The Effect of L-Glutamic Acid and Other Amino-acids in Hypoglycaemia

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While it is generally agreed that glucose is the only substrate able to maintain the function of the brain in intact animals under normal conditions, opinions are still divided on the part played by certain amino-acids and related substances in cerebral metabolism, particularly in the absence of glucose.

In vitro experiments by Quastel & Wheatley (1932) have shown that L-glutamic acid is oxidized by brain tissue in the absence of glucose. This work has been developed by Krebs (1935) with special reference to glutamine and glutaminase. Weil-Malherbe (1936) suggested that L-glutamic acid fulfils a specific function in relation to carbohydrate metabolism in cerebral tissue.

Using eviscerated animals Bollmann & Mann (1931) and Maddock, Hawkins & Holmes (1939) found that the intravenous injection of the substances known to be oxidized by the brain tissue *in vitro* does not maintain the electrical activity of the brain nor relieve the symptoms of hypoglycaemia. Klein & Olsen (1947) have shown that in cats anaesthetized with sodium amytal substances, such as lactate, pyruvate, L-glutamate and succinate, do not pass from the blood to the brain in significant quantity. Conversely, in the normal human subject a ready exchange of lactate and pyruvate takes place between the blood and the brain as demonstrated by Himwich & Himwich (1946).

The work of Nord (1926) should be mentioned here. We are indebted to Dr R. B. Fisher for drawing our attention to this after a preliminary publication of our results (Mayer-Gross & Walker, 1947). Working with rabbits, Nord produced a rise in the blood glucose by intravenous injection of glutamic and aminoacetic acids, an effect which was abolished after adrenalectomy. He concluded that this effect was due to hypersecretion of adrenalin and subsequent mobilization of glucose.

Further evidence of a direct effect of glutamic acid on cerebral function was provided by clinical observations. Price, Waelsch & Putnam (1943) observed that the administration of DL-glutamic acid hydrochloride reduced the number of *petit mal* attacks in epileptics but was without effect upon major seizures. Waelsch & Price (1944) subsequently showed that this effect was due to the L-glutamic acid fraction and not to any shift in the acid-base balance of the blood. Zimmermann & Ross (1944) and Zimmermann, Burgemeister & Putnam (1946), confirming these observations, have claimed a favourable influence of orally administered L-glutamic acid upon the ability of rats to learn a simple maze and upon the intellectual development of defective children. At the same time Unna & Howe (1945) have shown that the vomiting induced by the injection of L-glutamic acid is probably central in origin since it can be prevented by narcosis.

While the results of *in vitro* experiments could not be expected to be in complete agreement with those in the intact animal, the wide differences in the experiments quoted above suggested the desirability of a study of the immediate effect upon the human subject of relatively large quantities of L-glutamic acid. The original observation of Quastel & Wheatley (1932) that L-glutamic acid was only oxidized by brain tissue in the absence of glucose suggested that patients in a state of hypoglycaemic coma would prove to be suitable subjects for these experiments.

EXPERIMENTAL AND RESULTS

The subjects of the experiments were otherwise healthy patients undergoing Sakel's insulin coma treatment for schizophrenia. They had, except where otherwise mentioned, received the optimum dose of insulin to produce hypoglycaemic coma. Coma was defined as a state of unconsciousness from which the patient could not be roused by sensory stimulation; if stimulation provoked any movement, it was of a general nature and in no way local or purposeful. The blood-glucose levels, estimated by the method of King, Haslewood & Delory (1937), varied in coma between 7 and 12 mg./100 ml., the mean being 10 mg./100 ml. The results of this method closely approximate to true glucose values and we found it reproducible to ± 2 mg./100 ml. At the time of the onset of coma 1 mg. of atropine sulphate was injected subcutaneously to minimize the excessive gastric, bronchial and salivary secretion produced by insulin.

Glutamic acid

The results of the oral administration of L-glutamic acid hydrochloride (Waelsch & Price, 1944; Zimmerman & Ross, 1944; Zimmerman *et al.* 1946) suggested that it might be absorbed in sufficient amount from the intestinal canal to serve as a substrate for cerebral metabolism. The substance was first given in hypoglycaemic coma by stomach tube.

No effect upon consciousness could be observed in a series of 10 cases in which quantities of up to 100 g. of L-glutamic acid hydrochloride were administered.

In a further series of 14 experiments 20 g. of L-glutamic acid hydrochloride were added to the 200 g. of sucrose normally administered by stomach tube for the purpose of terminating hypoglycaemic coma; in 11 cases no effect upon the recovery of consciousness could be observed. The patients did not wake up more quickly than after glucose alone. The remaining three experiments, however, carried out on the same subject, did suggest that the L-glutamic acid hydrochloride might have some effect. This subject had never been known to recover from coma after the oral administration of sucrose. He recovered only after a subsequent intravenous injection of glucose. In these three experiments the addition of 20 g. of L-glutamic acid hydrochloride to the 200 g. of sucrose had the effect of rendering unnecessary the intravenous injection of glucose, consciousness being restored within 20 min. of the oral administration of the mixture. Without the 20 g. of L-glutamic acid hydrochloride intravenous injection of glucose was invariably necessary.

An injectable preparation of L-glutamic acid was prepared by cautious addition of 50% NaOH (w/v) to a saturated solution of L-glutamic acid hydrochloride. The two solutions were cooled with ice and mixed with constant stirring in a large mortar packed round with crushed ice. The L-glutamic acid at first precipitated was redissolved, and the pH was taken to 7·2 (pH meter). A solution which contained the equivalent of 25 g. of L-glutamic acid and 10–11 g. of NaCl/100 ml. was obtained, and was sterilized by filtration through a Ford 'Sterimat' grade SB.

This preparation (80 ml. ≡ 20 g. L-glutamic acid) was injected intravenously in a series of 45 experiments carried out on 31 patients in hypoglycaemic coma. The injection was made over a period of 2 min. Blood glucose was determined before the injection and at intervals of 3 min. from its termination. The subjects were closely watched for returning consciousness over a period of 15 min., and, if at the end of this period consciousness was not restored, an intravenous injection of glucose was administered. The criteria adopted as signs of consciousness were ability to obey spoken requests and to answer simple questions rationally. In some cases, where speech was delayed, the fact that the patient sat up and swallowed sips of water was considered a sign of consciousness.

In 26 of these 45 experiments the subject became sufficiently conscious to satisfy these criteria while in the remaining 19 cases consciousness was not restored. In many of the latter a considerable decrease in the depth of coma was noticeable, e.g. cessation of hyperventilation and return of normal respiration, disappearance of muscular spasm and twitchings, opening of the eyes, fixation and following the observers' movements, sighing, yawning, stretching, etc.

In all 45 experiments an increase in the blood glucose level was observed. In the 26 subjects recovering consciousness the blood-glucose level at the time of the recovery of consciousness, and in the remaining 19 cases the highest level reached, was noted. These values are set out in columns (1) and (6) of Table 1. The two sets of values are not greatly different from each other, the means being 25 and 22 mg./100 ml. respectively. The significance of the rise of blood glucose will be discussed later. There was the possibility that the large quantity of sodium chloride contained in the injected preparation of L-glutamic acid could have some effect upon the return of consciousness. For this reason a series of nine identical experiments was carried out in which the comatose subjects were given an intravenous injection of 80 ml. of a solution of sodium chloride containing 11 g./100 ml. In no case was there any effect upon the state of consciousness nor any significant effect upon the blood-glucose level, the highest level reached being 16 mg./100 ml. while the mean was 13 mg./100 ml. The values are set out in column (10) of Table 1.

Aminoacetic acid

The result of the experiments with L-glutamic acid suggested their extension to other amino-acids. Because of its ready availability aminoacetic acid was selected; and an injectable preparation containing 25 g./100 ml. was made, the pH being adjusted to 7·2 by means of a few drops of sodium hydroxide solution. The solution was sterilized by intermittent heating to 100° and stored at 37° as the amino-acid had a tendency to crystallize at lower temperatures.

A series of 26 experiments was carried out with this preparation under similar conditions to those in the experiments with L-glutamic acid, each subject receiving 20 g. of aminoacetic acid. In 13 experiments the patient became sufficiently conscious to satisfy the standard condition while in the remaining experiments this degree of consciousness was not reached although in many cases the coma became considerably less deep. The blood-glucose level at the time of the return of consciousness or, alternatively, the highest level reached in the time of the experiment was noted; these figures are set out in columns (2) and (7) of Table 1. The mean values were 24 and 20 respectively.

p-*Aminobenzoic acid*

A further series of experiments was carried out using a solution of the sodium salt of *p*-aminobenzoic acid, each subject receiving 20 g. of the acid. In four of these six experiments consciousness was restored while the remaining two did not reach this stage. The blood glucose values of this short series are set out in columns (3) and (8) of Table 1, the mean values being 22 and 24 mg./100 ml. respectively.

The significance of the rise of blood glucose

The result of these four series of experiments suggested that the injection of 20 g. of the amino-acid had been responsible for the return to consciousness of the subjects. The mechanism of this result was, however, in some doubt since the administration of

Table 1. *Relationship of blood glucose values and consciousness after the administration of amino-acids, glucose and sodium chloride*

Consciousness restored: blood glucose values (mg./100 ml.)					Remaining unconscious: blood glucose values (mg./100 ml.)				
Glutamic acid	Glycine	*p*-Amino-benzoic acid	Glucose only, oral	Glucose only, intravenous	Glutamic acid	Glycine	*p*-Amino-benzoic acid	Glucose oral and intravenous	NaCl
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
21	23	28	55	53	21	21	23	36	9
16	32	19	54	55	19	14	25	25	12
28	25	23	51	37	26	20	—	35	16
26	23	19	30	32	21	23	—	42	12
23	25	—	37	35	30	26	—	46	14
28	28	—	40	53	25	17	—	23	12
28	19	—	39	48	35	19	—	19	12
32	28	—	35	42	30	19	—	24	12
35	28	—	37	35	17	23	—	22	16
25	21	—	37	37	23	19	—	30	—
34	16	—	34	40	25	27	—	19	—
37	21	—	—	37	32	21	—	34	—
28	19	—	—	—	30	16	—	25	—
23	—	—	—	—	16	—	—	35	—
25	—	—	—	—	16	—	—	19	—
21	—	—	—	—	14	—	—	27	—
35	—	—	—	—	16	—	—	28	—
25	—	—	—	—	16	—	—	27	—
21	—	—	—	—	14	—	—	35	—
27	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—
Mean:									
25	24	22	42	42	22	20	24	29	13

the amino-acid had always been accompanied by a rise in the blood-glucose level above the average level of 10 mg./100 ml. found in hypoglycaemic coma. The work of Nord (1926) would suggest that the return to consciousness was entirely due to this increase in blood glucose which he proved to be a sympathomimetic effect. On the other hand, Nord had failed to obtain this effect when the quantity of amino-acid injected was less than 2 g./kg.; while in none of our experiments did the quantity of amino-acid exceed 0·4 g./kg. Furthermore, no clinical signs of hyperadrenalism such as a rise in pulse rate or in pulse pressure were seen. Previous investigations by ourselves (Mayer-Gross & Walker, 1945) had shown that consciousness was not restored until a blood-glucose level had been reached that was about double the average figures of 22–25 mg./100 ml. obtained in these experiments. For this reason it seemed probable that the elevation of blood glucose was not solely responsible for the return of consciousness. It seemed desirable, however, to arrange control experiments on an identical pattern in order to determine the critical level of blood glucose at which consciousness was restored.

In the first of two series of experiments 200 g. of sucrose were administered to subjects in hypoglycaemic coma by a stomach tube. Blood-glucose level was determined at 3 min. intervals, and the subject closely watched for the appearance of the signs of consciousness satisfying the previously mentioned criteria. The blood-glucose level at the time of the recovery of consciousness was noted.

In a second series 3 g. of glucose were injected intravenously into patients in coma and similar observations of blood glucose and consciousness were made. Out of a total of 42 experiments consciousness was restored in 23 while in 19 cases the patient remained in coma. The blood-glucose levels at which consciousness was restored are set out in columns (4) and (5) of Table 1, the mean values being 42 in each case. In the 19 cases where there was no restoration of consciousness the highest values determined within 15 min. after the administration of glucose were noted. These values are set out in column (9) of Table 1 the mean value being 29 mg./100 ml.

Statistical analysis of Table 1 shows that there are clearly no significant differences between the means of columns (1), (2) and (3), nor any significant differences

between those of columns (4) and (5). These columns may, therefore, be taken together. The grouped means are, for the amino-acids 24·53 and for the glucose, oral and intravenous, 41·34; the difference is 16·81, the standard error of difference is 1·89 and the ratio of the difference to its standard error 8·9. The chance probability of this is less than 0·001 and the difference between the means is highly significant.

It may, therefore, be concluded that the rise of blood glucose following injection of amino-acids was insufficient, in itself, to produce the return of consciousness. Taking column (9), which gives the blood-glucose values in experiments where consciousness was not restored, the group mean is 29·00, and the difference between this and the grouped mean of columns (1)–(3) is 4·46. The standard error of this difference is 1·96 and the ratio of difference to its error 2·28. The chance probability is less than 0·05 which attains a level of significance commonly accepted for clinical experiments. In other words, even in cases in which consciousness was not restored by glucose, the blood-glucose level was significantly higher than that of the cases in which it had been restored by amino-acids.

Effect of glutamic acid on the blood urea

An attempt was next made to determine whether the glucose responsible for the rise in blood-glucose level, after the injection of L-glutamic acid, was formed by the synthesis of glucose from the amino-acid. If this were so it would be necessary for deamination to occur, and, as a result of this, a rise in the blood-urea level. In 12 experiments in which 20 g. of L-glutamic acid were injected into patients in hypoglycaemic coma, parallel observations were made upon the blood-glucose level, the blood-urea level and upon the state of consciousness. The blood-urea levels were determined by the method of King *et al.* (1937) which we found reproducible to ± 1 mg./100 ml. The results are summarized in Table 2. In eight cases consciousness was restored at an average blood-glucose level of 28 mg./100 ml. with a slight rise in blood urea in four and a slight depression in the other four. Four cases did not recover consciousness, while their mean blood-glucose level rose to 25, in two with a slight rise in the blood-urea level and a slight fall in the remaining two.

Table 2. *Parallel observations on consciousness, blood glucose and blood urea after intravenous injection of 20 g. of L-glutamic acid in hypoglycaemic coma*

Conscious		Unconscious	
Blood glucose (mg./100 ml.)	Rise or fall in blood urea (mg./100 ml.)	Blood glucose (mg./100 ml.)	Rise or fall in blood urea (mg./100 ml.)
35	+2	23	−3
35	+2	25	−3
28	±2	25	+5
23	−3	28	+3
21	+6	—	—
32	−1	—	—
25	−5	—	—
25	−2	—	—
Mean 28		25	

Succinic acid

Succinic acid is one of the substances related to glutamic acid which may be converted in the body into glucose (Soskin & Levine, 1946). The problem was whether this could take place within the 15 min. period of our experiments and affect the hypoglycaemic condition. A solution of succinic acid suitable for intravenous injection was prepared by adding small quantities of succinic acid to a solution of sodium succinate until the pH was reduced to 7·2. The quantities were so adjusted that the final product contained the equivalent of 1 g. of succinic acid/10 ml.

In view of the expectation of very rapid oxidation of the succinic acid by the tissues of the body it was considered necessary to inject the preparation as rapidly as possible. An immediate difficulty was encountered, however, in the violent erythema that accompanied the intravenous injection of the substance, and, for this reason, it was deemed inadvisable to carry out the injection too quickly and to administer more than the equivalent of 10 g. succinic acid.

In a series of 14 experiments no change in the state of consciousness of the hypoglycaemic subject could be seen, nor was there any marked change in the concentration of the blood glucose, the greatest change in the latter being a rise of 5 mg./100 ml.

A number of determinations of the blood-succinic acid concentration were made by the method devised by Forssman (1941). The average concentration obtained was 11·6 mg./100 ml. the highest being 17·2 mg./100 ml. and the lowest 7·3 mg./100 ml. The results are summarized in Table 3.

Table 3. *The effect of intravenous injection of succinic acid upon blood-glucose and blood-succinic acid concentrations of subjects in hypoglycaemic coma*

(Subjects remained in coma.)

No.	Succinic acid injected (g.)	Rise in blood glucose (mg./100 ml.)	Blood succinic acid concentration immediately after injection (mg./100 ml.)
1	2	3	—
2	4	5	—
3	4	3	—
4	8	2	—
5	8	2	—
6	10	2	7·3
7	7	3	10·9
8	12	4	17·2
9	10	4	16·3
10	10	2	11·0
11	10	3	12·2
12	10	5	9·7
13	10	2	8·2
Mean		3	11·6

Individual reactions

Although the amino-acid experiments and glucose controls could not always be performed in the same individuals, parallel experiments and controls were possible in 10 subjects. While in some subjects the impression was gained that easy recovery from coma, or its persistence after injection of amino-acids was peculiar to the individual, critical examination of the experiments as a whole, and of the parallel tests in particular, showed that individual reaction patterns were rare. Success and failure in restoring consciousness seemed to depend mainly on the duration of coma before the administration of the amino-acid.

An interesting phenomenon occurred in one female patient who, in the normal process of recovery from hypoglycaemic coma after the oral administration of sucrose, invariably passed through a transient but violently emotional phase characterized by much weeping. When she was awakened by L-glutamic or aminoacetic acid the emotional phase did not occur. The patient became completely conscious and able to converse intelligently without emotional upset. Nevertheless, upon her subsequently drinking sucrose solution, the emotional reaction developed. This observation was made in four experiments on successive days.

Glutamic acid in the absence of hypoglycaemia

A series of nine experiments was made in which 20 g. of L-glutamic acid were injected intravenously to non-hypoglycaemic (fasting) subjects. The clinical condition and the blood-glucose level of the patient were closely watched for 20 min. after the injection. In spite of adminstration of atropine, as previously described, the vomitive effect of the L-glutamic acid (Unna & Howe, 1945) was invariably present and was much more marked than in the hypoglycaemic subjects. While there was no detectable effect upon the patient's mental state, there was a generalized sensation of tingling associated with the injection, which had always passed off before vomiting occurred. The effect on blood glucose was very variable; it was raised in four cases, depressed in four cases and not significantly changed in one case. The results which are summarized in Table 4 suggest that the observations of Quastel & Wheatley (1932) *in vitro* held true *in vivo* and that L-glutamic acid was not utilized in the presence of glucose.

Table 4. *Blood glucose (mg./100 ml.) before and after intravenous injection of 20 g. of L-glutamic acid to non-hypoglycaemic (fasting) subjects*

Before glutamic acid	After glutamic acid				Maximum rise or fall
	5 min.	10 min.	15 min.	20 min.	
83	83	83	93	83	+10
65	72	70	65	70	+7
79	67	79	77	79	-12
70	79	77	79	77	+9
101	95	98	88	88	-13
90	97	90	95	99	+9
100	89	72	98	95	-28
97	97	99	90	93	-7
82	80	84	82	82	+2

DISCUSSION

The work quoted in the introductory remarks suggests two explanations of the restoration of consciousness after the injection of amino-acids. Neither accounts for all our observations.

In the first place, the reaction of hypoglycaemic subjects could be due to oxidation of L-glutamic acid by the brain in the absence of glucose (Quastel & Wheatley, 1932). Identical reactions obtained with aminoacetic and *p*-aminobenzoic acids make this interpretation doubtful since Krebs (1935) found L-glutamic acid to be the only amino-acid oxidized by brain tissue.

The second explanation traces the effect of amino-acids to the invariable rise in blood glucose. This may be due to (1) general stimulation during injection, (2) conversion of amino-acid to glucose, or (3) sympathomimetic action.

(1) While a small rise of blood glucose may be produced in hypoglycaemic coma by any external stimulation the parallel experiments with 11% sodium chloride solution have shown that this rise is not equal to that produced by amino-acids.

(2) From the absence of change in blood urea, after the injection of glutamic acid and from the absence of a rise in blood glucose after injection of succinic acid, it seems improbable that glucose is derived from the amino-acid injected. Although it is feasible that glutamic and aminoacetic acids are converted into glucose within the body, this is unlikely in the case of *p*-aminobenzoic acid.

(3) Sympathomimetic action (Nord, 1926), although not observed clinically, cannot be ruled out in our experiments and may cause the rise of blood glucose. Whatever the cause of this rise, however, it cannot account for the recovery of consciousness in a large proportion of cases. Control experiments and statistical analysis of Table 1 have shown that it was too small for this purpose. Furthermore, the injection of glutamic acid into non-hypoglycaemic subjects has completely failed to produce the significant rise of blood glucose demonstrated in Nord's experimental animals. Similarly, the vomiting, much more marked without than with hypoglycaemia, points to a different action of glutamic acid in the two conditions. An explanation based only on the glucose rise therefore fails to account for our observations. Presumably, therefore, the amino-acids exert some additional influence on the metabolism of the nerve cell.

The nature of this influence is at present obscure. An excess of simple amino-acids may replace fractions of the protein molecule for some of their

functions thus releasing them for a more specific purpose in relation to carbohydrate metabolism. This might result in more efficient utilization of glucose and so in the recovery of consciousness at a lower level of blood glucose.

SUMMARY

1. Oral administration of L-glutamic acid to patients in hypoglycaemic coma was without effect except in one subject.

2. Intravenous injection of 20 g. of L-glutamic acid restored consciousness to subjects in hypoglycaemic coma in 26 of 45 experiments, and modified the depth of coma in the remaining 19 experiments.

3. Similar effects were produced by the injection of 20 g. of aminoacetic and *p*-aminobenzoic acids.

4. In all cases where amino-acids were injected intravenously into hypoglycaemic subjects there was a rise in blood glucose, which was, however, in itself inadequate for the restoration of consciousness.

5. Injection of L-glutamic acid into subjects in hypoglycaemic coma was without significant effect on blood urea.

6. Injection of succinic acid into hypoglycaemic subjects was without significant effect on blood glucose or on state of consciousness.

7. Intravenous injection of L-glutamic acid into non-hypoglycaemic subjects failed to produce any significant effect on blood glucose, but produced vomiting far more strongly than in hypoglycaemic subjects.

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Studies on the Metabolism of Semen

5. CITRIC ACID IN SEMEN

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The semen of man and certain other higher mammals is distinguished by a very high content of citric acid (Scherstén, 1929, 1936; Dickens, 1941; Huggins & Neal, 1942; Lardy & Phillips, 1945; Barron & Huggins, 1946*a*, *b*; Humphrey & Mann, 1948). The acid originates in the accessory glands of reproduction, chiefly the seminal vesicles, and in this respect it resembles another more recently discovered component of semen, namely fructose, which has similarly been shown to be secreted mainly in the seminal vesicles (Mann, 1946). The present study was undertaken primarily with the object of investigating the possibility that there may exist a link between the two substances with regard to their formation, distribution or function in the reproductive organs and semen. In the course of this study it was established that the process of generation and maintenance in semen of both fructose and