Distribution of Glutamine and Glutamic Acid in Animal Tissues

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It is generally accepted that glutamine and glutamic acid, apart from serving as structural units in proteins and peptides, play a special role in the metabolism of animals, plants and micro-organisms. A few specific functions have already come to light (see the reviews of Archibald, 1945, 1947), but it is probably correct to say that the chief functions are still unknown. It was thought that a survey of the occurrence of glutamine and glutamic acid in biological material might assist in elucidating the part played by the two substances in metabolism, and the two substances were, therefore, determined in a number of animal tissues. Surveys of the distribution of glutamine have been made by previous workers (Ferdman, Frenkel & Silakova, 1942; Hamilton, 1945), but data on the glutamic acid content of tissues are scanty because, until recently, no specific and convenient methods applicable to small quantities of material were available.

EXPERIMENTAL

The procedure described recently (Krebs, 1948) was used. All tissues except blood were frozen in liquid air as soon as possible, usually within 2 or 3 min. after death. A delay of about 5 min. was unavoidable in the case of sheep brain and of about 20–40 min. in the case of foetal material. A quantity of about 5 g., in some cases less, was weighed out in the frozen state, and crushed in a mortar with 2 vol. of 0·5 N-HCl and washed sand. Blood was collected from fasting hospital patients, mixed with heparin and immediately centrifuged. The plasma (10 ml.) was shaken *in vacuo* after addition of 0·25 ml. N-HCl to remove bicarbonate and CO_2. Two Warburg flasks were used for one analysis, both containing 4 ml. of plasma and 0·3 ml. of 3 M-acetate buffer pH 4·9 in the main compartment and one containing 0·5 ml. of washed bacterial cells in the side arm.

RESULTS

Glutamine and glutamic acid in animal tissues. Results obtained on various tissues, excluding blood and foetal tissues, are shown in Table 1. All tissues examined contained considerable quantities of glutamic acid and in most glutamine was also present. The sum of glutamic acid and glutamine in different samples of the same tissue varied less from animal to animal than the concentrations of the two components. In most tissues the concentration of glutamic acid + glutamine was much greater (*c.* 10 times) than the concentrations in the blood plasma, exceptions being adipose tissue, the crystalline lens and vitreous humour, all tissues whose metabolic activities are in general low. The highest concentrations were found in brain, mammalian heart and spleen (average concentrations between 10 and 15×10^{-6} mol./g. or 146–220 mg./100 g.). Relatively low values (average below 5×10^{-6} mol./g. or 73 mg./100 g.) were found in ovary, thyroid, lung and the tissues already given as having concentrations of the same order as blood plasma. Intermediate values (average between 5 and 10×10^{-6} mol./g. or 73–146 mg./100 g.) were found in the other tissues tested.

The proportion of glutamic acid to glutamine showed consistent differences from tissue to tissue. The mammalian heart was the only material in which glutamine regularly constituted the major part. In all other tissues examined including avian heart, glutamic acid usually predominated, though in varying degrees. In the spleen the ratio glutamic acid/glutamine was 6–10, in brain about 2, in most other tissues variable.

In foetal tissues (Table 2) the sum of glutamic acid and glutamine was generally lower than in the adult tissues, an exception being the lung. The ratio glutamic acid/glutamine was higher in most foetal tissues than in the adult tissue, especially in the heart. Among the foetal tissues thymus showed the highest concentration of glutamic acid.

Human blood plasma. Fifty-four specimens from fasting hospital patients were examined (Table 3). In all normal cases the average content of glutamic acid was 3·47 mg./100 ml. and of glutamine 5·78 mg./100 ml. No major deviations were found in the forty-three pathological specimens. In the group 'infectious diseases', the glutamic acid values seemed to be higher, and the glutamine values lower than in the other groups, but in view of the small number of cases no definite conclusions can be drawn. As in other materials, the concentration of glutamic acid + glutamine showed much less variation than did the concentrations of the two components separately. The average sum of all cases was 8·74 mg./100 ml., of which 42·6 % was glutamic acid and 57·4 % glutamine. The data are in general

Table 1. *Glutamic acid, glutamine and ammonia in animal tissues*

Tissue	Animal	Amounts of substance found (10^{-6} mol./g.) Glutamic acid	Glutamine	Glutamic acid + glutamine	Ammonia
Liver	Sheep	5·45	2·27	7·72	2·68
	Sheep	6·53	0	6·53	2·08
	Sheep	5·47	2·66	8·13	1·21
	Cat	2·86	3·66	6·52	4·08
	Pigeon	6·46	0	6·46	2·68
	Pigeon	5·53	5·93	11·46	2·50
	Pigeon	5·75	5·35	11·10	6·15
Spleen	Sheep	10·60	1·47	12·07	3·39
	Sheep	9·59	1·56	11·15	3·16
	Sheep	10·95	1·01	11·96	3·18
	Cat	9·55	1·38	10·93	3·39
Kidney cortex	Sheep	7·90	1·21	9·11	2·16
	Sheep	4·86	0	4·86	4·24
	Sheep	5·95	0·87	6·82	4·17
	Cat	9·41	1·12	10·53	4·55
Kidney medulla	Sheep	7·00	1·79	8·79	1·96
	Sheep	4·23	2·08	6·31	1·16
	Sheep	7·08	0	7·08	4·59
	Cat	5·30	3·70	9·00	4·19
Brain, grey matter	Sheep	11·1	4·19	15·29	2·71
	Sheep	9·93	3·36	13·29	4·39
Brain, white matter	Sheep	7·01	3·36	10·37	1·56
	Sheep	5·30	3·28	8·58	2·88
Brain, whole	Cat	9·93	5·27	15·20	2·05
	Pigeon	6·16	6·99	13·15	0·93
	Pigeon	13·95	4·52	18·47	5·71
	Pigeon	8·25	5·75	14·00	2·88
	Pigeon	10·28	5·60	15·88	6·06
Lung	Sheep	2·38	1·54	3·92	2·08
	Sheep	3·67	0	2·67	3·74
	Sheep	4·15	0·73	4·88	0·94
	Cat	5·17	1·85	7·02	6·37
	Pigeon	1·67	2·14	3·81	4·08
	Pigeon	3·19	0·80	3·99	4·77
Heart	Sheep	2·99	14·0	16·99	1·79
	Sheep	2·09	11·2	13·29	1·87
	Sheep	1·34	9·25	10·59	1·74
	Cat	5·17	9·89	15·06	8·37
	Pigeon	3·13	5·41	8·54	4·95
	Pigeon	7·80	2·05	9·85	2·08
	Pigeon	6·34	3·64	9·98	6·25
Pancreas	Sheep	4·87	1·94	6·81	2·05
	Sheep	3·01	0	3·01	3·14
	Sheep	5·66	2·41	8·07	3·39
	Pigeon	5·53	7·54	13·07	7·83
	Pigeon	9·56	3·14	12·70	7·20
Skeletal muscle	Sheep	5·58	2·41	7·99	2·50
	Sheep	6·73	7·08	13·81	2·08
	Cat	0·74	5·55	6·29	6·37
	Pigeon	0	3·28	3·28	7·70
	Pigeon	8·15	1·03	9·18	5·08
	Pigeon	3·30	2·48	5·78	6·81
Smooth muscle, gizzard	Pigeon	10·52	3·56	14·08	2·72
	Pigeon	6·69	1·78	8·47	2·61
	Pigeon	2·72	3·90	6·62	4·91
Testis	Sheep	4·28	4·48	8·76	2·48
	Sheep	7·82	2·14	9·96	3·92

Table 1 (*cont.*)

Tissue	Animal	Amounts of substance found (10^{-6} mol./g.) Glutamic acid	Glutamine	Glutamic acid + glutamine	Ammonia
Ovary	Sheep	1·32	1·63	2·95	1·36
	Sheep	4·21	0·58	4·79	1·94
Suprarenal gland (mainly cortex)	Sheep	3·55	1·52	5·07	4·91
	Cow	4·35	0·74	5·09	1·96
Thyroid gland	Sheep	0·80	0·47	1·27	3·52
	Cow	2·28	0	2·28	1·61
	Cow	4·28	0	4·28	2·68
Lymph gland	Sheep	6·09	2·28	8·37	4·35
	Cow	9·18	2·27	11·45	3·01
	Cow	6·60	1·65	8·25	0·40
Gastric mucosa	Sheep	2·70	0·94	3·64	3·18
Duodenal mucosa	Sheep	5·44	1·21	6·65	5·42
	Cat	5·03	3·60	8·63	6·34
Vitreous humour	Sheep	0·87	0	0·87	1·36
Lens	Sheep	1·07	0·58	1·65	0·67
Fat, peritoneal	Sheep	0·62	0·45	1·07	1·23

Table 2. *Glutamic acid, glutamine and ammonia in foetal tissues and placenta*

Tissue	Animal	Approx. age of foetus (weeks)	Amounts of substance found (10^{-6} mol./g.) Glutamic acid	Glutamine	Glutamic acid + glutamine	Ammonia
Liver	Calf	10	3·78	1·03	4·81	1·79
	Calf	30	2·74	3·90	6·64	1·47
	Sheep	6–7	3·68	1·87	5·55	4·42
Brain (whole)	Calf	10	4·76	1·05	5·81	1·56
	Calf	30	8·16	2·61	10·77	2·07
Brain	Sheep	6–7	2·34	2·28	4·62	1·87
Kidney	Calf	10	5·55	0	5·55	1·47
	Calf	30	4·82	0·62	5·44	2·03
Heart	Calf	10	7·85	2·49	10·34	1·16
	Calf	30	4·02	6·36	10·38	2·54
	Sheep	6–7	0·49	0	0·49	5·90
Lung	Calf	10	7·28	0·89	8·17	0·94
Thymus	Calf	10	9·85	0·94	10·79	5·34
	Calf	30	11·30	0·89	12·19	2·81
Placenta	Calf	10	7·30	1·92	9·22	1·63
	Calf	30	6·76	2·03	8·79	1·94
	Sheep	6–7	2·45	2·05	4·50	4·86
	Cat	Almost full term	2·54	5·17	7·71	1·87
Spleen	Calf	30	6·96	0·60	7·56	2·52
Bone marrow, femur	Calf	30	2·01	1·74	3·75	2·94

agreement with the glutamine determinations in blood plasma published by Harris (1943), Archibald (1944) and Prescott & Waelsch (1947), though the present average values are a little lower. The average values for glutamic acid, in contrast, are somewhat higher than those reported by Prescott & Waelsch.

DISCUSSION

Glutamic acid and glutamine in animal tissues. The literature does not contain many data which are comparable with those presented in this paper. Ferdman *et al.* (1942) examined the readily hydrolyzable 'amide nitrogen' in various tissues of the dog, cat, rabbit, pigeon and horse; the fresh tissue was frozen in liquid air, extracted with trichloroacetic acid and the increase of ammonia formed on hydrolysis (5–10 min.; 100°; 5 % sulphuric acid) was determined. Hamilton (1945) heated the picric acid extract of dog tissues to 100° at pH 6·5 for 90 min., and estimated the decrease in 'carboxyl nitrogen' by the ninhydrin method of Van Slyke, Dillon, MacFadyen & Hamilton (1941). The figures obtained by Ferdman *et al.* (1942) and Hamilton (1945) are of the same order as those reported in the present paper.

Table 3. *Glutamine and glutamic acid in human blood plasma*

(The results are expressed, in accordance with the practice of previous authors, as mg./100 ml. plasma. Glutamine is expressed as glutamic acid.)

	Plasma (mg./100 ml.)		
	Range	Mean	S.D.
Normal (11 cases):			
Glutamic acid	1·3– 5·9	3·41	1·39
Glutamine	2·7– 7·8	5·78	1·55
Total	7·6–10·1	9·19	0·84
Diseases of the circulatory system (13 cases):			
Glutamic acid	1·7– 5·3	3·74	1·13
Glutamine	3·5– 8·0	5·60	1·18
Total	6·2–10·6	9·34	1·17
Malignant tumours (5 cases):			
Glutamic acid	1·4– 4·6	2·94	1·12
Glutamine	2·6– 7·6	5·02	2·08
Total	7·2– 9·8	7·96	1·07
Infectious diseases (12 cases):			
Glutamic acid	2·4– 6·6	4·17	1·42
Glutamine	2·0– 6·8	4·05	1·58
Total	6·3–10·2	8·22	1·27
Miscellaneous (13 cases):			
Glutamic acid	0* – 7·2	3·96	2·14
Glutamine	2·0– 9·2	4·64	2·27
Total	5·6–11·4	8·60	2·11

* No glutamine was present in one case of thyrotoxicosis (basal metabolic rate +29 %).

Hamilton (1945) has already pointed out that in cardiac muscle of the dog glutamine contributes 50–60 % of the free total 'carboxyl nitrogen' of the tissue. A comparison of the present data with estimations of the total amino nitrogen in animal tissues (Van Slyke, 1913; Hamilton, 1945) indicates that in most tissues the sum of glutamic acid and glutamine represents 25–60 % of the total amino nitrogen.

Van Slyke (1913) was the first to note that tissues contain 5–10 times more amino nitrogen than blood plasma. Hamilton found a similar proportion for glutamine. The present data show that this is also true for glutamic acid.

The question may be raised whether the glutamic acid found in tissue suspension has, wholly or in part, arisen from glutamine after death, as a result of the action of glutaminase. The ammonia values given in Tables 1 and 2 are in most tissues much lower than the glutamic acid values. This applies especially to the tissues which are known to contain a glutaminase (liver, kidney, brain). Most of the glutamic acid found in these tissues must, therefore, have been preformed.

Ammonia in animal tissues. Many data are available on the ammonia content of blood and of other body liquids, but of the tissues only cardiac and striated muscle have been thoroughly studied, mainly by the schools of Parnas and Embden. As Parnas (1928) has pointed out, it is uncertain whether ammonia found in animal tissues, even in material treated with liquid air, is preformed or arises after death. This has to be borne in mind in the interpretation of results.

SUMMARY

1. The decarboxylase method (Gale, 1945; Krebs, 1948) has been used to determine separately glutamic acid and glutamine in twenty-four different animal tissues (including foetal material), and in fifty-four specimens of human blood plasma.

2. The sum of the concentrations of glutamic acid and glutamine was highest in brain cortex, heart, spleen and thymus (average $10–15 \times 10^{-6}$ mol./g.). Average values below 5×10^{-6} mol./g. were found in ovary, thyroid and lung, and below 1×10^{-6} mol./g. in most other tissues.

3. In the mammalian heart and in blood plasma glutamine was present in greater quantities than glutamic acid. In other tissues glutamic acid predominated as a rule.

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REFERENCES

Archibald, R. M. (1944). *J. biol. Chem.* **154**, 643.
Archibald, R. M. (1945). *Chem. Rev.* **37**, 161.
Archibald, R. M. (1947). *Rev. Euclides, Madrid*, **7**, 251.
Ferdman, D. L., Frenkel, S. R. & Silakova, A. I. (1942). *Biochimia*, **7**, 43.
Gale, E. F. (1945). *Biochem. J.* **39**, 46.
Hamilton, P. B. (1945). *J. biol. Chem.* **158**, 397.
Harris, M. M. (1943). *J. clin. Invest.* **22**, 569, 577.
Krebs, H. A. (1948). *Biochem. J.* **43**, 51.
Parnas, J. K. (1928). *Acta Biol. exp., Varsovie*, **1**, no. 3, 1.
Prescott, B. A. & Waelsch, H. (1947). *J. biol. Chem.* **167**, 855.
Van Slyke, D. D. (1913). *J. biol. Chem.* **16**, 187.
Van Slyke, D. D., Dillon, R. T., MacFadyen, D. A. & Hamilton, P. (1941). *J. biol. Chem.* **141**, 627.

A Method for Determining the Sedimentation Constant of Material of Low Molecular Weight: Studies on Oxidation Products of Insulin

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In a recent paper one of us (Gutfreund, 1948) has shown that insulin molecules probably consist of subunits of molecular weight 12,000; these units polymerize to molecules of weight 48,000 in neutral solutions of moderate insulin concentration (0·5–1%). The mean molecular weight is, however, dependent upon pH, temperature and concentration. From the determination of terminal amino groups of insulin, Sanger (1945) suggested that these submolecules, of molecular weight 12,000, are made up of four peptide chains bound together by disulphide linkages. Two of these chains have terminal glycyl residues and two have terminal phenylalanyl residues. Sanger (1947) has shown that the disulphide linkages can be split by oxidation with performic acid, without affecting any other part of the insulin molecule. Some preliminary studies on the peptides resulting from this oxidation of insulin have been reported by Sanger (1947).

It was the purpose of the work described in this paper to develop methods for the determination of sedimentation constants (S_{20}) less than 1×10^{-13}, and to study fractions from oxidized insulin both by ultracentrifugal sedimentation and by diffusion. Sanger (1947) suggested that the peptides obtained on oxidation of insulin should have a molecular weight of about 3000. This value is between the ranges of molecular weights which have been studied by methods suitable for macromolecules (osmotic pressure, sedimentation and diffusion, etc.) on the one hand, and those used for simpler compounds (freezing-point depression and similar procedures) on the other hand. It was necessary to modify the procedure of computing sedimentation constants to make it useful for the purpose of studying these polypeptides.

EXPERIMENTAL

Ultracentrifugal examinations were carried out in a Svedberg oil turbine ultracentrifuge, and the Philpot (1938) optical system was used for the observation of the boundaries. The speed of the centrifuge was about 1010 r.p.sec. Diffusion constants were determined by the method of Coulson, Cox, Ogston & Philpot (1948).

Dr F. Sanger kindly prepared for us all the oxidized insulin and fractions thereof used in this work. The material was prepared and fractionated as described by Sanger (1949). Crystalline zinc insulin (obtained from Boots Pure Drug Co. Ltd.) was used as starting material. Two fractions (*A* and *B*) were examined. Fraction *A* contains the peptides with terminal glycyl residues while fraction *B* contains those with terminal phenylalanyl residues. The purity of each fraction, as shown by end-group assay, was about 95%.

It was found that oxidized insulin diffused slowly through collodion or cellophan membranes on dialysis; as it was essential to get the solutions of oxidized insulin into equilibrium with a salt solution of known composition, these were dialyzed against a large volume of M-Na_2HPO_4. Up to half the nitrogenous material was lost from the solutions.