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 ($P > .4$).

In contrast, the MST of 15.2 days for 23 male B6 grafts contralateral to B6.*H-2*^{k} male grafts was significantly lower ($P < .005$).

If the immunogenicity of H-Y varies on different *H-2* backgrounds, as the above results suggest, then it should be possible to demonstrate this difference in single (unilateral) grafts. Accordingly, we used the congenic strains B10 and B10.BR (*11*) for the following experiment. Three groups of (B10 × B10.BR)F_1 hybrid females (*H-2*^{b}/*H-2*^{k}) received individual skin grafts from either B10 males, B10.BR males, or (B10 × B10.BR)F_1 males (Table 1). The B10.BR (*H-2*^{k}) male grafts (MST, 14.5 days) were rejected significantly faster than were B10 (*H-2*^{b}) male grafts (MST, 20.0 days) ($P < .005$). The MST of 17.5 days for male grafts of F_1 hybrid origin was intermediate between the values for male grafts from the two parental strains; this suggests a gene dosage effect.

The only histoincompatibility between donor and host in this experiment involves the H-Y antigen. Inasmuch as the origin of H-Y is identical in the congenic donors that we employed, and because the only difference between the grafts was their *H-2* genotype, *H-2* was in some way responsible for the disparate survival of B10 and B10.BR male grafts on the F_1 hybrid females. We conclude, therefore, that the immunogenicity of H-Y is influenced by the *H-2* locus; specifically, that H-Y antigens are more immunogenic on *H-2*^{k} cells than they are on *H-2*^{b} cells. On one hand, there could be a functional relation between the two loci such that the ontogeny of the H-Y antigen is in some way dependent on *H-2* (*12*). Alternatively, these differences might result from steric interference between *H-2* cell surface components and the binding of neighboring H-Y sites to anti-Y receptors. In any case, the effectiveness of rejection of incompatible grafts may depend not only on the ability of the host to respond to graft antigens, but also on the genetic background of the transplant donor.

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11. Females of strain B10.BR (*H-2*^{k}) reject B10.BR male skin grafts slowly or not at all. In contrast, B10 females (*H-2*^{b}) rapidly destroy B10 male skin grafts (MST, 19 days). This disparity is related to the *H-2* genotype of the responding female [D. L. Gasser and W. K. Silvers, *Transplantation* **12**, 412 (1971)].
12. One possible mechanism for the *H-2* dependence of the H-Y antigen could be that this antigen is coded for by an *H-2* linked gene that is turned on only in a male environment (D. C. Shreffler, personal communication). Until allelic differences at the *H-Y* locus are conclusively demonstrated so that it is possible to study linkage relationships of this locus, this hypothesis is probably not testable.
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Plasma Membranes: Isolation from Naturally Fused and Lysolecithin-Treated Muscle Cells

Abstract. *A medium containing bicarbonate and calcium was used to isolate plasma membranes of cultured muscle cells. Membranes from differentiated myotubes, as well as the labile, largely unfused, lysolecithin-treated cells from the same culture could be isolated by identical manipulations. Adenylate cyclase of high specific activity was assayed in plasma membranes from both types of cells. Lysolecithin treatment apparently interferes with the metabolism and turnover of membrane phospholipids and thus prevents the natural fusion of muscle cells.*

Skeletal muscle cells differentiating in vitro present a useful model for studying the role of the plasma membrane (*1*) in cellular growth and differentiation. In this system, single muscle cells (myoblasts) naturally fuse into myotubes, and the multinucleate skeletal muscle fibers can eventually be formed by subsequent fusion of myoblasts and myotubes (*2*). The plasma membrane of these muscle fibers can be further studied during synapse formation with dissociated cells of neural origin, also grown in culture (*3*). The isolation of plasma membrane from cultured muscle cells at various stages of differentiation offers advantages in studying specialized membrane structures since replicate plates from muscle cell cultures can also be used for electrophysiological and cytological measurements (*4*).

We now report a method of isolating plasma membranes from rat muscle cells cultured in regular medium and exhibiting extensive fusion, as well as muscle cells cultured in lysolecithin-containing medium and exhibiting confluent but unfused cellular growth. Selected biochemical comparisons between the two preparations are also presented.

The characteristic growths of rat muscle cells in a mixture of common culture media (R) or media treated with lysolecithin (LL) (at a concentration of 150 μg per milliliter of medium) have been described (*5*). After the LL mixture was replaced with regular medium (LLR) at any time during culture, the fusion inhibition, imposed by lysolecithin, was completely reversible and occurred over a 6- to 8-hour interval (*5*). The muscle cells were sampled after 7 days in culture—when cell confluency and myotube formation were well established (between days 5 to 6)—in petri plates containing regular medium (R). Half the culture plates were treated with lysolecithin starting from day 3 of culture until day 7. Culture medium was changed every other day.

After repeated washing in Hanks solution, the cells were collected by gently scraping the culture plates (*5*). This method was preferable to the use of enzymes which could have caused loss of membrane components. After being scraped in ice-cold medium, the cells were centrifuged at 1000 rev/min for 10 minutes, and the collection medium, consisting of 0.3*M* sucrose, 5 m*M* Tricine [*N*-tris (hydroxymethyl)-methylglycine], *p*H 7.5, 1 m*M* $CaCl_2$, and 5 m*M* KCN, was discarded. The cells were homogenized with 12 to 15 strokes in a 5-ml-capacity Dounce