THE ACTION OF GLUTAMIC ACID IN HYPOGLYCAEMIC COMA.*

By H. WEIL-MALHERBE.

Runwell Hospital, Wickford, Essex.

THE loss of consciousness in hypoglycaemia is generally regarded as a direct consequence of the fact that the brain cells are being increasingly deprived of glucose, their principal fuel. The prompt relief of symptoms by glucose administration led to a number of investigations on the effect of other substrates known to sustain the respiration of surviving brain slices *in vitro*. Amongst these are various mono- and disaccharides, and such acids as lactic, pyruvic, succinic or glutamic acid which may be formed from glucose in the course of its metabolism. It appeared, however, that, in contrast to their *in vitro* action, most of these substances, including glutamic acid, were unable to relieve the symptoms of hypoglycaemia in eviscerated or hepatectomized animals (Bollmann and Mann, 1931 ; Maddock, Hawkins and Holmes, 1939). Similarly, lactic and pyruvic acids were found to have no effect on the oxygen consumption of the brain or the comatose state of hypoglycaemic patients undergoing insulin shock therapy (Wortis and Goldfarb, 1940 ; Goldfarb and Wortis, 1941). It has been shown for several substrates, including glutamic acid, that their rate of diffusion from the blood stream into brain tissue was markedly slower than that of glucose, and that therefore the concentration necessary for the maintenance of nervous function was not reached (Klein, Hurwitz and Olsen, 1946 ; Klein and Olsen, 1947). In harmony with this are the observations of Friedberg and Greenberg (1947), and of Waelsch, Schwerin and Bessman (1949) that intravenously injected glutamic acid is not taken up by brain tissue. The differences between the *in vitro* and *in vivo* results seemed to be adequately explained by these experiments.

The demonstration by Mayer-Gross and Walker (1947, 1949) that the coma of some hypoglycaemic patients may be terminated by an intravenous injection of glutamic acid was therefore unexpected. To reconcile it with the facts already known one of the following two assumptions may be made : (1) that there exists a species difference between the experimental animals previously used and the human subject, and that in consequence of this the effective concentration of glutamic acid is either lower, or reached more readily in human than in animal brain ; or (2) that the inactivity of glutamic acid in the animal experiments was due to the absence of the viscera, especially liver and adrenals. In this case the effect would not be due to a direct action of glutamic acid on the brain cells, be it as fuel or in connection with some specific metabolic function of glutamic acid, but would ensue from a primary effect elsewhere, e.g. conversion to glucose in the liver or hormonal stimulation.

* A Paper read at the Quarterly Meeting of the Royal Medico-Psychological Association on 23 February, 1949.

https://doi.org/10.1192/bjp.95.401.930 Published online by Cambridge University Press

A direct utilization of glutamic acid as fuel for the brain cells is unlikely as explanation for the effect. It would be difficult to understand why closely related metabolites such as lactic, pyruvic or succinic acid, all like glutamic acid readily oxidized by brain slices, should not be equally effective. The inactivity of succinic acid, which is in the direct path of oxidation of glutamic acid, has been shown by Mayer-Gross and Walker and confirmed in this hospital. On the other hand, Mayer-Gross and Walker have found that glycine and p-aminobenzoic acid have effects similar to that of glutamic acid, yet there is no evidence that these substances can be utilized by brain cells *in vitro*.

In the same way the absence of any correlation between the activity of a substance and its glycogenic properties argues against the effect being due to the conversion of glutamic acid into glucose by the liver. Moreover, since the liver is the locus of the supposed transformation, feeding of glutamic acid by nasal tube should be even more effective than intravenous injection, yet a number of feeding experiments have shown negative results, confirming similar experiments reported by Mayer-Gross and Walker. Finally, conclusive evidence will be presented showing that the injection of small doses of glutamic acid elicited a rise of blood sugar far in excess of the limits which could have been reached had the glucose originated from the injected glutamic acid.*

The conclusion arrived at in this communication is that the effect of glutamic acid in hypoglycaemic coma is entirely due to adrenergic stimulation. It is based on the following observations:

(1) The injection of glutamic acid in hypoglycaemic coma induced the classical triad of adrenaline action, rise of blood pressure, rise of pulse rate and rise of blood sugar.

(2) The minimum effective dose of glutamic acid was found to be much smaller than the dose given by Mayer-Gross and Walker, suggesting a catalytic mechanism.

(3) Blood taken from hypoglycaemic patients after glutamic acid injection showed increased adrenergic activity compared with blood taken before the injection.

(4) Intravenous injection of adrenaline produced the same effects as glutamic acid. In particular the rise of blood sugar was of the same order of magnitude.

Experimental.

The monosodium salt of glutamic acid was used for injection. To avoid the undesirable admixture of large quantities of NaCl the salt was prepared from free glutamic acid by the addition of the calculated amount of sodium hydroxide solution. The solutions were 25 per cent. with respect to free glutamic acid.

* Mayer-Gross and Walker also cite the absence of change in blood urea as evidence against the conversion of glutamic acid into glucose. This is, however, a doubtful argument, as the absorption of glutamic acid does not lead to increased urea formation (Leuthardt and Glasson, 1946).

Adrenaline was injected in solutions containing 5 mgm. in 100 ml. of saline (1:200,000) at a rate of 1–2 ml. per minute.

Solutions for injection were sterilized by filtration through Seitz filters and stored in sealed ampoules.

Blood sugar was determined in duplicate by the method of Nelson (1944) on 0·2 ml. samples of capillary blood. Samples were collected before and 5 minutes after the injection, in the case of adrenaline 10 minutes after the beginning of the injection.

Consciousness was regarded as fully restored when the patient was able to drink from a cup the full amount of glucose routinely given by nasal tube to terminate the coma (about 1 pint). These cases are denoted in the tables by +. When the patient was sufficiently awake to respond when spoken to, but was unable to drink sufficiently, the case is denoted by (+).

Other experimental details will be described in the following section.

Results.

The effect of intravenous injection of glutamic acid.—Table I contains some typical results obtained with glutamic acid in cases of hypoglycaemic coma. Mayer-Gross and Walker found a positive response in a little over half the injected cases; a similar proportion was found in this hospital. It should be pointed out that, whereas in some cases the response seems to be consistently either negative or positive, it may be variable from day to day in others. This is hardly surprising if, as we believe, the positive response depends on such a variable quantity as the level of mobilizable liver glycogen.

Table I shows that the dose of 80 ml., chosen by Mayer-Gross and Walker, is unnecessarily large. Positive results were obtained with 5 ml. and, in favourable cases, even with 2 ml. Injection of 1 ml., however, had no effect on consciousness, although even with that dose a blood-sugar rise was noticeable. The 2 ml. dose contained 0·5 gm. of glutamic acid, which could, under the most favourable conditions, have yielded no more than about 0·3 gm. of glucose, i.e. assuming that 100 per cent. of the 3-carbon residue of glutamic acid would have been converted to glucose. Yet this dose elicited a blood-sugar rise of 20 mgm. per cent. or more. Assuming a blood volume of 7 l., this corresponds to the appearance of 1·4 gm. of glucose, and probably more if the extracellular tissue fluids are taken into account. The blood-sugar rise is therefore at least 4 times greater than can be accounted for by the amount of glutamic acid injected.

The injection of glutamic acid usually caused a marked increase of blood pressure and—unless it was accelerated even before the injection—of pulse rate. The blood-pressure rise, which sometimes amounted to 50 mm. Hg. or more, was of the typical fleeting form associated with adrenaline action and usually subsided within 1–2 minutes (Figs. 1–4). In the writer's opinion these phenomena were not the result of unspecific stimulation. The observations were extended over a considerable period before injection and, in the majority of cases, the blood pressure did not show any large fluctuations. Muscular spasms sometimes produced a blood-pressure rise, but phases of rigidity were

TABLE I.—*Effect of Intravenous Injection of a 25 per cent. Glutamic Acid Solution on Patients in Hypoglycaemic Coma.*

Patient.	Sex.	Ml. injected.	Blood pressure (mm. Hg.).		Pulse rate/minute.		Blood sugar (mgm. %).		Restoration of consciousness.
			Before injection.	Maximum after injection.	Before injection.	Maximum after injection.	Before injection.	5 minutes after injection.	
B. K—	M.	10	128/65	144/65	70	97	..	..	+
		5	130/78	180/110	72	112	19	38	+
		5	..	..	..	..	10	30	+
		2	130/88	156/90	70	96	12	33·5	(+)
		2	136/90	148/80	84	98	13	27	(+)
		1	130/74	112/68	84	100	9	14	−
L. F—	M.	100	126/66	170/90	68	96	..	..	+
		10	112/78	154/82	72	72	22	43	+
		5	124/78	164/90	72	80	20	38	+
		5	134/86	170/84	78	102	30	53	+
		2	114/67	166/94	80	104	23	44	+
		1	134/80	130/80	80	88	28	34	−
E. S—	M.	100	136/70	180/80	68	84	..	..	−
		10	134/64	176/78	68	80	13	19	−
		5	..	..	..	..	11	23	−
S. C—	F.	5	140/70	200/100	100	112	7	27	+
		2	130/80	165/80	118	130	6	21	−
D. J—	F.	5	120/80	168/110	116	96	14	23·5	+
W. R—	F.	5	115/70	160/100	93	105	11·5	19	(+)
U. R—	F.	5	120/70	150/90	90	104	22	30·5	+
M. S—	F.	5	125/86	140/70	114	120	17	20·5	−

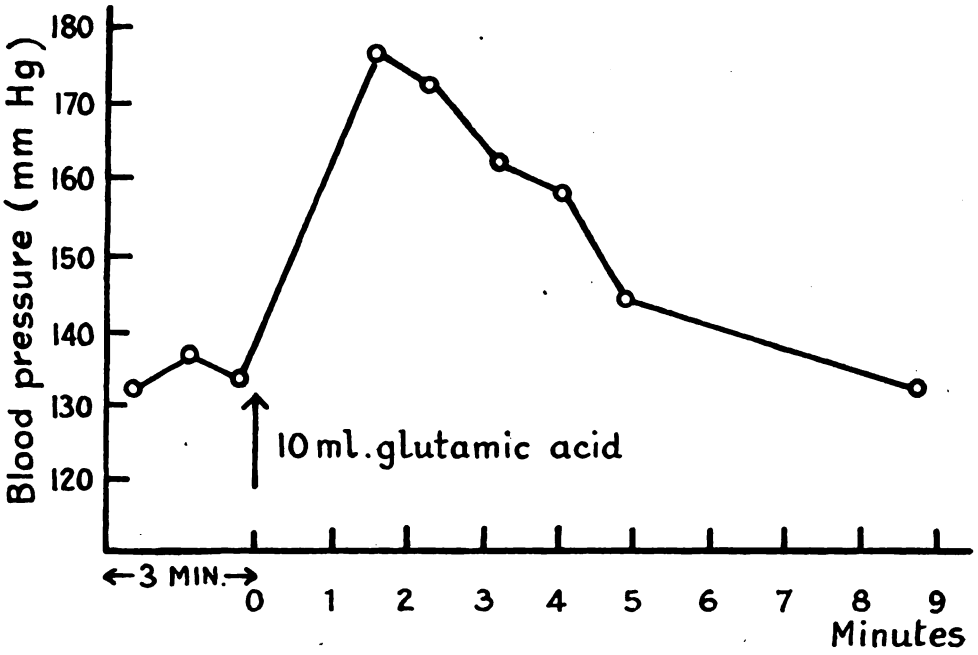


FIG. 1.

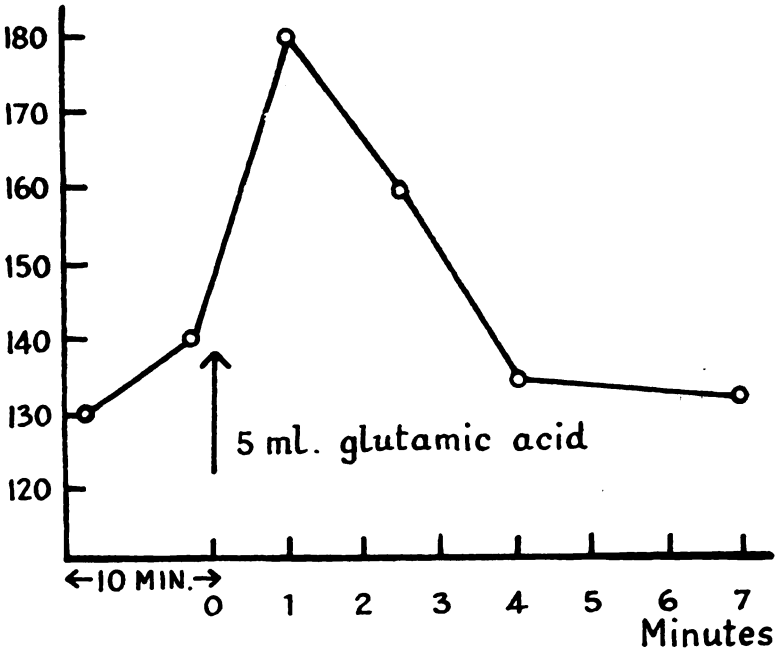


FIG. 2.

FIGS. 1–4.—Effect of glutamic acid injections on the blood pressure of patients in hypoglycaemic coma.

https://doi.org/10.1192/bjp.95.401.930 Published online by Cambridge University Press

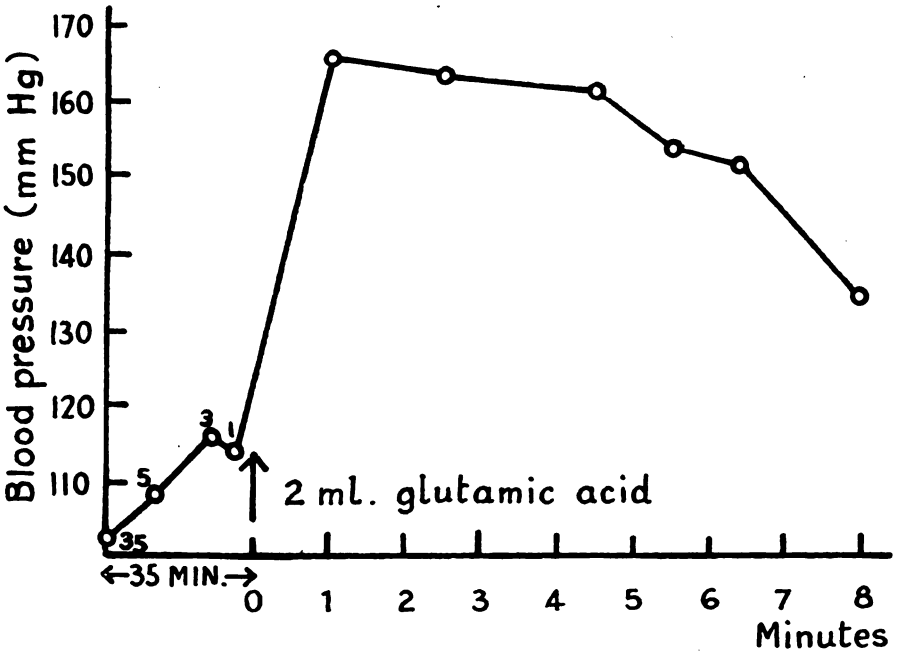


FIG. 3.

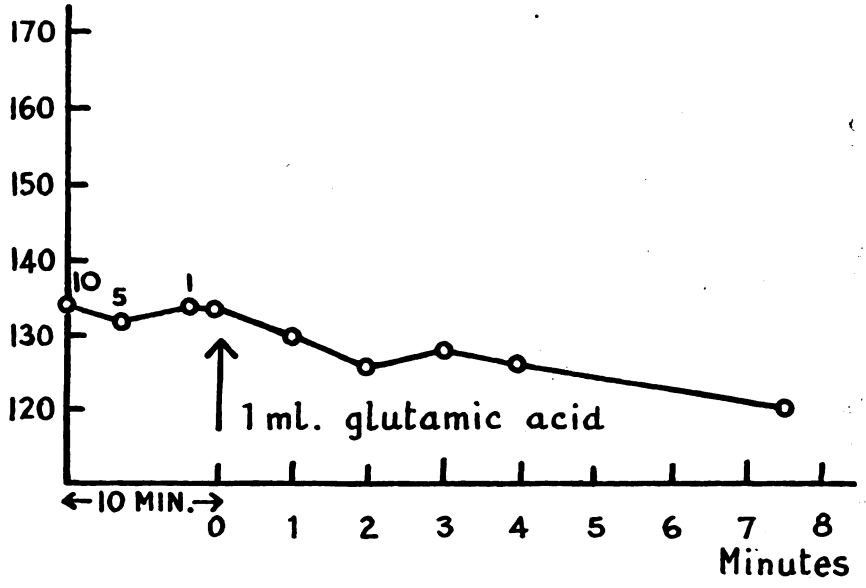


FIG. 4.

https://doi.org/10.1192/bjp.95.401.930 Published online by Cambridge University Press

always allowed to pass before the injection was started. The injection of glutamic acid did not produce rigidity; on the contrary, it usually led to a marked relaxation and decrease of restlessness. The volume of the injected solutions was small and, on that score, did not constitute any undue stress. Furthermore, there is some correlation between blood-pressure rise and the efficacy of the injection. Whereas 5 or 2 ml. still produced a blood-pressure rise, 1 ml. failed to do so. Results, reported in Table III, with the protein hydrolysate "casydrol" show that the injection of much larger volumes of this solution had a much smaller effect on the blood pressure.

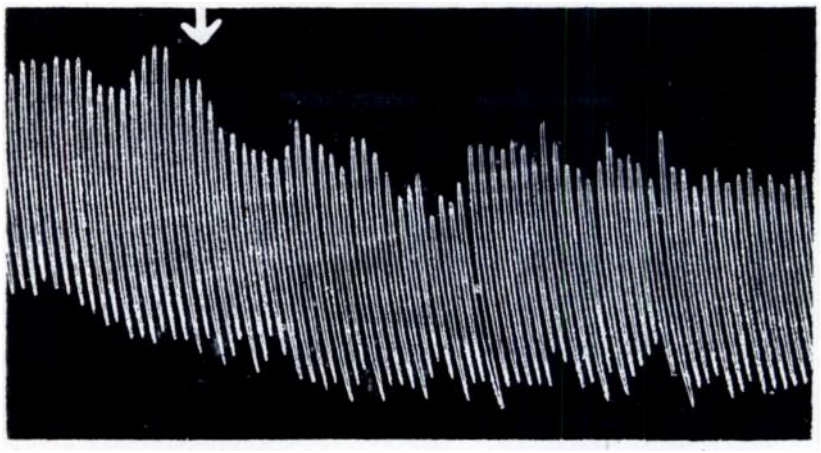
Presence of adrenergic substances in blood.—For the detection of adrenergic substances in blood a preparation of rabbit's intestine was used. A strip of jejunum, 1–1·5 cm. long, was attached to a recording lever and suspended in 100 ml. Krebs' bicarbonate Ringer solution containing 0·1 per cent. glucose at a temperature of 37° C. A slow stream of O_2/5 per cent. CO_2 mixture was led through a sintered glass plate into the solution. Provision was made to empty and flush out the container between tests without disturbing the tissue preparation. The solution to be tested was added in a volume of 1 ml.

Venous blood samples were withdrawn before and 5 minutes after the intravenous injection of 5 ml. glutamic acid; heparin was used as anti-coagulant.

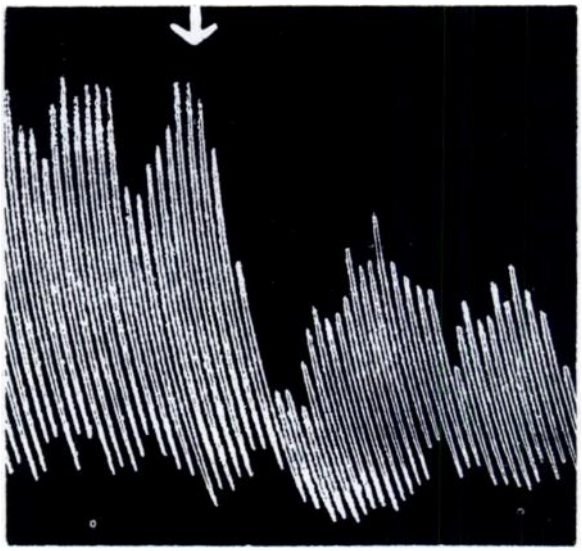
No clear effect was obtained when either whole blood or plasma was tested. With both fluids there was frequently an initial inhibition, followed by a stimulation of contractions, but there was no significant difference between samples collected before or after the injection of glutamic acid. When, however, a solution of washed and laked blood corpuscles was used, a clear effect was frequently observed. The haemolysate was prepared by washing the corpuscles three times with saline, suspending them in distilled water up to the original blood volume and twice freezing the resulting solution. Two typical results are reproduced in Figs. 5 and 6. In the first case (Fig. 5) addition of the pre-injection sample had only a very slight effect, whereas the post-injection sample produced a considerable and lasting relaxation. In the second case (Fig. 6) there was a transient relaxation after the addition of the pre-injection sample. On adding the post-injection sample a strong and lasting relaxation occurred, which closely simulated the effect of 0·5 μgm. of adrenaline (Fig. 6, *c*).

It is well known that adrenaline added to blood *in vitro* is partly incorporated into the corpuscles, and may become biologically active only after laking. The blood cell adrenaline concentration may be 4–5 times greater than the plasma adrenaline concentration, especially when the concentrations are low (Bain, Gaunt and Suffolk, 1937). It need not be assumed, however, that adrenaline was absent from plasma in our experiments, as its action may have been masked by the presence of antagonists (*cf.* Gaddum, Peart and Vogt, 1949). What is surprising is the fact that the combination with cell constituents should have been so rapid, since the equilibrium is established only after a considerable time *in vitro*.

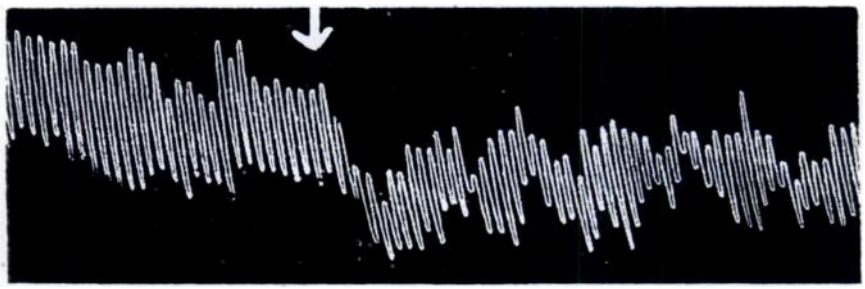
The effect of intravenous injection of adrenaline.—If glutamic acid acts by stimulating adrenaline secretion, it should be possible to obtain the same



a.



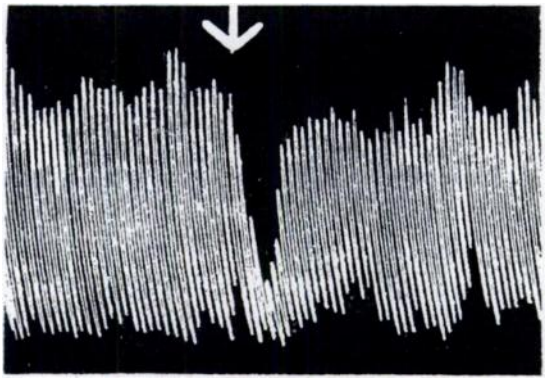
b.



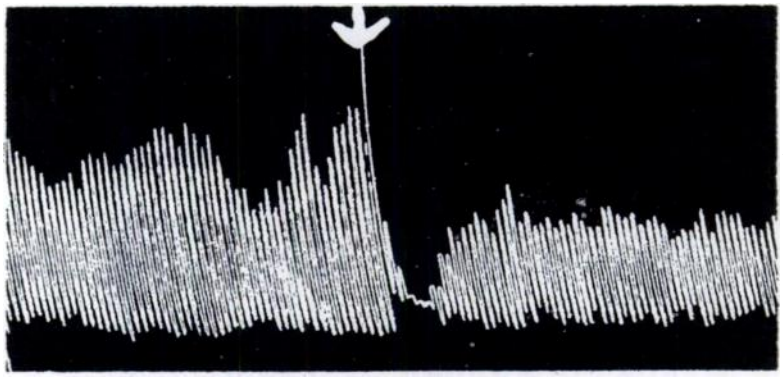
c.

FIG. 5.—Effect of the addition of 1 ml. of corpuscle lysates on the isolated rabbit intestine. (*a*) Lysate prepared from blood of patient in hypoglycaemic coma before injection of glutamic acid. (*b*) and (*c*) Lysate from blood of the same patient 5 min. after injection of 5 ml. glutamic acid solution. Samples added at time marked by arrow.

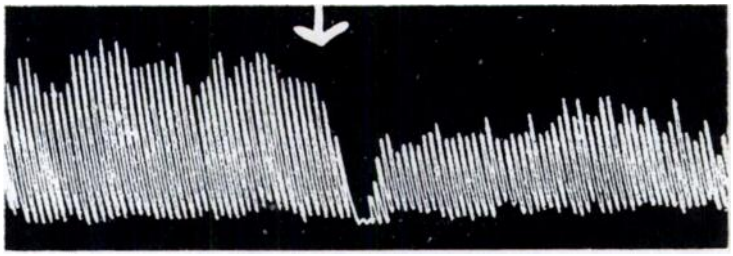
https://doi.org/10.1192/bjp.95.401.930 Published online by Cambridge University Press



a.



b.



c.

FIG. 6.—Effect of corpuscle lysates (1 ml. samples) on the isolated rabbit intestine. (*a*) Lysate withdrawn from patient in hypoglycaemic coma before injection of glutamic acid. (*b*) Lysate from blood 5 minutes after injection of 5 ml. glutamic acid solution. (*c*) Addition of 1 ml. of adrenaline solution containing 0·5 µgm.

https://doi.org/10.1192/bjp.95.401.930 Published online by Cambridge University Press

effects with adrenaline. To avoid the complications of local vasoconstriction it was decided to administer adrenaline by slow intravenous injection at the rate of about 50 μgm. per minute. A continuous control of blood pressure and pulse was maintained during the injection. Except for a pronounced pallor no alarming symptoms were observed. Both blood pressure and pulse rate were increased during the injection, but quickly returned to the norm. The patients chosen for these experiments had previously been found to respond to the injection of glutamic acid. With two exceptions they responded to adrenaline in a similar way (Table II). The return of consciousness seemed even accele-

TABLE II.—*Effect of Intravenous Injection of Glutamic Acid and Adrenaline on Patients in Hypoglycaemic Coma.*

Patient.	Sex.	Glutamic acid solution. (ml. injected.)	Adrenaline solution. (ml. injected.)	Blood sugar (mgm. %). Before injection.	Blood sugar (mgm. %). After injection.	Blood sugar (mgm. %). Increase.	Restoration of consciousness.
R. F—	M.	5	..	18	27·5	9·5	(+)
		..	10	14·5	33·5	19	+
R. G—	F.	5	..	0	23·5	23·5	(+)
		..	5	5	14·5	9·5	−
H. R—	M.	5	..	17	27	10	(+)
		..	5	12·5	46·5	34	+
J. H—	M.	10	..	12·5	40	27·5	+
		..	6	11	38·5	27·5	+
H. W—	F.	10	..	18	42·5	24·5	+
		..	8	32	54·5	22·5	+
D. P—	M.	5	..	18	33	15	(+)
		..	5	17	41·5	24·5	+
J. Ho—	M.	10	..	19·5	32	12·5	+
		..	6	18	35	17	+
C. C—	M.	10	..	23.5	32·5	9	+
		..	9	18.5	30	11·5	+
De P—	M.	10	..	18	37	19	+
		..	10	23	45	22	+
A. S—	F.	10	..	13	29	16	+
		..	8	11	22	11	+
A. St—	M.	10	..	9	24	15	+
		10	..	5·5	15·5	10	−
			6	19	19	0	−

rated, and the patients changed from coma to full alertness without a stage of confusion and noisiness which so often intervenes after the usual glucose feed.* One of the two negative cases had previously shown a slight, and the other a variable reaction to glutamic acid. If the action of adrenaline depends on the level of liver glycogen, such variations are to be expected.

The blood-sugar rise was in some cases almost identical with that produced

* Patients injected with glutamic acid were also frequently seen to wake up without passing through a phase of confusion or anxiety. Their greater mental brightness and alertness during the rest of the day was repeatedly the subject of spontaneous comment by nursing and occupational therapy staff.

https://doi.org/10.1192/bjp.95.401.930 Published online by Cambridge University Press

by glutamic acid. Where differences exist they do not seem significant, and the maximum effect of adrenaline on the blood sugar did not exceed that which was found possible for glutamic acid in favourable cases.

The effect of intravenous injection of a protein hydrolysate.—In view of Mayer-Gross and Walker's results with glycine and p-aminobenzoic acid, the question arose whether the action of glutamic acid can be accounted for simply by an increase of blood amino-N. The effect of "casydrol," a 5 per cent. enzymic digest of casein, was therefore investigated. A preparation free of glucose was obtained on request from the manufacturers. Its analysis showed that it contained 0·52 per cent. amino-N and 1·22 per cent. total N, corresponding to a hydrolysis of 42 per cent. The concentration of free glutamic acid was found to be 0·35 per cent.

For the estimation of free glutamic acid the dicarboxylic acid fraction was isolated chromatographically by the method of Fromageot, Jutisz and Lederer (1948) and oxidized with $KMnO_4$. The solution was then subjected to continuous ether extraction for 12 hours, and the silver salt of succinic acid titrated according to Arhimo and Laine (1939).

The results (Table III) show that, although there was some effect on blood pressure and blood sugar, and although the depth of coma was decreased in some cases, full restoration of consciousness could not be achieved with the doses given, and this in spite of the fact that the content of amino-N of the higher doses was more than twice as high as that of the minimum effective dose of glutamic acid. It may be concluded that the effect of glutamic acid is not merely a function of its amino-N.

Discussion.

The possibility that the effect of glutamic acid is based on an adrenergic mechanism was discussed by Mayer-Gross and Walker, but was considered improbable, mainly because these authors had found that after the injection of glutamic acid, consciousness is restored at a lower level of blood glucose than after the administration of glucose. This argument implies that, if consciousness could be restored by a purely adrenergic mechanism, e.g. by the injection of adrenaline itself, the blood-sugar rise would have to be higher than after the injection of glutamic acid. This is not the case; it has been shown that after a successful adrenaline injection the blood-sugar rise is not materially different from that found after glutamic acid injection.

The objections against an adrenergic mechanism of the glutamic acid effect therefore lose much of their force, and if there is indeed a difference in the blood-sugar level at which consciousness returns, it must be concluded that, after the injection or mobilization of adrenaline, auxiliary factors operate which allow the return of consciousness at a lower blood-sugar level than after glucose administration. At least two functions of adrenaline are known which may be important in this connection: (1) As shown by Rein (1937), slow infusion of adrenaline in physiological doses raises the cerebral blood flow and may thus increase the utilization of glucose. (2) Adrenaline has a direct action on nervous tissues: it facilitates impulse transmission and

TABLE III.—*Effect of Intravenous Injection of "Casydrol" on Patients in Hypoglycaemic Coma.*

Patient	Sex.	Ml. injected.	Blood pressure (mm. Hg.).		Pulse rate/minute.		Blood sugar (mgm. %).		Restoration of consciousness.
			Before injection.	Maximum after injection.	Before injection.	Maximum after injection.	Before injection.	5 minutes after injection.	
L. F—	M.	10	134/80	154/100	72	78	16	23	—
S. C—	F.	5	190/80	190/80	132	134	16	25	—
		20	160/60	210/90	120	156	5	23	(+)
J. G—	F.	10	120/30	130/70	70	80	20	31	—
		25	135/75	140/70	102	116	16·5	31	—
M. S—	F.	10	110/70	110/70	..	..	11	21	—
W. R—	F.	25	134/60	144/70	80	112	8	11	—

potentiates the action of acetylcholine (Burn, 1945). This effect in particular may assist in the restoration of cortical functions.

The response of patients in hypoglycaemic coma to relatively small doses of adrenaline may seem surprising to those who hold that insulin hypoglycaemia entails a state of constant and extreme stimulation of the adrenal system. Without denying that there is some degree of adrenal stimulation in hypoglycaemic coma, it may be pointed out that attempts to demonstrate an increased level of adrenaline in the blood of hypoglycaemic patients have yielded neither uniform nor impressive results (Heilbrunn and Liebert, 1939; Tietz and Birnbaum, 1942). However this may be, the present results show that the susceptibility to a sudden adrenergic shock has not been lost.

Another fact which may seem unexpected is the presence in such a large proportion of cases of sufficient reserves of liver glycogen to allow for blood sugar rises of 20 mgm. per cent. or more after the onset of coma. For a blood volume of 7 l. this would correspond to the mobilization of 1·4 gm. of glucose. Assuming a liver weight of 1500 gm., the glycogen content of the liver must have amounted to 0·09 per cent. if the glycogen stores were completely emptied, or to 0·18 per cent. if they were halved. These are not impossible figures even in hypoglycaemic coma, and are of an order similar to those found by Höpker (1947) in hypoglycaemic rats, having regard to the large variations between species, between individuals, and possibly in the same individual from day to day.

Finally, the adrenergic activity of glutamic acid, and possibly other amino-acids, requires some comment. It has been known for some time that the level of amino-acids in human and animal blood is lowered during insulin hypoglycaemia. According to Davis and Van Winkle (1934) this fall no longer occurs after adrenalectomy and it is therefore attributed by these authors to the secondary action of adrenaline. This opinion is shared by Luck and Morse (1933), who found that adrenaline injection decreases the blood amino-acid level. On the other hand, Nord (1926–27) showed that the injection of glycine or glutamic acid causes a pronounced hyperglycaemia in rabbits, which exceeds the blood-sugar rise after the injection of an equivalent dose of glucose, and that these amino-acids are able to antagonize the hypoglycaemic action of insulin. Both effects were abolished after adrenalectomy, and they were interpreted by Nord as resulting from a powerful stimulation of adrenaline secretion. In a footnote Nord mentions that the chromaffinity of adrenal medulla is diminished in rabbits which had received injections of glutamic acid or glycine. There is thus ample experimental evidence for a mutual interaction between the adrenal system and some amino-acids.

Recently, reports of favourable results achieved with oral administration of glutamic acid in the treatment of mental deficiency (Zimmerman *et al.*, 1946, 1947, 1948) have aroused great interest. It may be suggested that, in this instance too, an adrenergic mechanism may be worth serious consideration. It is not claimed by its originators that glutamic acid therapy causes an actual increase of intelligence, but that it enables the mentally deficient subject to reach the " ceiling " of his intellectual powers. The psychological effects of amphetamine and related adrenergic drugs, such as increase of mental effi-

ciency, of capacity to concentrate, of spontaneity, initiative and sociability (Reifenstein and Davidoff, 1939; Nathanson, 1937), are very reminiscent of those claimed for glutamic acid in mentally retarded children.

Summary.

The effects of intravenous injection of a 25 per cent. neutral solution of glutamic acid have been studied after the onset of hypoglycaemic coma in patients undergoing insulin shock therapy and the following observations are recorded:

(1) The minimum effective dose of glutamic acid solution necessary for the restoration of consciousness in comatose patients was found to be 5 ml., and in favourable cases 2 ml. The blood-sugar rise produced by these doses is greater than can be accounted for by the amount of glutamic acid injected. It is concluded that the effect is due neither to a direct utilization of glutamic acid as fuel for the brain cells nor to its conversion into glucose by the liver, and it is suggested that only a catalytic mechanism can account for the high degree of activity of glutamic acid.

(2) The injection of glutamic acid induced the classical triad of adrenaline action: a fleeting rise of blood pressure and pulse rate (the latter only when it was not accelerated before the injection) and a rise in blood sugar.

(3) Blood samples taken before and after the injection of glutamic acid were examined for adrenergic activity by the rabbit's intestine method. No effect was found when whole blood or plasma was tested, but when a solution of washed and laked blood corpuscles was used the post-injection sample frequently showed a clear increase of adrenergic activity over the pre-injection sample.

(4) Intravenous injection of adrenaline at the rate of 50 μgm./min. duplicated in every respect the effect of glutamic acid in terminating the coma and in producing a blood-sugar rise.

(5) Injection of "casydrol," a 5 per cent. enzymic digest of casein, in doses which contained more than twice as much amino-N as the minimum effective dose of glutamic acid, had some effect on blood-pressure and blood sugar and in some cases lessened the depth of coma, but full restoration of consciousness was not observed. It is concluded that the effect of glutamic acid is not merely a function of its amino-N.

The results are discussed and the opinion is expressed that the objections against the adrenergic mechanism of the glutamic acid effect are without foundation. It is also suggested that an adrenergic mechanism may account for the favourable results of glutamic acid therapy in mental deficiency.

I am greatly indebted to the Medical Superintendent, Dr. R. Ström-Olsen, not only for his constant inspiration and encouragement, but also for his practical co-operation in the clinical part of this investigation. I also wish to acknowledge the expert technical assistance of Mr. A. D. Bone.

References.

ARHIMO, A. A., and LAINE, T. (1939), *Suomen Kemistilehti*, **12 B**, 18.
BAIN, W. A., GAUNT, W. E., and SUFFOLK, S. F. (1937), *J. Physiol.*, **91**, 233.
BOLLMANN, J. L., and MANN, F. C. (1931), *Amer. J. Physiol.*, **96**, 683.
BURN, J. H. (1945), *Physiol. Rev.*, **25**, 377.
DAVIS, B. L., and VAN WINKLE, W. (1934), *J. biol. Chem.*, **104**, 207.
FRIEDBERG, F., and GREENBERG, D. M. (1947), *ibid.*, **168**, 411.
FROMAGEOT, C., JUTISZ, H., and LEDERER, E. (1948), *Biochem. et Biophys. Acta*, **2**, 487.
GADDUM, J. H., PEART, W. S., and VOGT, M. (1949), *J. Physiol.*, **108**, 467.
GOLDFARB, W., and WORTIS, J. (1941), *Proc. Soc. Exp. Biol. and Med.*, **46**, 121.
HEILBRUNN, G., and LIEBERT, E. (1939), *Endocrinology*, **25**, 354.
HÖPKER, W. (1947), *Klin. Wochschr.*, **24/25**, 337.
KLEIN, J. R., HURWITZ, R., and OLSEN, N. S. (1946), *J. biol. Chem.*, **164**, 509.
Idem., and OLSEN, N. S. (1947), *ibid.*, **167**, 1.
LEUTHARDT, F., and GLASSON, B. (1946), *Helv. Chim. Acta*, **29**, 1344.
LUCK, J. M., and MORSE, S. W. (1933), *Biochem. J.*, **27**, 1648.
MADDOCK, S., HAWKINS, J. E., and HOLMES, E. (1939), *Amer. J. Physiol.*, **125**, 551.
MAYER-GROSS, W., and WALKER, J. W. (1947), *Nature, Lond.*, **160**, 334.
Iidem (1949), *Biochem. J.*, **44**, 92.
NATHANSON, M. H. (1937), *J. Amer. Med. Ass.*, **108**, 528.
NELSON, N. (1944), *J. biol. Chem.*, **153**, 375.
NORD, F. (1926/27), *Acta Med. Scand.*, **65**, 1, 61.
REIFENSTEIN, E. C., Jr., and DAVIDOFF, E. (1939), *Amer. J. Psychol.*, **52**, 56.
REIN, H. (1937), *Verhandl. deut. Ges. Kreislaufforsch.*, 10. Tagung, 27.
TIETZ, E. B., and BIRNBAUM, S. M. (1942), *Amer. J. Psychiat.*, **99**, 75.
WAELSCH, H., SCHWERIN, P., and BESSMAN, S. P. (1949), *Fed. Proc.*, **8**, 264.
WORTIS, J., and GOLDFARB, W. (1940), *Amer. J. Physiol.*, **129**, 502.
ZIMMERMAN, F. T., BURGEMEISTER, B. B., and PUTNAM, T. J. (1946), *Arch. Neurol. and Psychiat.*, **56**, 489.
Iidem (1947), *Psychosom. Med.*, **9**, 175.
Iidem (1948), *Amer. J. Psychiat.*, **104**, 593.