Studies on the Glutamine and Glutamic Acid Content of the Rat Brain during Insulin Hypoglycaemia

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There is good experimental evidence that the brain *in vivo* normally derives its energy for functional activity from the oxidation and glycolysis of glucose. During a physiological emergency such as insulin hypoglycaemia, when the supply of glucose is limited, it is likely that the brain will also oxidize other metabolites. Thus the consumption of oxygen by the brain in insulin coma is higher than would be necessary for the oxidation of the glucose removed from the blood during its passage through the brain (Kety, Woodford, Harmel, Freyhan, Appel & Schmidt, 1948). It is well known that one of the substances oxidized by brain tissue *in vitro* is glutamic acid (Weil-Malherbe, 1936). The present investigation was designed to determine whether glutamic acid can be utilized in the brain *in vivo* during insulin hypoglycaemia. It was found that in hypoglycaemic rats the glutamic acid content of the brain was reduced below the normal level, suggesting that oxidation of this substance does indeed occur.

The passage of glutamic acid from the blood into the brain seems to be a slow process, for it has recently been shown that the injection of glutamate into animals has no measurable effect on the level of glutamic acid in the brain (Waelisch, 1949). Moreover, the changes in the electroencephalogram produced by progressive hypoglycaemia are not abolished by glutamate injection, although restoration to the normal pattern is quickly brought about by glucose (Maddock, Hawkins & Holmes, 1939). Recent work has demonstrated that patients in insulin coma can be restored to consciousness by glutamic acid injection (Mayer-Gross & Walker, 1949). It has, however, been shown that this action of glutamic acid is due to adrenergic stimulation, and not to a direct utilization of the injected glutamate by the brain cells (Weil-Malherbe, 1949).

METHODS

Young rats of the Wistar albino strain, fed on a normal mixed diet, were used throughout the investigation. They were killed by decapitation, and the heads immediately immersed in liquid oxygen. The frozen whole brain was dissected out, powdered in a previously cooled crusher, and then introduced into a weighed, stoppered centrifuge tube containing 3.8 ml. of 0.5N-HCl. After weighing and centrifuging, the free NH_3 present in a sample of the supernatant solution was

estimated without delay, as even at room temperatures glutamine in the extract would slowly decompose, producing NH_3 . Experiments in which the extraction was done with trichloroacetic acid, which was then removed with ether, were unsatisfactory, owing to interference with the enzymic determinations described below.

Glutamine and glutamic acid determinations. These substances were estimated by the method of Krebs (1948), using the glutaminase and glutamic acid decarboxylase contained in a special strain of *Clostridium welchii*. The organisms were grown anaerobically in a tryptic digest of casein, as recommended by Gale (1945). It was found that a washed suspension of bacteria, when frozen solid, could be stored for 3 months without appreciable deterioration of its enzymic properties. The CO_2 evolved from a 2 ml. sample of the supernatant solution (see above) was measured in the Warburg apparatus. The acetate buffer used by Krebs (1948) was replaced by a citric acid- Na_2HPO_4 buffer of the same pH, as it was found that the volatility of the acetic acid interfered with the micromethod of NH_3 determination used in these investigations. NH_3 was estimated by the microdiffusion method of Conway (1947), with the modifications used by Richter & Dawson (1948). Samples (1 ml.) of the brain extract and of the final Warburg solution were used, respectively, for the determination of free NH_3 and the NH_3 produced by glutaminase activity. A correction for the hydrolysis of glutamine during the microdiffusion was applied in the determination of free NH_3 . A comparison between the values obtained for the glutamine content of brain, as determined by enzymic and hydrolytic methods (Richter & Dawson, 1948), showed that these two methods give practically identical results. The water content of the brain was assumed to be 80%.

Protein and amino-acid nitrogen determinations. The frozen and powdered brain was extracted with 10% (w/v) trichloroacetic acid, and after centrifuging the precipitate was washed with 10 ml. of 5% trichloroacetic acid, followed by 10 ml. of water. The residue was then dehydrated with acetone and extracted three times with 10 ml. of boiling lipid solvent (25% ether, 75% ethanol, v/v). After drying at room temperature the precipitate was hydrolysed by refluxing for 24 hr. with 10 ml. of 20% (w/v) HCl. Control observations showed that hydrolysis for a longer period liberated no more free amino-acid nitrogen. The hydrolysate was slowly evaporated down to about 2 ml. and the solution then made up to 10 ml. with water. A direct 2 ml. sample was assayed for glutamic acid. By running L-glutamic acid standards in the series it was possible to calculate a correction for the slow racemization to the D-isomer during the hydrolysis; the latter isomer is not decarboxylated by the enzyme. After suitable dilution the NH_3 produced was estimated by the Conway (1947) procedure; it represented

the protein amide nitrogen, together with small amounts of NH_3 produced by the hydrolysis of serine and threonine (Rees, 1946) and possibly nucleic acids (Markham & Smith, 1949). Amino-acid nitrogen was determined in the trichloroacetic acid extract and in the protein hydrolysate by the colorimetric method of Moore & Stein (1948). This was found to give results accurate to about 5%. Glutamic acid was used as a standard, and all results given are referred to this standard. A correction was applied for the colour produced by the NH_3 present in the solutions. As the trichloroacetic acid extracts were kept for some time before analysis, and it is known that glutamine readily decomposes in solution, the extracts were heated for 75 min. at 70° to complete this decomposition (Richter & Dawson, 1948). The glutamine is largely converted into NH_3 and pyrrolidonecarboxylic acid, so that it is not included in the free amino-acid nitrogen figures.

Adrenal demedullation. Removal of the adrenal medulla in young rats was performed by Dr J. Crossland by the method of Evans (1936). The animals were treated with cortical extract and given 1% salt solution to drink for a few days after the operation, during which time regeneration of the damaged cortex occurred. The success of the operation was verified by post-mortem and histological examination.

Hypoglycaemia. Rats were injected intraperitoneally with 12–60 units/kg. of pure insulin (Burroughs Wellcome) and allowed no further access to food. They were killed at times up to 2 hr. after injection; the physiological state of the animal at the time of killing was roughly classified as depressed, semi-coma or coma. Control experiments showed that the injection of saline was without effect on the concentration of glutamic acid or glutamine in the brain.

RESULTS

Normal series. The results obtained for the concentration of glutamic acid and glutamine in the normal rat brain (Table 1) were in good agreement

with those of Waelsch (1949), who used a chemical method of estimation, and also with those of Krebs, Eggleston & Hems (1949), who used the decarboxylase method on sheep, cat and pigeon brain. The recorded ammonia content of the brain was much higher than the true *in vivo* level due to the rapid liberation of ammonia following decapitation (Richter & Dawson, 1948). Estimations carried out on brains which were not frozen in liquid oxygen until some minutes after decapitation showed that the glutamic acid and glutamine levels were not significantly changed, although the free ammonia content had increased (Table 1). This showed that post-mortem protease activity does not liberate free glutamine or glutamic acid immediately after death, unless it is assumed that these are removed at an identical rate by other metabolic processes.

Hypoglycaemic rats. It is seen from the results presented in Table 2 that insulin hypoglycaemia caused a reduction in the content of free glutamic acid in the rat brain. The result was statistically significant ($P < 0.01$ when tested by Student's 't' test). Although in the series of hypoglycaemic rats the mean glutamine level in the brain was slightly higher than that of normal rats, the difference was not significant.

It was possible that an extract of hypoglycaemic brain could in some way interfere with the enzymic assay of glutamic acid, but further experiments showed that: (1) insulin in high concentrations had no effect on the recovery of carbon dioxide from glutamic acid or glutamine standards; (2) the bacterial suspension would not ferment glucose, and the lowering was still observed when glucose was

Table 1. *Ammonia, glutamine and glutamic acid content of the normal rat brain*

Wt. of rat (g.)	Sex	Ammonia (mg. NH_3 / 100 g. tissue)	Glutamine (mg. glutamic acid/100 g. tissue)	Free glutamic acid (mg./100 g. tissue)	Total non-protein* glutamic acid (mg./100 g. tissue)	Remarks
108	F	0.8	82	144	226	—
44	M	0.8	74	165	239	—
99	M	—	64	152	216	—
99	M	1.0	62	144	206	—
156	F	1.0	63	140	203	—
122	M	1.2	61	153	214	—
62	M	0.6	71	155	226	—
57	M	0.8	84	156	240	Starved 12 hr.
114	M	1.3	76	162	238	—
48	M	1.4	93	193	286	Starved 12 hr.
44	M	1.3	87	163	250	Starved 8 hr.
Mean		1.0	74	157	231	
56	M	2.0	78	132	210	Starved 8 hr.; 5 min. post-mortem†
68	M	1.6	75	125	200	Starved 12 hr.; 5 min. post-mortem†
68	M	1.8	83	148	231	7 min. post-mortem†
54	M	1.8	76	153	229	Starved 12 hr.; 4 min. post-mortem
32	M	2.0	65	160	215	Starved 12 hr.; 4 min. post-mortem
Mean		1.8	75	142	217	

* Excluding glutamic acid combined in peptides.

† Brains were removed before freezing.

Table 2. *Ammonia, glutamine and glutamic acid content of rat brain during insulin hypoglycaemia*

(Insulin 12-60 units/kg., depending on age and period of food deprivation, injected intraperitoneally.)

Wt. of rat (g.)	Ammonia (mg. NH ₃ /100 g. tissue)	Glutamine (mg. glutamic acid/100 g. tissue)	Free glutamic acid (mg./100 g. tissue)	Total non-protein glutamic acid (mg./100 g. tissue)	Remarks
90	1.2	75	113	188	Depressed
78	1.3	63	102	165	In convulsions
96	1.0	68	112	180	Semi-coma
80	0.8	79	98	178	Coma
64	0.9	94	130	224	Depressed
104	1.0	82	115	197	Depressed
48	1.0	107	123	230	Semi-coma. Starved 12 hr. previously
56	1.3	71	128	199	Semi-coma. Starved 8 hr. previously
52	1.0	44	108	152	In convulsions. Starved 8 hr. previously
52	0.9	79	120	199	Coma. Starved 12 hr. previously
Mean	1.0	76	115	191	

Table 3. *Effect of glucose feeding on the ammonia, glutamine and glutamic acid content of the brain in normal rats and in rats injected with insulin*

Wt. of rat (g.)	Ammonia (mg. NH ₃ / 100 g. tissue)	Glutamine (mg. glutamic acid/100 g. tissue)	Free glutamic acid (mg./100 g. tissue)	Total non-protein glutamic acid (mg./100 g. tissue)	Remarks	
40	0.9	82	150	232	Rats fed on glucose only for 22 hr.	
50	0.9	79	147	226		
54	0.6	71	184	255		
66	0.5	84	162	246		
Mean	0.7	79	161	240		
56	0.8	76	160	236	Rats injected with insulin 30 units/kg. and allowed access to glucose	Killed 2 hr. later
37	0.6	71	144	215		Killed 1.5 hr. later
37	0.5	68	141	209		Killed 2 hr. later
50	0.4	83	158	241		Killed 1.5 hr. later
Mean	0.6	74	151	225		
60	0.9	70	128	198	In insulin coma. Revived by glucose injection and killed	

added to an extract from hypoglycaemic brain; (3) the recovery of glutamic acid added to an extract from hypoglycaemic brain was quantitative. The lowering of the glutamic acid level could either occur through a direct action of insulin, or as a result of the associated hypoglycaemia. In order to test these alternatives, rats were injected with a large dose of insulin, and allowed to eat glucose; they showed none of the usual signs of the hormone administration, and were killed 1.5-2 hr. after injection. The results given in Table 3 show that in these rats the level of glutamic acid in the brain was within normal limits. As it can be expected that the glucose ingested by the rats would markedly reduce the degree of hypoglycaemia, the lowering of the glutamic acid level in the brains of animals treated with insulin must therefore be associated with the resultant hypoglycaemia. The results presented in Table 3 show also that the feeding of rats for 22 hr. solely on glucose produced no change in the con-

centration of glutamic acid and glutamine in the brain.

Davis & Van Winkle (1934) have demonstrated that the well defined lowering of the blood amino-acid level by insulin is a result of the secretion of adrenaline caused by the hypoglycaemia. It was therefore desirable to know whether this mechanism was in any way connected with the lowering of the glutamic acid level observed in the brain. Estimations were therefore carried out on the brains of rats after adrenal demedullation, with and without insulin administration. The results (Table 4) showed that insulin still produced a lowering of the glutamic acid level in the brains of these rats. The secretion of adrenaline produced by the hypoglycaemia cannot therefore be responsible for the change observed.

Although it is known that glutamic acid is the only amino-acid oxidized by brain tissue, some of the other amino-acids are connected with glutamic acid through the transamination system which is present

Table 4. *Ammonia, glutamine, and glutamic acid content of the brain in rats after adrenal demedullation*

Wt. of rat (g.)	Time since operation (days)	Period of starvation (hr.)	Ammonia (mg. NH ₃ /100 g. tissue)	Glutamine (mg. glutamic acid/100 g. tissue)	Free glutamic acid (mg./100 g. tissue)	Total non-protein glutamic acid (mg./100 g. tissue)	Remarks
52	14	12	1.0	66	161	227	—
58	8	12	0.7	63	157	220	—
45	8	8	0.7	63	152	215	—
Mean			0.8	64	157	221	
Hypoglycaemic* rats							
55	14	12	0.6	73	111	184	Semi-coma
52	8	12	1.1	56	111	167	In convulsions
52	8	8	0.6	63	124	187	Semi-coma
Mean			0.8	64	115	179	

* Insulin 25 units/kg. injected intraperitoneally.

Table 5. *Protein and free amino-acid nitrogen content of the brain in normal and hypoglycaemic rats*

Wt. of rat (g.)	Free amino-acid N (mg./100 g. tissue)	Protein hydrolysate NH ₃ (mg./100 g. tissue)	Protein glutamic acid (g./100 g. tissue)	Protein amino-acid N (g./100 g. tissue)	Remarks
Normal rats					
50	39.7	146	1.39	1.02	—
48	41.9	144	1.39	0.98	—
48	44.0	139	1.26	0.96	—
48	47.1	130	1.32	0.88	—
29	44.8	123	1.37	0.95	—
52	—	145	1.36	0.90	—
Mean	43.5	138	1.35	0.95	
Hypoglycaemic rats					
56	37.2	142	1.34	0.90	Semi-coma
49	40.3	132	1.34	0.92	Semi-coma
46	44.0	140	1.38	0.98	Coma
29	40.8	138	1.32	0.92	Semi-coma
52	—	146	1.38	0.88	In convulsions
60	38.0	—	—	—	Coma
Mean	40.1	140	1.35	0.92	

Table 6. *Ammonia, glutamine and glutamic acid content of the brain in rats injected with sodium L-glutamate*

(Sodium L-glutamate solution (1 ml. of 20%) injected intraperitoneally.)

Wt. of rat (g.)	Time after injection (min.)	NH ₃ (mg./100 g. tissue)	Glutamine (mg. glutamic acid/100 g. tissue)	Free glutamic acid (mg./100 g. tissue)	Total non-protein glutamic acid (mg./100 g. tissue)
140	5	0.8	71	165	236
114	12	1.5	82	139	221
110	15	0.9	65	167	232
98	30	0.9	74	153	227
Mean		1.0	73	156	229

in brain (Cohen & Hekhuis, 1941). Estimations were therefore made of the free amino-acid nitrogen present in normal and hypoglycaemic rat brains to determine whether the lowering of the glutamic acid level was reflected in changes in the other amino-acids. The results in Table 5 indicate that although the free amino-acid nitrogen level was slightly reduced in the brains of hypoglycaemic rats, the reduction could wholly be accounted for by the observed lowering of the glutamic acid level.

As the bulk of the glutamic acid present in brain is stored in the proteins, determinations were made of the glutamic acid, ammonia and amino-acid nitrogen produced by acid hydrolysis of these proteins. No difference was observed in the results of estimations made on normal and hypoglycaemic rat brains (Table 5).

The effect of glutamic acid injection on the glutamic acid and glutamine content of rat brain was also investigated. The results presented in

Table 6 confirm those of Waelsch (1949), who showed that the injection of glutamic acid has no effect on the level of these substances in the rat brain.

DISCUSSION

The present results clearly indicate that during insulin hypoglycaemia the level of free glutamic acid in the rat brain is reduced. This loss of some of the glutamic acid from the brain could be caused either by metabolic utilization or by the passage of glutamic acid from the brain to the blood. Stern, Eggleston, Hems & Krebs (1949) have recently shown that slices of brain cortex are able to withdraw L-glutamic acid from a saline medium containing glutamate, providing glucose, lactate or pyruvate are also present in the medium. This transport of glutamic acid occurs against a concentration gradient of this substance, the necessary energy being derived from the oxidation of glucose, lactate or pyruvate. How far this mechanism would apply to the hypoglycaemic brain *in vivo* it is difficult to say, but even when the animal is in convulsions and the blood-sugar level is approaching zero there is still a considerable concentration gradient of glutamic acid between the brain and the blood. It is known that the permeability of the membrane of the brain cells is different from that of the blood-brain barrier which is highly impermeable to anions. In the same investigation Stern *et al.* (1949) show that the amount of glutamate metabolized in brain tissue is reduced by the presence of oxidizable substrates, such as lactate, pyruvate and glucose. In a hypoglycaemic emergency the amounts of these substances present in the brain are considerably reduced, and one would therefore expect glutamic acid to be oxidized. If this oxidation does occur, the ammonia produced must in some way be metabolized or pass out of the brain, for the ammonia content of the brain does not increase during insulin hypoglycaemia. It is possible that ammonia is detoxicated in the brain by conversion to glutamine, which can act as an ammonia carrier between the tissues. The blood-brain barrier is relatively permeable to glutamine (Waelsch, 1949). The synthesis of glutamine requires energy, and it is therefore difficult to say whether it takes place in the brain during hypoglycaemic conditions. It is known that the brain maintains its glutamine level in a variety of physiological states (Richter & Dawson, 1948), and insulin coma appears to be no exception.

The amount of energy derived from the oxidation of the glutamic acid which disappears from brain tissue during insulin hypoglycaemia would be small compared with the total energy requirements of the brain. It is interesting, however, to speculate on the physiological effects of a reduction in the brain glutamic acid level. Although the change for the

whole brain is comparatively small, it is not known whether the reduction is in any way anatomically localized, for example in the cortex. Moreover, the results of Stern *et al.* (1949) show that in the absence of metabolic substrates for oxidation during hypoglycaemia there is likely to be a redistribution of glutamic acid between the intracellular and extracellular spaces.

It is known that glutamic acid is able to reactivate the choline acetylase system, present in brain extracts, which synthesizes acetylcholine under anaerobic conditions (Nachmansohn, John & Waelsch, 1943). Although the effect of glutamine is much less, it is not yet clear how specific glutamic acid is in this reactivation. Welsh (1943) has observed that the 'free acetylcholine' present in rat brain is reduced during insulin coma, although other investigators could find no change in the total quantity present in brain (Cortell, Feldman & Gellhorn, 1941).

Small quantities of glutamic acid are also known to have pronounced effects on the glycolysis of brain tissue (Weil-Malherbe, 1938). Another property of glutamic acid recently discovered by Krebs & Eggleston (1949) is its ability to prevent the loss of potassium from slices of brain cortex. It is known that during insulin convulsions potassium is lost from the neurones of the cerebral cortex (Colfer, 1947). In the nerves of certain invertebrates the loss of potassium is a phenomena closely related to the electrical activity of the nerve (Hodgkin & Huxley, 1947).

The observed lowering of the brain glutamic acid level appears to be unrelated to the lowering of the amino-acid level in the blood produced by the injection of insulin. Davis & Van Winkle (1934) have shown that this latter change is not observed after administration of insulin to rabbits from which the adrenal medullas had been removed. Moreover, the reduction of the amino-acid level in the blood after insulin injection appears to be independent of the associated hypoglycaemia, for it occurs when the blood-sugar level is maintained by carbohydrate administration or by adrenaline injection (Luck & Spaulding, 1929; Luck & Morse, 1933). This result, however, is not in agreement with recent work of Bone & Reid (1948), who found that the lowering of blood amino-acid level produced in cats by insulin was prevented by carbohydrate ingestion.

The present investigation has given good evidence that during the physiological emergency of insulin hypoglycaemia the brain metabolizes part of its free glutamic acid for the production of energy.

SUMMARY

1. The content of free L-glutamic acid in the rat brain decreases after insulin administration.

2. The decrease is a consequence of the hypoglycaemia produced by the insulin.

3. The decrease is not related to the release of adrenaline evoked by the hypoglycaemia as it occurs in rats after adrenal demedullation.

4. No change could be detected in the concen-

trations of the other free amino-acids or of the proteins of the brain during insulin hypoglycaemia.

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REFERENCES

- Bone, A. H. & Reid, C. (1948). *J. Physiol.* **107**, 5P.
 Cohen, P. P. & Hekhuis, G. L. (1941). *J. biol. Chem.* **140**, 711.
 Colfer, H. F. (1947). *Epilepsy*, p. 98. Baltimore: Williams and Wilkins.
 Conway, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*. London: Crosby Lockwood.
 Cortell, R., Feldman, J. & Gellhorn, E. (1941). *Amer. J. Physiol.* **132**, 588.
 Davis, B. L. & Van Winkle, W. (1934). *J. biol. Chem.* **104**, 207.
 Evans, G. (1936). *Amer. J. Physiol.* **114**, 297.
 Gale, E. F. (1945). *Biochem. J.* **39**, 46.
 Hodgkin, A. L. & Huxley, A. F. (1947). *J. Physiol.* **106**, 341.
 Kety, S. S., Woodford, R. B., Harmel, M. H., Freyhan, F. A., Appel, K. E. & Schmidt, C. F. (1948). *Amer. J. Psychiat.* **104**, 765.
 Krebs, H. A. (1948). *Biochem. J.* **43**, 51.
 Krebs, H. A. & Eggleston, L. V. (1949). *Biochem. J.* **44**, vii.
 Krebs, H. A., Eggleston, L. V. & Hems, R. (1949). *Biochem. J.* **44**, 159.
 Luck, J. M. & Morse, S. W. (1933). *Biochem. J.* **27**, 1648.
 Luck, J. M. & Spaulding, J. (1929). *Proc. Soc. exp. Biol., N.Y.*, **26**, 631.
 Maddock, S., Hawkins, J. E. & Holmes, E. (1939). *Amer. J. Physiol.* **125**, 551.
 Markham, H. & Smith, J. D. (1949). *Nature, Lond.*, **164**, 1052.
 Mayer-Gross, W. & Walker, J. W. (1949). *Biochem. J.* **44**, 92.
 Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
 Nachmansohn, D., John, H. M. & Waelsch, H. (1943). *J. biol. Chem.* **150**, 485.
 Rees, M. W. (1946). *Biochem. J.* **40**, 632.
 Richter, D. & Dawson, R. M. C. (1948). *J. biol. Chem.* **176**, 1199.
 Stern, J. R., Eggleston, L. V., Hems, R. & Krebs, H. A. (1949). *Biochem. J.* **44**, 410.
 Waelsch, H. (1949). *Lancet*, ii, 1.
 Weil-Malherbe, H. (1936). *Biochem. J.* **30**, 665.
 Weil-Malherbe, H. (1938). *Biochem. J.* **32**, 2257.
 Weil-Malherbe, H. (1949). *J. ment. Sci.* **95**, 930.
 Welsh, J. H. (1943). *J. Neurophysiol.* **6**, 329.

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The observations reported in the previous paper (Dawson, 1950) give evidence that the brain utilizes glutamic acid during insulin hypoglycaemia. This led to the question of whether this effect was specific for the brain or was shown also by other tissues. Experiments were therefore carried out to determine whether the glutamic acid content of the liver was affected by insulin hypoglycaemia. Glutamic acid, with its complex interrelations with other amino-acids, plays a central role in the amino-acid and protein metabolism of the liver. The present results show that during insulin hypoglycaemia there is a pronounced reduction in the concentration of free glutamic acid and glutamine in the liver.

METHODS

Young rats (30–60 g.) were decapitated and the carcass immediately frozen in liquid oxygen. The frozen liver was removed, crushed to a powder, and introduced into 3.8 ml.

of 0.5N-HCl. The subsequent procedure and methods of estimation, etc., were the same as those used on the brain tissue and described in the previous paper (Dawson, 1950).

RESULTS

Preliminary experiments showed that there is an appreciable variation in the glutamic acid and glutamine content of the normal rat liver. It is possible that the levels of these substances in the liver are affected by the protein intake of the animal. Experiments of Dent & Schilling (1949) have shown that during protein digestion the levels of the various free amino-acids in the portal blood are considerably raised. It is also known that after the injection of rats with glutamine or glutamic acid the levels of these substances in the liver are greatly increased (Waelsch, 1949). It was therefore necessary to control dietary factors of this kind in determining whether the concentration of glutamic acid and