The animals surviving alpha-toxin injection, exhibited a decrease of the Na^+-K^+-ATPase activity by 30–50 per cent within 30 min after injection. The enzyme level was then gradually reaching a normal level.

Marked inhibition of Na^+-K^+-ATPase activity in rabbits killed by the alpha-toxin and in animals surviving lower doses, suggests occurrence in their organs of an inhibition of sodium and potassium active transport. It cannot be concluded at present whether this is a primary effect. Nevertheless, the observed inhibition of Na^+-K^+-ATPase activity may produce serious disturbances of sodium-potassium equilibrium in cells and be responsible for brain bioelectric activity changes observed before.[3] Rahal *et al.*[8] have described an inhibition of activite sodium transport using isolated toad bladder. Both observations may suggest importance of active transport inhibition in the mechanism of lethal action of staphylococcal alpha-hemolysin.

National Institute of Hygiene,
24 Chocimska Street,
Warszawa, 36, Poland

S. SZMIGIELSKI
K. KWARECKI
J. JELJASZEWICZ
C. ZAK

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Monosodium L-glutamate—Inhibition of glucose uptake in brain as a basis for toxicity*

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RECENT reports have implicated monosodium L-glutamate as the causative agent in a variety of pathological and physiological lesions. Oral or parenteral administration of glutamate in amounts in excess of 3 g may be associated with symptoms of burning, facial pressure, chest pain and headache collectively known as the Chinese restaurant syndrome.[1–3] Parenteral administration of glutamate to neonatal mammals has been reported to cause degenerative changes in the inner layer of the retina[4–7] and in the hypothalamus,[8,9] the latter effect probably accounting for the skeletal stunting, obesity and sterility observed.[8,9] There has been controversy over some of these reports,[10,11] but one practical result has been the discontinuation of the practice of adding glutamate to commercial baby foods.

Our own interest in this compound stems from its action as an antagonist of the antimitotic alkaloid, vinblastine.[12,13] There is a competitive relationship between vinblastine and glutamate for entry into human leukocytes,[14] despite their great disparity in chemical structure, and the alkaloid also inhibits

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the transport of other amino acids and uridine.[15] Because of these findings, it occurred to us that glutamate also might inhibit the transport of other natural metabolites as did vinblastine, and that this could be a basis for its toxicity. Among the metabolites tried was glucose. In our human leukocyte system, either at rest or during phagocytosis, glutamate failed to affect the uptake of glucose. However, the critical dependence of cerebral function upon glucose led us to study the effect of parenteral glutamate on glucose uptake by the brain. The present data from adult mice indicate that a significant if brief depression of the uptake of this sugar does indeed occur.

Adult Swiss mice (CD-1; 30 g) received intraperitoneal injections of monosodium L-glutamate (300 mg/kg; 9 mg in a 0·3-ml injection volume) or isotonic saline as a control, followed after a 15-min interval by glucose-2-^{14}C (0·62 mc/m-mole; 1 μc) given by the same route. At intervals thereafter, the mice were killed, their brains removed into ice-cold saline, and blood samples were collected from the hearts. Brains were washed free of blood, homogenized with perchloric acid (0·5 M), heated at 80° for 20 min, cooled and centrifuged. Radioactivity in the supernatant was measured with a Packard Tri-Carb liquid scintillation counter using a toluene–ethanol based fluor. The precipitate

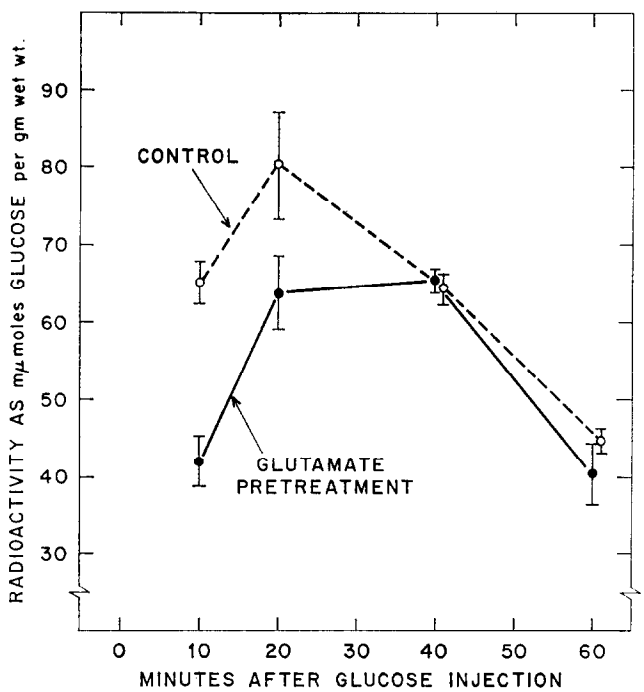

FIG. 1. Effect of glutamate on the levels of radioactivity in brain derived from glucose-^{14}C. Glutamate (9 mg) was injected 15 min before glucose-2-^{14}C which was given at time zero. Procedures were as described in the text. Standard errors of the means (six animals per point) are indicated.

was suspended in NaOH solution (0·2 N) and counted similarly. Data for the acid-soluble fraction (Fig. 1) showed that at 10 and 20 min after injection of glucose, the ^{14}C levels in the brain, expressed as glucose equivalents, were significantly lower in the glutamate-treated mice ($P < 0·005$ at 10 min, $< 0·01$ at 20 min, by the t-test). By 40 min, however, there was no difference between the groups. The acid-insoluble residue contained between 13·3 and 17·4 per cent of the total brain radioactivity, and its behavior closely paralleled that of the soluble fraction. Thin-layer chromatography of the neutralized 10-min acid extracts (six mice per group) on Silica gel H (Merck) with n-butanol–formic acid–water (77:10:13) as solvent showed that 64·2 $\pm$ 0·7 per cent of the control and 59·7 $\pm$ 1·4 per cent of the treated ^{14}C migrated as unchanged glucose. This small reduction, although significant ($P = 0·02$), is unlikely to explain a 35·5 per cent decrease in brain radioactivity; indeed such a reduction in the glucose component might be expected if the amount of glucose entering the brain declines without changes in its rate of utilization for metabolic processes.

The blood samples (0·02 ml) were plated out with NaOH solution on stainless steel planchettes, dried at room temperature, and counted in a Nuclear Chicago gas-flow counter without a window. There was no difference between the blood levels of ^{14}C in control and glutamate-treated mice (Fig. 2); the bulk of this radioactivity migrated as glucose in the early samples.

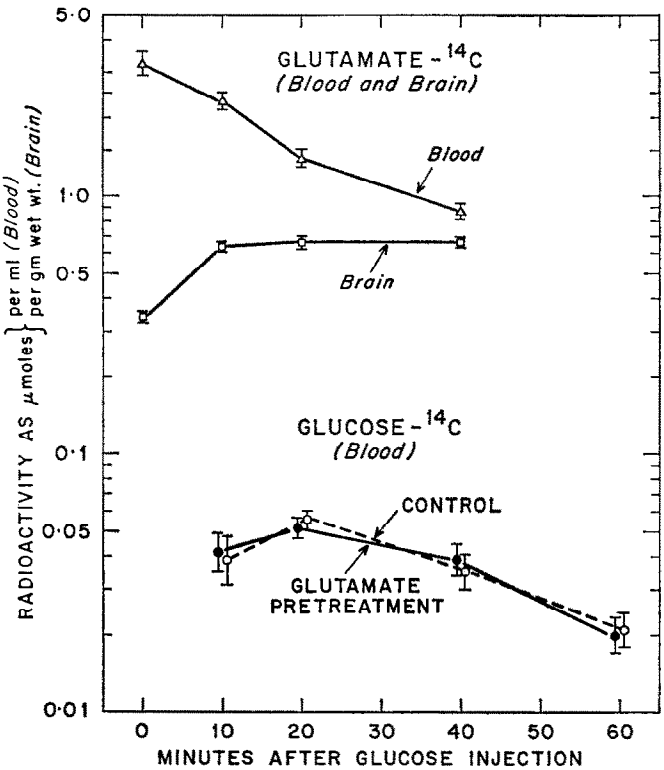

FIG. 2. Radioactivity derived from glutamate-^{14}C (blood and brain) or from glucose-^{14}C (blood). Glutamate, either labeled or unlabeled (9 mg), was given 15 min before zero time, when glucose was injected. Levels were determined as described in the text. Standard errors of the means (six animals per point are indicated.

In order to determine the levels of glutamate in the blood needed to achieve an effect on glucose uptake, intraperitoneal injections of glutamate-3,4-^{14}C (9 mg and 1 μc/mouse) were given and the blood and brain levels of radioactivity measured as for glucose-2-^{14}C. The results (Fig. 2) indicate that the excess blood levels of glutamate over those occurring normally—for mouse plasma reported as 33·3 μg/ml or 0·27 μmole/ml[16]—fell from an initial 3·26 to 1·42 μmoles/ml during the time that an effect on glucose transport was evident.

Thus, large doses of glutamate in adult mice can reduce the uptake of glucose into the whole brain by up to 35·5 per cent. That the overall effect is dose-dependent was shown by another experiment in which twice the amount of glutamate (600 mg/kg) produced an inhibition of 64·1 per cent. Neither glycine nor sucrose (300 mg/kg) affected the entry of glucose into the brain to any significant extent when compared with saline.

One might speculate that, since some areas of the brain are likely to be more sensitive to glutamate than others, the differences in glucose uptake in critical tissues may be even greater. There is evidence for the existence of centers within the lateral and ventromedial regions of the hypothalamus that control feeding behavior,[17] and which appear to take up labeled glucose more readily than surrounding areas.[18] In addition, gold thioglucose exerts a selective toxic effect on the hypothalamus that is not shown by the other related agents such as gold thiomalate, and apparently depends on the ability

of the thioglucose moiety to facilitate transport of gold into the sensitive areas.[19,20] The ventral hypothalamus is especially affected, including both lateral areas and such ventromedial structures as the ventromedial nucleus, the arcuate nucleus, the supraoptic nucleus and the median eminence.[19] Damage from monosodium glutamate is prominent in the region of the arcuate nucleus, the pre-optic nucleus and the median eminence.[8] Aspartic acid and cysteine, but no other amino acid, produce similar lesions.[21]

In previous studies, 0·5–4 g/kg of glutamate produced brain damage or developmental abnormalities[7,8] and similar amounts given daily over 10–18 days resulted in retinal lesions;[4–6] these dosages are far larger than those used in this study. The amounts of glutamate needed to induce the Chinese restaurant syndrome in man varied widely, but were about 3 g in the most sensitive individuals. Some subjects had thresholds of 7–12 g, corresponding to 100–170 mg/kg. These doses approach those needed to affect glucose uptake and, in addition, man, whose plasma glutamate level is little more than one-fifth that of the mouse,[16] may be more sensitive to this amino acid. Interference with glucose uptake is thus one plausible basis for the toxicity of monosodium L-glutamate.

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WILLIAM A. CREASEY
STEPHEN E. MALAWISTA*

Departments of Internal Medicine and Pharmacology,
Yale University School of Medicine,
New Haven, Conn. 06510, *U.S.A.*

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