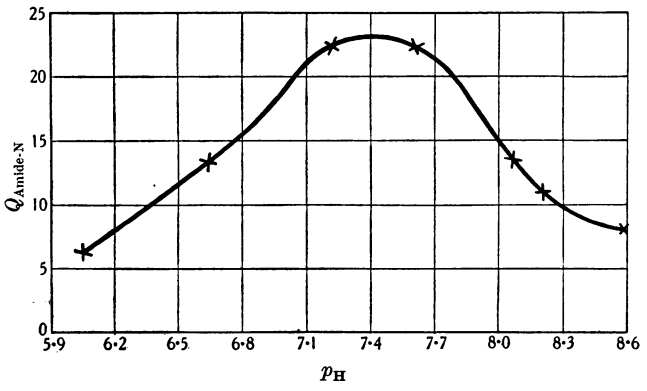

Fig. 2. Influence of p_H on the synthesis of glutamine (guinea-pig kidney).

B. *Concentration of glutamic acid.* It is only when the solution contains excess of glutamic acid that ammonia reacts completely to form glutamine in kidney (Tables III and X). In brain or retina however no excess of glutamic acid is

Table X. *Influence of the concentration of* l(+)*-glutamic acid on the synthesis of glutamine.*

Guinea-pig kidney; 3 ml. phosphate saline: initial concentration of ammonium chloride 0·00148 *M*; amount of tissue 4–6 mg.; time 80 min.; 37·5°.

Concentration of *l*(+)-glutamic acid *M*	Q_{O_2}	Q_{NH_3}	$Q_{\text{Amide-N}}$
0	11·5	+ 0·36	1·67
0·0033	29·1	−10·9	8·80
0·0066	28·7	−16·0	15·4
0·022	44·0	−24·2	26·0

necessary; if glutamic acid is added in small quantities and ammonia in excess, practically the whole of the glutamic acid is converted into glutamine. The amount of ammonia which disappears is approximately equivalent to the amount of glutamic acid present (Table XI).

Table XI. *Disappearance of ammonia in the presence of small amounts of* l(+)*-glutamic acid and excess of ammonia.*

Sheep retina (about 30 mg. per experiment); 37·5°; 2 hours; bicarbonate saline with 0·3% glucose; 5% CO_2 in O_2.

Glutamic acid added	NH_3 in the liquid (μl.)		Change in NH_3	Total NH_3 consumed by the tissue, corrected for blank	Amide-N found
μl.	Initial	Final	μl.	μl.	μl.
112	350	264	− 86	104	72
224	350	140	−210	228	158
0	0	18	+ 18	—	2

C. *Influence of glucose.* The synthesis of glutamine in kidney is unaffected by glucose; in retina and brain however the rate of synthesis is very small in the absence of glucose. All the figures recorded in Table VIII were obtained in glucose-containing saline. Table XII shows some examples of the effect of glucose on the rate of glutamine synthesis in brain and retina.

Table XII. *Influence of glucose on the rate of disappearance of ammonia and rate of synthesis of glutamine.*

Experimental conditions as in Table VIII.

	No glucose		*M*/50 glucose	
Tissue	Q_{NH_3}	$Q_{\text{Amide-N}}$	Q_{NH_3}	$Q_{\text{Amide-N}}$
Retina, sheep	−0·46	+0·58	−6·03	+3·67
Brain, rat	−0·70	+1·40	−3·75	+3·72

D. *Grinding and extracting.* Ground kidney, suspended in 10 vols. of saline, does not synthesise glutamine. The synthesis is bound up with the structure of the cell, as might be expected since it requires energy.

E. *Relation to energy-giving reactions.* In kidney, respiration is practically the only reaction which can provide energy for endothermic reactions. It would

therefore be expected that inhibition of respiration would inhibit the synthesis of glutamine. This is actually the case. Under strictly anaerobic conditions, glutamine is not synthesised in kidney. Hydrocyanic acid inhibits the glutamine synthesis approximately in the same degree as it inhibits respiration (Table XIII).

Table XIII. *Inhibition by hydrocyanic acid.*

Guinea-pig kidney; phosphate saline; $M/50$ $l(+)$-glutamic acid; $M/400$ ammonium chloride; 37·5°.

Concentration of HCN (M)	Q_{O_2}	Q_{NH_3}	$Q_{\text{Amide-N}}$
0	−44·0	−24·2	26·0
10^{-4}	−17·6	− 5·66	6·65
10^{-3}	− 4·30	− 3·1	2·1

Unlike kidney cortex, brain and retina possess a second very active energy-providing system, namely lactic acid fermentation. It seemed of interest to test whether lactic acid fermentation can provide the energy for the synthesis of glutamine. 30·7 mg. of brain cortex were shaken for 130 min. in bicarbonate saline solution (0·027 M bicarbonate, 0·02 M $l(+)$-glutamic acid, 0·002 M ammonium chloride, 0·002 M pyruvic acid, 5 % CO_2 in N_2) under strictly anaerobic conditions (wet yellow phosphorus in the inner cup of the vessel). After 130 min. 16 μl. amide-nitrogen were found in the solution. This corresponded approximately to the preformed amide-nitrogen so that no synthesis of glutamine had occurred.

Retinae of pig and pigeon however synthesise glutamine under strictly anaerobic conditions (Table XIV). In pig retina the rate of synthesis is smaller

Table XIV. *Synthesis of glutamine in retina under anaerobic conditions.*

$M/50$ $l(+)$-glutamic acid; 0·4 % glucose; $M/500$ pyruvate; $M/40$ bicarbonate; 5 % CO_2 in the gas mixture; 3 ml. solution.

Animal	Tissue mg.	Conditions	Time mins.	Initial conc. of NH_3 M	Amide-N found μl.	$Q_{\text{Amide-N}}$
Pig	20·65	N_2, phosphorus	80	0·002	93·5	2·78
Pigeon	8·03	N_2	60	0·0022	68·0	8·5
,,	7·82	O_2	60	0·0022	88·0	11·2
Pigeon	10·29	N_2, phosphorus	40	0·0012	30·2	4·42
,,	9·00	O_2	40	0·0012	33·0	5·50
Pigeon	9·56	N_2, phosphorus	40	0·0018	33·5	5·25
,,	10·87	O_2	40	0·0018	46·2	6·35

anaerobically than aerobically whilst in pigeon retina no appreciable difference is found in oxygen and nitrogen. The respiration of pigeon retina is extremely small; in bicarbonate saline or serum there is no measurable respiration [Krebs, 1927] (for technical reasons) whilst in phosphate saline a respiration of 7·5 is found (Table XV). The glycolysis (Q_M) of pigeon retina amounts to about 150. Pigeon

Table XV. *Respiration of pigeon retina.*

Phosphate saline; 0·4 % glucose; O_2; inner cup: 0·3 ml. NaOH, 38°.

Number	Q_{O_2}
1	7·50
2	6·88
3	7·82

retina obtains its energy preferentially from the anaerobic lactic acid fermentation and therefore it is not surprising that the synthesis of glutamine, as an energy-requiring reaction, does not depend on the presence of oxygen. Accordingly $10^{-3}M$ HCN does not inhibit the formation of glutamine in pigeon retina.

F. *Inhibition by* d(−)*-glutamic acid.* If *d*(−)-glutamic acid and ammonia are added to kidney, no glutamine is formed. Racemic glutamic acid gives about half the yield of glutamine and half the increase in respiration given by *l*(+)-glutamic acid under the same conditions (Table XVI). This halving effect

Table XVI. *Inhibition of glutamine synthesis by* d(−)*-glutamic acid.*

Guinea-pig kidney; phosphate saline; 37·5°.

Substrates added (final concentrations)	Q_{O_2}	Q_{NH_3}	$Q_{\text{Amide-N}}$
l(+)-Glutamic acid (*M*/80); NH_4Cl (*M*/300)	−31·2	−25·3	29·0
dl-Glutamic acid (*M*/40); NH_4Cl (*M*/300)	−21·0	− 7·3	12·1
NH_4Cl (*M*/300)	−13·0	—	0·5
l(+)-Glutamic acid (*M*/80); NH_4Cl (*M*/150)	−29·0	−16·1	25·8
dl-Glutamic acid (*M*/40); NH_4Cl (*M*/150)	−19·6	− 6·65	11·1
NH_4Cl (*M*/150)	−12·0	—	—
l(+)-Glutamic acid (*M*/50); NH_4Cl (*M*/370)	−44·1	−24·2	26·0
l(+)-Glutamic acid (*M*/50); *d*(−)-glutamic acid (*M*/50); NH_4Cl (*M*/370)	−31·3	−14·6	13·1
l(+)-Glutamic acid (*M*/150); *d*(−)-glutamic acid (*M*/150); NH_4Cl (*M*/370)	−33·4	−17·5	17·1
l(+)-Glutamic acid (*M*/150); *d*(−)-glutamic acid (*M*/50); NH_4Cl (*M*/370)	−29·5	− 8·9	7·6
l(+)-Glutamic acid (*M*/150); NH_4Cl (*M*/370)	−28·7	−16·0	15·4
NH_4Cl (*M*/370)	−11·5	+ 0·4	1·7
d(−)-Glutamic acid (*M*/150), NH_4Cl (*M*/370)	−12·5	+ 1·0	1·3

is due to specific inhibition of the glutamine-synthesising system by *d*(−)-glutamic acid. Addition of *d*(−)-glutamic acid to *l*(+)-glutamic acid has the same result as addition of *dl*-glutamic acid. The figures in Table XVI show that it is not the absolute concentration of *d*(−)-glutamic acid which determines the degree of inhibition but the ratio $\frac{\text{concentration of } l(+)\text{-glutamic acid}}{\text{concentration of } d(-)\text{-glutamic acid}}$. For instance *M*/150 *d*(−)-glutamic acid inhibits the amide-nitrogen formation by 50 % if the concentration of the antipode is *M*/150, but only by 34 % if the concentration of the antipode is *M*/50.

The inhibition by *d*(−)-glutamic acid can be explained by the assumption that both isomerides combine with the enzyme and that the affinities of the enzyme for both are equal; only *l*(+)-glutamic acid however reacts to form glutamine.

Neither α-ketoglutaric acid, *dl*- and *l*(−)-aspartic acid nor *dl*-alanine influences the rate of glutamine formation. A slight inhibition is found when synthetic *dl*-β-hydroxyglutamic acid is added (Table XVII).

Table XVII. *Inhibition of the glutamine synthesis by* dl-β*-hydroxyglutamic acid.*

Guinea-pig kidney, phosphate saline, 37·5°.

Substrates added (final concentrations)	Q_{O_2}	Q_{NH_3}	$Q_{\text{Amide-N}}$
l(+)-Glutamic acid (*M*/60), NH_4Cl (*M*/184)	28·9	−25·3	+26·5
l(+)-Glutamic acid (*M*/60), NH_4Cl (*M*/184) + *dl*-β-hydroxyglutamic acid (*M*/60)	26·7	−19·3	+16·9

G. *Bicarbonate and phosphate buffers.* Varying the concentration of bicarbonate between *M*/40 and *M*/270, or replacing bicarbonate with phosphate does

not essentially influence the rate of glutamine synthesis in guinea-pig kidney if the p_H is kept constant (Table XVIII).

Table XVIII. *Synthesis of glutamine in bicarbonate and phosphate buffers.*

Guinea-pig kidney; $M/50$ $l(+)$-glutamic acid; $M/150$ NH_4Cl; p_H 7·4.

Buffer	Q_{NH_3}	$Q_{Amide\text{-}N}$
Phosphate ($M/100$)	−24·8	+26·0
Bicarbonate ($M/40$); CO_2 (5 vol. %)	−18·5	+22·8
Bicarbonate ($M/270$); CO_2 (0·8 vol. %)	−19·0	+25·0

6. *Enzymic hydrolysis of glutamine into glutamic acid and ammonia.*

If glutamine is added to extracts of brain, retina or kidney, the reaction described in the previous sections is reversed and glutamine is split into glutamic acid and ammonia (Table XIX). This reaction is not dependent on the cell structure and can be conveniently investigated in aqueous tissue extracts.

Table XIX. *Activities of asparaginase and glutaminase in kidney and liver extracts of rabbit and guinea-pig.*

Each test-tube contains 4·5 ml. veronal buffer (p_H 7·7; $M/10$), 1·5 ml. water, 0·5 ml. $M/5$ substrate (asparagine or glutamine) and 1 ml. tissue extract (1 part fresh tissue ground extracted with 10 parts water, centrifuged and supernatant fluid used), 1 hour; 37·5°.

Tissue	Substrate	NH_3 found after 1 hour's incubation μl.	NH_3 formed (corrected for blank) μl.
Rabbit liver	Asparagine	331	312
,,	Glutamine	92	73
,,	—	19	—
Rabbit kidney	Asparagine	18	3
,,	Glutamine	291	276
,,	—	15	—
Guinea-pig liver	Asparagine	1520	1484
,,	Glutamine	368	332
,,	—	36	—
Guinea-pig kidney	Asparagine	95	51
,,	Glutamine	225	181
,,	—	44	—

Enzymic hydrolysis of glutamine was observed by Hunter and Geddes [1928] and by Grassmann and Mayr [1933] in yeast extracts (see also Luck [1924]) when they were studying the specificity of asparaginase (which hydrolyses asparagine to aspartic acid and ammonia). These authors reached no conclusion as to whether glutamine and asparagine are split by one and the same enzyme. It can be shown however that there is a specific "glutaminase" in some tissues: rabbit kidney splits glutamine rapidly but hydrolyses asparagine very slowly (Table XIX). Rabbit liver on the other hand splits glutamine about five times more slowly than asparagine (under the same conditions). The ratio $\frac{\text{activity of asparaginase}}{\text{activity of glutaminase}}$ is about 1/100 for rabbit kidney and about 4 for rabbit liver extracts. If one enzyme were responsible for the breakdown of both amides, the above ratio should be constant. It follows from these experiments that glutaminase is a specific enzyme.

When glutamine is converted into ammonia and glutamic acid apparent amino-nitrogen must disappear from the solution, since 90% of the total

nitrogen of glutamine reacts in Van Slyke's method (the reaction time being 5 min.). Hence the disappearance of amino-nitrogen as determined by Van Slyke's method should amount to 80% of the ammonia formed. The experimental result agrees with the predicted figure within the limits of error: 0·6 ml. *M*/10 glutamine was mixed with 0·9 ml. brain extract (rat brain extracted with 10 vols. water) and 0·15 ml. *M* sodium bicarbonate. Immediately after mixing 785 μl. amino-nitrogen were found in 0·5 ml. solution. After one hour's incubation (37·5°) 341 μl. ammonia were found in 0·5 ml. Another 0·5 ml. was mixed with 1 ml. borate buffer, p_H 9·5, and evaporated *in vacuo* to 0·5 ml. in order to remove ammonia. The amount of amino-nitrogen found in this solution was 480 μl. Thus 305 μl. amino-nitrogen had disappeared, whilst 341 μl. ammonia appeared. The loss of amino-nitrogen is somewhat higher than calculated (89% found, 80% calculated). This might be expected since slight decomposition of glutamine during the evaporation, necessary for the removal of ammonia, is unavoidable.

Glutamine, heated (100°) in neutral solution, yields pyrrolidonecarboxylic acid and ammonia [Chibnall and Westall, 1932]. In this case two amino-groups disappear for one equivalent of ammonia formed; thus enzymic splitting and neutral heat hydrolysis yield different products.

7. *Some properties of glutaminase.*

A. *Specificity.* As mentioned in the previous paragraph glutaminase does not hydrolyse asparagine. Phenacetylglutamine and benzoylglutamine, kindly supplied by Dr N. W. Pirie, are not attacked by kidney or brain extracts. Glutamine peptides (glutaminylglycine and glutaminylglutamic acid) which Prof. Chibnall kindly gave me did not yield ammonia in the presence of rat brain extracts. *iso*Glutamine (also from Prof. Chibnall) is slowly hydrolysed, the velocity being only a few % of the rate of splitting of glutamine (Table XX).

Table XX. *Action of rat brain extract on glutamine derivatives.*

Brain extract: one part brain ground with 10 parts *M*/10 $NaHCO_3$. Each test-tube contained 0·5 ml. extract and 0·3 ml. *M*/10 neutralised substrate solution. p_H 8·5. Blank controls for brain extract and for the substrates. Incubation 2 hours; 37·5°.

Substrate added	NH_3 formed (corrected for blank) μl.
Glutamine	590
*iso*Glutamine	35
Benzoylglutamine	0
Phenacetylglutamine	0
Glutaminylglycine	12
Glutaminylglutamic acid	0

B. *Inhibition by glutamic acid.* A peculiar phenomenon is observed when the course of hydrolysis of glutamine is followed in extracts of kidney, brain or retina. The hydrolysis starts with a high velocity (Table XXI, Fig. 3), but the rate soon falls off. Initially 156 μl. ammonia were formed per mg. dry kidney per hour, but when only one-sixth of the total amount of glutamine was split the velocity fell to one-third of the initial rate, and when half of the substrate was decomposed the velocity was about 6 μl. per mg. per hour, that is 3·8% of the initial rate. This decrease of activity is not due to destruction of the enzyme, since addition of fresh extracts does not restore the initial rate, but increases

Table XXI. *Course of action of glutaminase.*

5 ml. phosphate buffer; p_H 7·8; 1 ml. pig kidney extract (1 part minced kidney cortex extracted with 4 vols. of water, centrifuged; 3 ml. glutamine ($M/20$); 1 ml. water. Control tube water instead of glutamine solution; 1 drop octyl alcohol. From time to time 1 ml. or 0·5 ml. analysed; calculated for complete splitting; 338 μl. per 1 ml.

Time min.	NH_3 in 1 ml. μl.	Time min.	NH_3 in 1 ml. μl.
2·5	26·4	100	154
5	52	380	216
10	68	2880	313
20	96	5760	340
40	127		

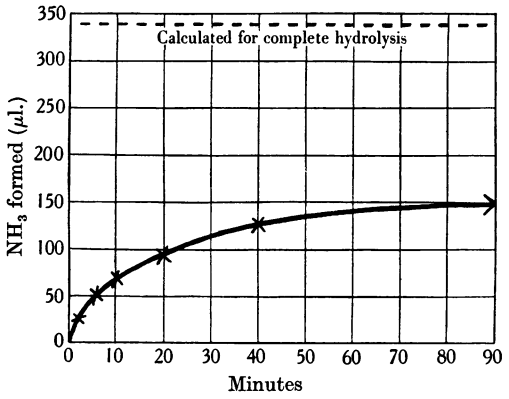

Fig. 3. The course of the hydrolysis of glutamine (pig kidney extract).

the rate only in proportion to the enzyme concentration. The diminishing activity is due to inhibition of glutaminase by the glutamic acid formed in the hydrolysis.

This inhibition of glutaminase by glutamic acid is shown directly in Table XXII. $2·5 \times 10^{-2} M$ $l(+)$-glutamic acid produces 98 % inhibition of the

Table XXII. *Inhibition of glutaminase by glutamic acids.*

Each test-tube contains 2 ml. phosphate buffer (p_H 7·8), 0·5 ml. pig kidney extract (see Table XXI), 1 ml. glutamine solution (0·035 M) and 0·5 ml. of the solution given in the first column; control tube water instead of glutamine.

Solution added	NH_3 per test-tube after 40 min. incubation at 37·5° μl.	NH_3 formed (corrected for blank) μl.
Water	485	437
$l(+)$-Glutamic acid ($M/5$)	57	9
$d(-)$-Glutamic acid ($M/5$)	57	9
Control	48	—

splitting of $0·87 \times 10^{-2} M$ glutamine. Both optical isomerides of glutamic acid are equally active as inhibitors. This inhibition is not due to thermodynamic equilibrium between glutamine and ammonium glutamate; the equilibrium of the reaction lies at practically complete hydrolysis of the amide (in physiological solutions). This is shown by the facts that the splitting of glutamine

goes to completion even in the presence of glutamic acid (though extremely slowly), and that no trace of glutamine is formed from glutamic acid and added ammonia in the presence of glutaminase.

The inhibition of glutaminase by glutamic acid could be explained however by a competition of glutamine and glutamic acid for the enzyme. Competitive inhibitions by compounds which are chemically similar to the substrate are known (*e.g.* in the cases of xanthine oxidase and of sucrase), but asparaginase is not inhibited by aspartic acid. It is remarkable that the inhibition of glutamic acid requires very small amounts of glutamic acid, indicating that the affinity of glutamic acid for the enzyme is much greater than that of glutamine. From the experiment given in Table XXI and Fig. 3, it may be calculated that the rate falls to 50 % of the initial rate when 18–20 % of the glutamine is hydrolysed. Hence the enzyme is equally distributed between glutamine and glutamic acid if the ratio of concentrations of glutamine to glutamic acid is 4 to 1.

The inhibitory action of glutamic acid is specific except that *dl*-β-hydroxyglutamic acid causes a slight inhibition (Table XXIII). Other amino-acids, or glutathione or α-ketoglutaric acid have no effect. The fact that *d*(−)-glutamic acid and *dl*-β-hydroxyglutamic acid inhibit glutamine synthesis and

Table XXIII. *Inhibition of glutaminase by* dl-*β-hydroxyglutamic acid.*

Each test-tube contained 0·5 ml. veronal buffer, 0·5 ml. *M*/10 glutamine, and 1 ml. brain extract (rat brain extracted with 10 parts water; 37·5°; 30 min.).

Solution added	NH_3 formed (μl.)
0·5 ml. water	144
0·5 ml. *M*/5 *dl*-β-hydroxyglutamic acid	112

glutamine hydrolysis to the same extent is evidence in favour of the identity of the enzymes concerned with the synthesis and the hydrolysis.

C. *Dry enzyme preparations.* To minced pig kidney 5 vols. of acetone were added. Extracts of the dried precipitate hydrolysed glutamine, though more slowly than extracts of fresh material. The activity was about the same whether the dry powder was extracted with water or with *M*/10 sodium bicarbonate. Acid extraction (1 % acetic acid) gave less active enzyme solutions (Table XXIV).

Table XXIV. *Glutaminase in acetone preparations of pig kidney cortex.*

0·5 g. powder extracted for 15 min. with 10 ml. solution;
1 ml. extract + 1 ml. *M*/10 glutamine + 0·2 ml. $NaHCO_3$ (*M*); p_H 8·5.

Solution used for extraction	NH_3 formed in 30 min.; 37·5° (corrected for blank) μl.
Water	33·7
M/10 $NaHCO_3$	36·8
M/60 acetic acid	23·7

Table XXV. *Influence of enzyme concentration.*

Guinea-pig brain extracted with 10 parts water; *M*/75 glutamine; bicarbonate buffer; p_H 8·5; total volume 3·2 ml.

Volume of brain extract ml.	NH_3 formed in 30 min.; 37·5° μl.
2·0	79
1·0	45
0·5	20
0·25	14

D. *Enzyme concentration.* The initial velocities are approximately proportional to the enzyme concentration as shown in Table XXV.

E. *Influence of glutamine concentration.* The inhibition by glutamic acid—a combination between catalyst and a product of the catalysis—complicates the kinetics of glutaminase. It is not proposed to analyse the kinetics exhaustively in this paper, but a few figures will be given showing the activity of glutaminase under different conditions. Table XXVI shows the influence of glutamine con-

Table XXVI. *Influence of glutamine concentration.*

Guinea-pig brain extracted with 10 parts water; 2 ml. extract + 0·3 ml. *M* $NaHCO_3$ + 2 ml. glutamine solution in each tube; p_H 8·5; 37·5°.

Final concentration of glutamine *M*	NH_3 formed in 30 min. μl.
0·02	188·0
0·01	69·5
0·005	20·5

Table XXVII. p_H *curve of brain, kidney and liver glutaminase.*

Buffer final *M* concentrations				NH_3 formed (corrected for blank) μl.		
Na_2CO_3	$NaHCO_3$	Vol. % CO_2 in gas	p_H	Brain	Kidney	Liver
0·09	0·01	—	10·8	38·5	53	10
0·05	0·05	—	9·8	134·0	215	21
0·01	0·09	—	8·8	525·0	288	72
—	0·1	5	8·1	230·0	312	75
—	0·025	5	7·5	32·0	190	218
—	0·005	5	6·8	10·0	145	191
—	0·005	20	6·2	4·0	116	26

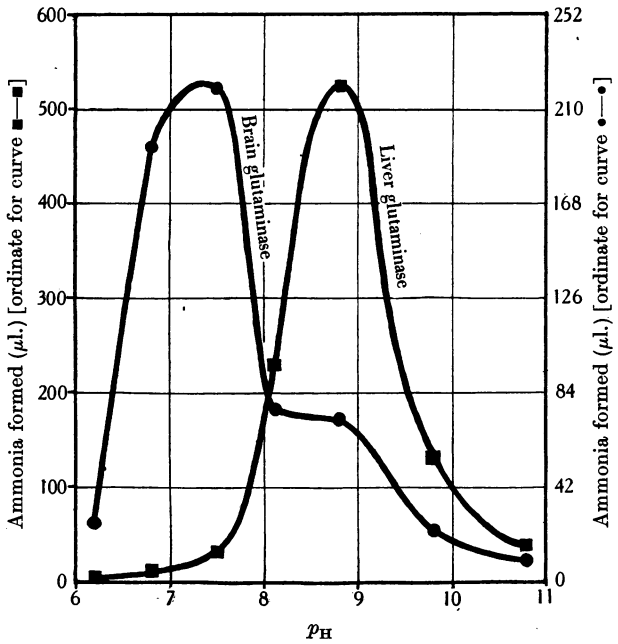

Fig. 4. p_H curve of brain and liver glutaminase.

centration on the initial velocity of hydrolysis. As expected from the preceding experiments an increase of the concentration of glutamine increases the velocity of the hydrolysis considerably.

F. *Influence of* p_H. 3 g. guinea-pig brain were ground with sand and extracted with 24 ml. water. 1 ml. of the centrifuged extract was mixed with 0·8 ml. *M*/10 glutamine solution and 0·2 ml. buffer. The flasks were incubated for 40 min. at 37·5°; ammonia was then determined. Blanks were carried out to measure the ammonia formation in the absence of added glutamine. The blank was found to be independent of p_H; it amounted to 11 μl. NH_3 per flask. The results are given in Table XXVII and Fig. 4. Similar experiments were carried out with kidney and liver extracts. Sheep kidney was extracted with 4 vols. of water. The liver extract was prepared by extracting guinea-pig liver with five parts of water. The results are also recorded in Table XXVII.

Owing to the complications in the kinetics before mentioned the curves in Fig. 4 give only an approximate picture of the influence of p_H on glutaminase. The optimum of brain glutaminase lies between p_H 8 and 9. The curves for kidney, liver and brain glutaminase are not identical. In the next paragraph further evidence will be adduced showing that the splitting of glutamine in various tissues is due to different "glutaminases".

G. *Glutaminase in various tissues. Existence of different glutaminases.* Glutaminase is found in those tissues in which the synthesis of glutamine occurs, but it is also found in some tissues which do not synthesise glutamine, for instance in spleen and liver of the guinea-pig and in pig kidney. Table XXVIII shows the relative activities of extracts from various tissues.

Table XXVIII. *Glutaminase in various tissues. Influence of glutamic acid.*

Veronal buffer; p_H 8·5; 37·5°.

Tissue	Water used for extract parts	Vol. of extract used ml.	Total vol. of fluid ml.	*M* conc. of glutamine	*M* conc. of *l*(+)-glutamic acid	Time min.	NH_3 formed (corrected for blank) μl.
Pig retina	30	1	2·2	0·0023	0	120	225
,,	,,	,,	,,	,,	0·046	,,	~0
Pig liver	4	1	2·5	0·02	0	30	238
,,	,,	,,	,,	,,	0·04	,,	238
Pig kidney	4	1	2·5	0·02	0	30	91
,,	,,	,,	,,	,,	0·04	,,	18
Guinea-pig spleen	12	1	2·5	0·02	0	60	27
,,	,,	,,	,,	,,	0·04	,,	3
Guinea-pig liver	5	1	2·5	0·02	0	60	740
,,	,,	,,	,,	,,	0·04	,,	740
Guinea-pig lung	5	1	2·5	0·02	0	60	68·5
,,	,,	,,	,,	,,	0·04	,,	3
Guinea-pig muscle	5	1	2·5	0·02	0	60	8
,,	,,	,,	,,	,,	0·04	,,	6
Guinea-pig testicle	5	1	2·5	0·02	0	60	0
Guinea-pig blood (laked)	3	3	7·0	0·02	0	120	27
,, ,,	,,	,,	,,	,,	0·04	,,	31
Rat brain	5	1	2·5	0·02	0	60	186
,,	,,	,,	,,	,,	0·04	,,	57
Rat liver	4	1	2·5	0·02	0	60	173
,,	,,	,,	,,	,,	0·04	,,	153
Rat muscle	8	2	4·0	0·02	0	140	7
,,	,,	,,	,,	,,	0·04	140	7

The most characteristic property of glutaminase found so far is the inhibition of the enzyme by glutamic acid. But this inhibition is not found in all tissue extracts in which a glutaminase is found. Guinea-pig liver for instance hydrolyses glutamine rapidly, but glutamic acid does not inhibit the reaction (Table XXVIII). This proves again (see the preceding paragraph) that the glutamine-splitting enzyme in guinea-pig liver is different from the enzyme in kidney, brain or retina. There are at least two types of glutaminase distinguishable by their p_H optima and their inhibitions by glutamic acid ("brain type" and "liver type"). Some tissues, for instance rat kidney, seem to contain both types of glutaminase, since the splitting of glutamine is partly inhibited by glutamic acid and the p_H curves show two maxima.

The second glutaminase ("liver type"), too, is different from asparaginase. Guinea-pig liver splits both amides, asparagine and glutamine, and the rates of splitting are of the same order of magnitude. But blood serum from guinea-pig does not attack glutamine whereas it splits asparagine rapidly [Clementi, 1922]: 1 ml. serum hydrolyses up to 30 mg. asparagine per hour (p_H 7·4, 37·5°). Asparaginase is thus found in nature separately from the enzymes which split glutamine. This proves that asparaginase is not concerned in the splitting of glutamine.

8. *Glutamine as precursor of ammonia in tissues.*

Most animal tissues are capable of forming ammonia by anaerobic reactions when incubated at body temperature. The rate of ammonia formation is often much greater after destruction of the cells. Thus red blood corpuscles of birds form hardly any ammonia as long as the cells are intact and the medium contains oxygen and sugar; but laked cells, or cells which have been deprived of their substrates for energy-giving reactions, form ammonia. A difference between intact and ground tissues as regards ammonia formation has been demonstrated for muscle by Embden *et al.* [1928] and by Parnas and Mozołowsky [1927], for brain by Schwarz and Diebold [1932] and by Riebeling [1934]. I find it also in kidney, testicle and intestinal wall.

The precursors of the ammonia derived from anaerobic sources in tissues are not all known. In some tissues, adenylic acid seems to be the chief precursor. In blood (see Klisiecki and Heller [1935]), brain and other tissues the amount of adenylic acid is too small to account for the total ammonia formed.

Other possible sources of ammonia formed anaerobically are asparagine and glutamine. I have used the inhibitory effect of glutamic acid on glutaminase to decide whether some of the ammonia formed in mashed tissue is due to the action of glutaminase ("brain type"). Brain, retina and kidney (pig and guinea-pig) were divided into two equal fractions; the first fraction was ground with water, the second with $M/50$ $l(+)$-glutamic acid. The same amount of ammonia was found however in each fraction after incubating the tissues and the extracts at 38° at p_H 8·5 or 7·4. Hence we may conclude that glutaminase ("brain type") is not concerned with spontaneous ammonia formation in the tissues examined.

9. *Effect of* $l(+)$*-glutamic acid on the formation of ammonia in brain and retina.*

Slices of brain tissue or retina, when suspended in saline, produce considerable amounts of ammonia (for references see Dickens and Greville [1933]. $l(+)$-glutamic acid reduces the output of ammonia in brain and retina as shown in

Table XXIX. In some experiments no ammonia at all could be detected when $l(+)$-glutamic acid was present; but instead of ammonia amide-nitrogen was found. The sum of ammonia *plus* amide-nitrogen is approximately the same in

Table XXIX. *Formation of ammonia and amide-nitrogen in brain and retina in the presence of* $l(+)$*-glutamic acid.*

Tissue	Substrates added	Q_{NH_3}	$Q_{\text{Amide-N}}$	$Q_{NH_3}+Q_{\text{Amide-N}}$
Guinea-pig brain	—	1·68	0·71	2·39
,,	$M/50$ $l(+)$-glutamic acid	~0	2·67	2·67
Guinea-pig brain	—	1·62	0·25	1·87
,,	$M/50$ $l(+)$-glutamic acid	0·21	1·48	1·69
Guinea-pig brain	$M/70$ glucose	0·19	0·44	0·63
,,	$M/70$ glucose + $M/50$ $l(+)$-glutamic acid	0·02	1·03	1·05
Rabbit brain	—	1·52	0·67	2·19
,,	$M/100$ $l(+)$-glutamic acid	0·83	1·63	2·46
Cat brain	—	1·51	~0	1·51
,,	$M/50$ $l(+)$-glutamic acid	0·70	0·76	1·46
Pig retina	—	0·63	0·18	0·81
,,	$M/50$ $l(+)$-glutamic acid	~0	1·34	1·34
Pig retina	—	1·20	0·59	1·79
,,	$M/50$ $l(+)$-glutamic acid	0·35	1·46	1·81
,,	$M/70$ glucose	0·99	0·86	1·85
,,	$M/70$ glucose – $M/50$ $l(+)$-glutamic acid	~0	1·12	1·12
Ox retina	—	1·25	0·33	1·58
,,	$M/50$ $l(+)$-glutamic acid	0·14	0·77	0·91
,,	$M/90$ glucose	0·43	0·33	0·76
,,	$M/90$ glucose – $M/50$ $l(+)$-glutamic acid	0·28	0·67	0·95

the presence as in the absence of $l(+)$-glutamic acid. Thus whilst glutamic acid causes a change in the ratio $\frac{\text{ammonia}}{\text{amide-nitrogen}}$ it does not increase the sum ammonia *plus* amide-nitrogen. This is remarkable seeing that glutamic acid causes an increase in the oxygen uptake of brain and retina [Krebs, 1935].

The non-natural $d(-)$-glutamic acid does not inhibit the formation of ammonia in brain. This shows that the "inhibition" of ammonia formation by $l(+)$-glutamic acid is not due to inhibition of glutaminase, since glutaminase is equally affected by the stereoisomerides. The "inhibition" must be explained by the secondary disappearance of the ammonia through the synthesis of glutamine.

Warburg *et al.* [1924] and Dickens and Greville [1933] have found that slices of kidney, brain, and retina form less ammonia in the presence of glucose than in glucose-free saline. But glucose, unlike glutamic, acid does not increase the yield of amide-nitrogen (see the last experiment in Table XXIX).

10. *The fate of glutamine in brain and retina.*

When the amount of amide-nitrogen which is formed in brain or retina is compared with the amount of ammonia which has disappeared (see Table VIII) it will be seen that, in some cases, more ammonia is used than amide-nitrogen is found. The deficit is very considerable in some experiments on retina. Two explanations for this deficit suggest themselves: either ammonia may be used for another reaction, or a part of the glutamine formed may disappear by a secondary reaction. The following experiments favour the second explanation.

Retina was suspended in glutamine-containing saline at p_H 7·4. After incubating for one or two hours the tissue was well washed and the washings were

added to the experimental solution. Ammonia and amide-nitrogen were then determined in the solution. Only a small amount of ammonia was found, hardly more than in controls to which no glutamine was added. The amide-nitrogen on the other hand was considerably decreased (Table XXX). The sum of ammonia

Table XXX. *Disappearance of glutamine from retina.*

For each experiment 3 ml. bicarbonate saline, 0·4% glucose, 5% CO_2 in O_2; 35·0°. The deficit shown in the last column has not been corrected for blanks and therefore represents a minimum value.

Animal	Tissue mg.	Glutamine added μl.	Time min.	NH_3 formed μl.	Amide-N found μl.	Deficit μl.
Sheep	33·95	162	100	3·5	122	36·5
,,	24·48	610	120	22·0	544	44·0
,,	16·54	212	120	29·0	132	51·0
,,	30·0	308	120	16·5	154	137·0
Pigeon	7·0	141	120	12·0	96	33·0

and amide-nitrogen as compared with the added glutamine shows a marked deficit. These results indicate that the tissue utilises glutamine but not by splitting it into glutamic acid and ammonia. The nature of the products of the conversion of glutamine remains to be investigated.

11. *Some experimental details.*

The methods used in this paper—tissue slices, manometric procedure, determination of ammonia—were essentially the same as in the preceding paper [Krebs, 1935]. Certain additional details are given in this section.

Glutamine was prepared according to Schultze and Winterstein from sugar beet or mangold wurzels [see Vickery *et al.*, 1935]. In the beginning of this investigation, I had at my disposal a large sample prepared by Dr N. W. Pirie.

Preparation of dl-*glutamic acid.* 50 g. of *l*(+)-glutamic acid were heated in an oil-bath for 50 min. at 170–180° and for 50 min. at 220°. The *dl*-pyrrolidonecarboxylic acid formed was hydrolysed with 3 times its weight of concentrated hydrochloric acid for 4 hours at boiling temperature. The *dl*-glutamic acid was then isolated in the usual way. Yield 17 g. recrystallised *dl*-glutamic acid. $[\alpha]_D + 1{\cdot}84°$ (in 2*N* HCl); amino-N 9·85%. This procedure is the method of Abderhalden and Kautsch [1910], except for the time of heating. The racemisation was very incomplete when the directions of Abderhalden and Kautsch were followed.

Preparation of d(−)-*glutamic acid.* 10 g. *dl*-glutamic acid were fermented with yeast according to Ehrlich [1914]. Yield 1·6 g. recrystallised *d*(−)-glutamic acid. $[\alpha]_D - 34{\cdot}6°$ (0·1936 g. in 20 ml. 2*N* HCl). Amino-N 9·43%.

Solutions. "Phosphate saline" and "bicarbonate saline" were made up as described previously [Krebs, 1933, 1]; 2–4 ml. were used for each flask. All substrate solutions which were added to the experimental fluid were neutralised with sodium hydroxide before they were added; ammonia was added as ammonium chloride.

Determination of amide-nitrogen (*glutamine*). The free ammonia was removed and determined in the apparatus of Parnas and Heller. The ammonia-free solution was transferred to a test-tube, neutralised with 14*N* sulphuric acid and mixed with one-tenth volume of 30% trichloroacetic acid. The volume of the fluid was now measured. The solution was filtered and an aliquot part of the filtrate was acidified with one-tenth of its volume of 50% sulphuric acid, heated for 5 min. in a boiling water-bath (see Table VI), cooled and made alkaline with 50% sodium hydroxide (thymolphthalein). Ammonia was then determined in Parnas's apparatus. Known amounts of glutamine were recovered by this method with an accuracy of 1–2%.

Blanks. Since glutamine solutions decompose spontaneously (though at a very slow rate), blanks were carried out in all experiments on the enzymic hydrolysis of glutamine to measure the spontaneous decomposition. Between p_H 7 and 9 about 0·2–0·4% of the glutamine dissolved

decomposed in 1 hour at 38° as determined by ammonia formation. This figure includes the hydrolysis which may occur during the determination of ammonia.

Tissue extracts were made by grinding the tissue with sand and water and centrifuging. The supernatant fluid was used as "extract".

Units. In order to make the metabolic changes comparable with respiration all substances are expressed in terms of gas volumes. 17 mg. NH_3, or 14 mg. amide-N, or 14 mg. amino-N are taken as equivalent to 22400 μl. at N.T.P.

12. *Discussion.*

A. *Reversibility of the glutamine synthesis.* The synthesis of glutamine in tissues can be reversed *in vitro*, for instance by changing the p_H. However, two facts indicate that the synthesis of glutamine is practically not reversed in the living cell. (1) The hydrolysis occurs in practice only outside the physiological range of p_H. (2) Glutamine disappears from brain or retina without forming ammonia. Thus there appears to be a cycle of ammonia in nervous tissue in which the conversion of ammonium glutamate into glutamine is one step. Nothing is known about the other stages.

B. *Physiological significance of glutaminase in brain and retina.* In a previous section (7 B) it has been shown that one and the same enzyme is probably concerned in the synthesis and the hydrolysis of glutamine in kidney, brain and retina. In the preceding paragraph it is suggested that the glutaminase found in extracts of these tissues is concerned *in vivo* with the synthesis only. It may well be that several hydrolysing enzymes found in tissue extracts are only components of synthesising systems. For instance hippuricase (histozym), or proteolytic and lipolytic enzymes are found in extracts of those tissues known to perform the respective syntheses *in vivo*.

C. *Glutamine synthesis and energy-giving reactions.* If the energy-giving reactions are inhibited (section 5 E) synthesis of glutamine ceases. This makes it evident that the system which synthesises glutamine consists of glutaminase and of an additional factor concerned with the transmission of energy. The transmission of energy results in a change in the thermodynamic equilibrium between ammonium glutamate and glutamine in favour of the latter. If this occurs the enzyme catalyses the attainment of the new equilibrium.

D. *Significance of the "glutamine system".* Nothing definite is known at present about the physiological function of the glutamine synthesis. Certain experiments suggest a connection between the system and the energy-giving reactions: *l*(+)-glutamic acid is the only amino-acid which increases respiration in brain and retina; unlike other amino-acids it inhibits anaerobic lactic acid fermentation. These experiments will be described in a later paper.

Summary.

1. Brain cortex and retina of vertebrates and kidney of rabbit and guinea-pig convert ammonium glutamate into glutamine. Under optimum conditions, kidney synthesises 10–20 % of its dry weight of glutamine per hour, retina 5–7 %, brain cortex 1–2 % (37·5°).

2. The synthesis of glutamine is an endothermic reaction and therefore depends on energy-giving reactions. Respiration supplies the energy in kidney and brain. In retina the energy can be derived from anaerobic lactic acid fermentation.

3. Extracts from those tissues which synthesise glutamine contain a specific enzyme which hydrolyses glutamine to ammonium glutamate ("glutaminase").

4. The synthesis of glutamine and the hydrolysis of glutamine are specifically inhibited by the non-natural *d*(−)-glutamic acid.

5. Some properties of glutaminase are described. Characteristic is the specific inhibition by glutamic acid.

6. Liver of mammals (pig, guinea-pig, rat) contains a glutamine-splitting enzyme which is not inhibited by glutamic acid and shows a p_H optimum different from the optimum of glutaminase from brain, kidney and retina. Both glutaminases ("brain type" and "liver type") are distinct from asparaginase.

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