($P < .01$). The mean latency to eat following 2DG is also significantly shorter in the intact hepatic portally infused rabbit than in the other two preparations ($P < .01$ in both comparisons). The latencies are shown in Fig. 2.

The increments in food consumption after 2DG injections into the jugular vein or into the hepatic-portal system of the vagotomized rabbit correspond with those reported by others. Houpt and Hance (*8*) observed a 90 percent increase and Smith and Epstein (*6*) observed a 104 percent increase in eating in animals not deprived and administered 2DG peripherally. Also, Balagura and Kanner reported an increase of only 79 percent after administration of 2DG directly into the lateral hypothalamus of the rat (*2*). Our observations showed 2DG producing an increase of 103 or 106 percent, respectively, in the vagotomized rabbit with administration into the portal system and after administration into the jugular vein in the intact rabbit. In contrast, the mean increase in food intake that follows 2DG in the intact, portally infused subject was 209 percent. In addition, the latencies we observed are with one exception the shortest that have been reported (*10*). Thus, the results of our experiments suggest the existence of hepatic glucose utilization receptors that are innervated by the vagus nerves and that are important in the initiation of feeding. While the hyperglycemia that is known to result from 2DG injections might eventually cause increased insulin secretion, the latency to eat after insulin administration is quite lengthy (*6*). Although we have not yet looked at the changes in glucose or hormone concentrations after 2DG administration, the short latency feeding response observed suggests that these are probably not involved.

Nothing in our experiments argues against the existence of central glucoreceptors. Moreover, the fact that 2DG in the vagotomized animal still increased food intake supports the notion of extrahepatic receptors or at least receptors not innervated by the vagus nerves. However, our results suggest that there are, in addition, peripheral receptors which in response to decreased glucose utilization promote a state of hunger followed by immediate food ingestion.

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10. S. Nicolaidis, A. N. Epstein, J. Le Magnen, *J. Physiol. Paris* **65**, 150a (1972); S. Nicolaidis and M. J. Meile, *ibid.*, p. 151a. These investigators, using rats with intra-auricular cannulas, observed latencies to eating (range 1 to 9 minutes), after 2DG infusions, that were comparable to and probably shorter than the latencies we observed after hepatic-portal 2DG infusions. One might, on the basis of these data, argue against the significance of our observation of short latencies (with respect to jugular vein infusions). Several points, however, should be made. One obvious difference between experiments is species, rats as compared to rabbits. Their doses were higher, approximately 300 mg/kg, compared to 250 mg/kg in our experiments. Also, we believe they measured latencies from the end of the injections, whereas we measured latencies from the beginning. The most significant difference, we believe, is that, while the absolute rates of injections were the same (1 ml/min) on a weight basis, their animals were getting 2DG considerably faster than ours; that is, our subjects after 2 minutes had 50 mg/kg, whereas their rats had approximately 300 mg/kg by that time.
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Glutamate Uptake by the Isolated Toad Brain

Abstract. *The isolated toad brain accumulates* L-*glutamate against strong concentration gradients until a tissue-to-medium concentration ratio of about 3000 : 1 is attained. The accumulated glutamate does not equilibrate with most of the endogenous tissue glutamate but is converted rapidly to glutamine and released into the medium. This mechanism may be involved in the preservation of low extracellular levels of cerebral glutamate.*

L-Glutamate, at concentrations of 2 to 20 m*M*, is one of the most abundant amino acids in tissues of the nervous system (*1*). Considerable evidence suggests that glutamate may be an excitatory neurotransmitter (*2–4*). An essential requirement of any synaptic neurotransmitter is that it must be rapidly inactivated subsequent to its release, by destruction or removal from the synaptic cleft (*5*). Removal of a transmitter from the synaptic cleft may be mediated by re-uptake into the presynaptic terminal, uptake into the postsynaptic cell, or uptake into surrounding glial tissue. It is reasonable to assume that a substance should be maintained at very low levels in the extracellular fluid if it is to function efficiently as a transmitter.

The present study shows that the isolated toad brain has the capacity to remove glutamate rapidly from the extracellular environment against enormous concentration gradients. This capacity exceeds that shown for mammalian brain slices (*6*) and glial cell preparations (*7*).

Toads (*Bufo boreas*) weighing 25 to 50 g were anesthetized by chilling them in crushed ice for 20 minutes. The brain of each toad was exposed by removing the upper portion of the cranium; the spinal cord was ligated and the brain was excised. The dura was removed and the brain was blotted and transferred to a tared weighing bottle containing an oxygenated, ice-cold Ringer-like medium composed of NaCl (100 m*M*), $NaHCO_3$ (25 m*M*), KCl (2 m*M*), KH_2PO_4 (3 m*M*), $MgCl_2$ (1 m*M*), Na_2SO_4 (1 m*M*), $CaCl_2$ (1 m*M*), glucose (10 m*M*), and urea (10 m*M*). The final *p*H of the medium was 7.3. The brain was weighed and transferred to a test tube containing 2 ml of the medium to which L-glutamate (0.04 m*M* or 0.10 m*M*) and L-[U-^{14}C]glutamate (0.02 μc, New England Nuclear Corp., 100 mc/mmole) were added previously. The brain weights ranged from 55 to 85 mg with a mean weight of 69.7 mg. The brains were incubated at 25°C for 10 to 120 minutes. During incubation, a mixture of 95 percent O_2, 5 percent CO_2 was bubbled into the medium to oxygenate and mix the system. After incubation each brain was rinsed twice with cold medium (containing no glutamate), blotted, weighed, and homogenized in 1 ml of cold 3 percent perchloric acid, and the tissue extract was separated by centrifugation. The content and specific radioactivity of glutamate and glutamine in the tissue extract and the incubation medium were measured by methods described elsewhere (*8*).

When toad brains were incubated as described, glutamate in the medium was taken up rapidly by the brain tissue. Within 30 minutes, more than two-thirds of the glutamate was removed from the medium (Fig. 1A), even though the volume of the medium was approximately 30 times that of the brain. Glutamate continued to be taken up until the concentration in the medium was reduced to about 0.002 m*M* (Fig. 1A). It has been reported that when glutamate is applied to the external surfaces of neurons, a concentration of about 0.1 m*M* is required to elicit neuronal excitation (*1, 3*). Hence our findings demonstrate that central nervous system tissues in the toad have the capacity to remove glutamate from the extracellular environment to a level far below that which induces excitation. Although we have not been able to measure reliably the concentration of glutamate in the cerebrospinal fluid of toads, it is known that glutamate in the cerebrospinal fluid of mammals is maintained at a very low level, approximately 0.01 m*M* (*9*), which is about one-thousandth of the level in mammalian brain tissues. Thus the low level of glutamate in the cerebrospinal fluid of mammals may reflect an uptake process similar to that in the toad brain. An active transport process for the uptake of glutamate has been demonstrated for mammalian brain slices (*6*) and glial cell preparations (*7*); however, the tissue-to-medium concentration ratio of 3000 : 1 (Fig. 1B) generated by the toad brain exceeds by a factor of 10 or more the ratios obtained with the other preparations. This difference may be due in part to the tissue damage incurred during preparation of mammalian brain slices and glial cells.

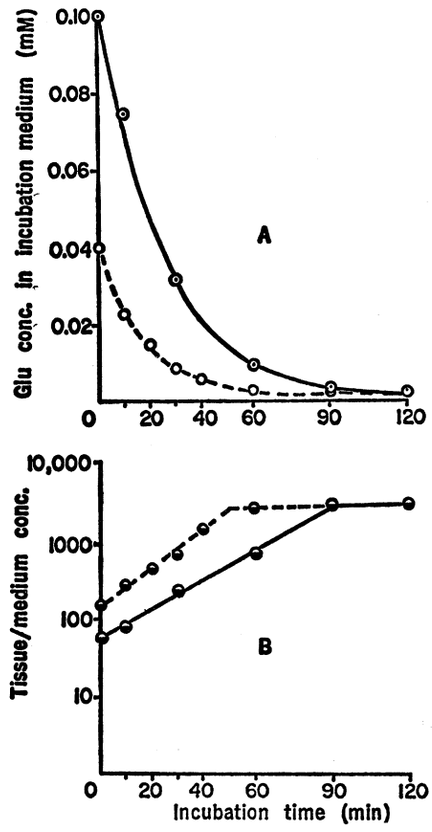

Fig. 1. Changes in glutamate concentrations during incubation. (A) Decline of glutamate (glu) concentration (micromoles per milliliter) in incubation medium. Each point is an average obtained in two to four experiments. The dotted and solid lines represent experiments in which the initial glutamate concentration of the incubation medium was 0.04 m*M* or 0.10 m*M*, respectively. (B) Semilog plot of the ratio of the glutamate concentration in the tissue to glutamate concentration in the medium. At the end of each incubation period, the glutamate content in the tissue (micromoles per gram of wet tissue) was divided by the glutamate concentration (micromoles per milliliter) in the medium. For values at zero time, the glutamate content in nonincubated brains was divided by the initial concentration of glutamate in the medium.

Recently, high affinity transport systems for the uptake of glutamate into glial cell ($K_m \sim 0.01$ m*M*) and synaptosomal ($K_m \sim 0.02$ m*M*) preparations of rat central nervous system tissues have been reported (*7, 10*). The uptake capacity for glutamate found in the whole toad brain might involve similar high affinity transport systems and possibly other carrier mechanisms with a lower affinity.

The glutamate taken up by toad brain was rapidly and extensively converted to glutamine (Fig. 2A), much of which was released back into the medium (Fig. 2A). When the brains were incubated for 60 minutes or longer, glutamine in the tissue and medium together accounted for about one-half of the total initial radioactivity in the [^{14}C]glutamate. In the tissue, glutamate never contained more than one-sixth of the initial radioactivity. A large accumulation of exogenous glutamate within the brain tissues might have decreased glutamate uptake; however, as a result of the rapid conversion of glutamate to glutamine, such an accumulation was not observed in our experiments.

At all time periods studied the specific radioactivity of glutamine in the tissue was two to three times higher than that of glutamate (Fig. 2B). Furthermore, the specific activity of the glutamine released into the medium was two to three times higher than that of glutamine in the tissue (Fig. 2B). These results indicate that most of the glutamate removed from the medium and taken up by the tissue did not equilibrate with the bulk of the tissue glutamate. Such data can be explained by assuming that the labeled glutamate was transported extensively into a tissue compartment containing only a small portion of the endogenous glutamate

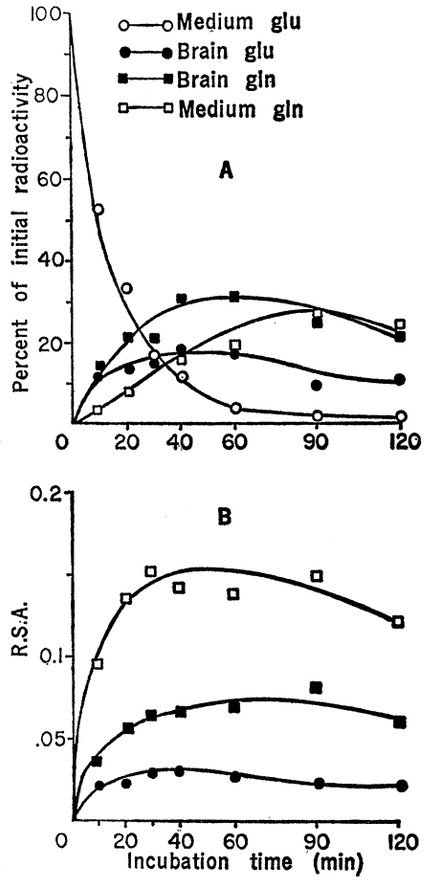

Fig. 2. Changes with time of radioactivity in the glutamate (glu) and glutamine (gln) of incubation medium and brain tissue. (A) Expressed as a percentage of the total radioactivity present exclusively in the glutamate of the medium at zero time. Each point represents an average of two to four experiments. These data do not include radioactivity in glutamate and glutamine incorporated into protein. (B) Relative specific activities (R.S.A.). The specific activity of tissue glutamate, tissue glutamine, and medium glutamine expressed as a fraction of the specific activity present at zero time in the glutamate of the medium. The glutamate of the medium was assigned an arbitrary specific activity of 1.0 at zero time. The results shown in Fig. 2 are from experiments in which the initial concentration of glutamate in the medium was 0.04 m*M* and the [^{14}C]glutamate added to the 2 ml of incubation medium was 0.02 μc. Similar results were obtained when the initial glutamate concentration was 0.10 m*M*.

and then rapidly converted to glutamine. Compartmentation of glutamate and glutamine into two or more metabolic pools in vertebrate central nervous system tissues has been demonstrated previously (*1, 11*). A large pool of glutamate which is derived primarily from glucose and is not extensively converted to glutamine is thought to be present in neurons (*1, 11, 12*). A small pool which may equilibrate slowly with the larger pool (*13*) is present in another tissue compartment, possibly glia (*1, 12*). In this small pool, glutamate is converted extensively and rapidly to glutamine (*1, 11*).

If exogenous [^{14}C]glutamate were taken up extensively into the larger glutamate pool of brain tissue, subsequent transfer of radioactivity into the glutamine pool would be slight. Also, the specific activity of glutamate should be higher than that of glutamine in the tissue. However, the opposite result was obtained in our experiments with toad brain. The [^{14}C]glutamate was converted rapidly to glutamine (Fig. 2A) and the specific activity of glutamine in the tissue was always higher than that of the glutamate (Fig. 2B). These results suggest that it is an initial transport of glutamate into the compartment containing the small pool of glutamate which is instrumental in maintaining low extracellular levels of glutamate.

At present, there is no evidence to indicate that glutamate is enzymatically inactivated or metabolized on the external surface of neurons. It seems plausible, therefore, that a rapid uptake mechanism for glutamate such as the one that has been described for mammalian central nervous system tissue (*7, 10*) and now for whole toad brain also may play a part in the inactivation of neurotransmitter glutamate, after its release from presynaptic terminals of "glutamatergic" neurons.

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Male-Specific Antigen: Modification of Potency by the H-2 Locus in Mice

Abstract. *Skin grafts from C57BL/10 (B10) male mice survive significantly longer than do B10.BR male skin grafts on (B10 × B10.BR)F_1 females. This indicates that the* H-2 *locus influences the potency of the male-specific antigen in mice.*

The rejection of male skin grafts by females of the same inbred strain has been attributed to the occurrence of a Y chromosome-determined antigen (H-Y) in the grafts (*1, 2*). Although this antigen appears to be expressed in the tissues of all normal males (*3*), females of some strains do not reject male skin grafts (*2, 4*). The ability to reject male grafts is correlated with the *H-2* genotype of the recipient female (*4*). For example, females of strains possessing the *H-2^b* allele almost invariably destroy intrastrain male skin grafts, whereas females of strains which possess the *H-2^k* allele usually do not (*5*). These differences can be explained on the basis of an immune response gene closely linked to the *H-2* locus (*4*).

In addition to genetically determined immune response differences, there are apparent strain differences in the potency of the H-Y antigen. Thus, (C57BL/6 × CBA)F_1 hybrid females reject CBA male skin grafts much more vigorously than they reject those from C57BL/6 (B6) males (*6*). In light of evidence that males of these strains possess the same H-Y antigen (*3*), this observation suggests that H-Y is more strongly expressed in CBA males than it is in B6 males (*7*). Initial studies in our laboratories indicate that this difference in immunogenicity may be related to the *H-2* genotype of the cell bearing the H-Y antigen (*8*). This is now confirmed by two experiments in which we utilized congenic strains of mice having the same H-Y antigen and differing only at the *H-2* locus. The C57BL/10 (B10) congenic pair, B10 (*H-2^b*) and B10.BR (*H-2^k* on a B10 background), was obtained from the Jackson Laboratory, Bar Harbor, Maine; the other congenic pair, B6 and B6.*H-2^k* (*H-2^k* on a B6 background) was from colonies maintained by E. A. Boyse, Memorial Sloan-Kettering Cancer Center, New York.

Table 1. Survival of male skin grafts on (B10 × B10.BR)F_1 female recipients (± standard deviation).

Male donor	Recipients (No.)	MST (days)
B10 (*H-2^b*)	30	20.0 ± 1.4
B10.BR (*H-2^k*)	30	14.5 ± 1.3
(B10 × B10.BR)F_1	14	17.5 ± 1.3

Transplantation was performed according to procedures described elsewhere (*9*), and graft survival was appraised daily. Donors and recipients were virgin adults 90 to 120 days of age. Median survival times (MST's) were computed by the graphic method of Litchfield (*10*). Significance was determined by the *t*-test applied to mean survival times.

In our first experiment, we exposed female B6 mice to skin grafts from B6 males and simultaneously to contralateral skin grafts from B6.*H-2^k* males. A second group of B6 females received grafts from B6 males and contralateral grafts from B6.*H-2^k* females. A third group of B6 females received bilateral grafts from B6 males. The MST for 15 male B6 grafts made contralateral to B6.*H-2^k* female grafts was 21.9 days, not significantly different from the MST of 23.0 days for 24 bilateral B6 male grafts on B6 females ($P > .4$).