relatively insensitive cells, but a more thorough search is required.

Autoradiography of cultures exposed to ^{125}I-labeled α-bungarotoxin, a molecule that binds specifically to ACh receptors (*10*), supports the electrophysiologic findings (Fig. 3). The paucity of grains over active fibers, compared to grains over inactive cells, is striking. There are dense patches of grains over inactive fibers [see (*2, 11*)] which may correspond to the peaks of sensitivity detected by ACh iontophoresis.

In conclusion, we have demonstrated that activity of muscle fibers that develop in vitro from isolated myoblasts is sufficient to reduce the sensitivity of the surface membrane to ACh. It remains to be demonstrated that activity is also a necessary condition. The finding that fibers innervated in vitro by added spinal cord neurons but which remain relatively inactive are as sensitive as controls (*2, 9*) suggests that it is.

Our initial impression is that peaks as well as the "background" level of chemosensitivity are reduced or eliminated after stimulation of uninnervated fibers. It will be important to confirm this finding and to determine whether similar peaks that are found on innervated fibers near nerve terminals (*2, 9, 12*) are maintained under the same conditions.

It might be possible, in this in vitro system, to further analyze the roles played by the complex membrane phenomena and the muscle twitch associated with "activity" in altering membrane chemosensitivity by uncoupling excitation from contraction.

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Mobility of Potassium Ion in Frog Muscle Cells, both Living and Dead

Abstract. *The diffusion coefficient of potassium ion (D_K) in frog muscles was studied by a new method. In normal cytoplasm D_K averaged one-eighth of the value in the free solution. Arrest of metabolism or injury caused an increase in D_K. In muscles killed with iodoacetate, D_K rose to three-quarters of the value in free solution. The data support the association-induction hypothesis.*

Widely cited in support of the membrane theory (*1*) is the demonstration by Hodgkin and Keynes (*2*) of a nearly normal self-diffusion coefficient of K^+ labeled with ^{42}K in giant squid axons. More recently, Kushmerick and Podolsky (*3*) measured the diffusion coefficients of K^+ and other solutes in small segments of single frog muscle fibers. They concluded that all of these solutes (except Ca^{2+}) are free in living frog muscle cells and that it is the mechanical barrier effect of intracellular proteins which impartially reduces virtually all diffusion rates by a factor of 2.

Since K^+ mobility is crucial in the controversy over what constitutes the physicochemical nature of a living cell, and since criticisms can be made of the above-mentioned experiments (*4, 5*), we have developed a technique to reinvestigate the mobility of K^+ in sartorius muscle cells of northern North American leopard frogs (*6*). At least a major portion of the K^+ in the sartorius muscle was exchanged with ^{42}K. This substantial exchange of cell K^+ with ^{42}K insured that the monitored K^+ mobility was truly representative of the bulk K^+ (*4*). The muscle, thus labeled with ^{42}K, was drawn through a close-fitting slit in a silicone rubber plug, which closed one end of a glass tube; the tube was filled either with a mixture of O_2 (95 percent) and CO_2 (5 percent) or with purified petroleum jelly. The tibial end of the muscle protruding beyond the plug was then cut off (*7*) at about 0.5 to 1 mm from the outer edge of the slit (see Fig. 1A). The muscle in the tube was approximately 20 mm long and was maintained in a straight but relaxed length. The extreme tip of the cutoff tibial end, about 2 mm, was cut off and discarded. The remainder of the cutoff end was analyzed for the initial ^{42}K content of the muscle. The cut end of the muscle in the tube was then exposed to a large amount (1 liter or more) of well-stirred, sterile Ringer-GIB medium (*8*) kept at 25° ± 1°C. After a specific length of time, the muscles were frozen in liquid nitrogen at their natural lengths and cut into 10 to 12 sections; their ^{42}K and water contents were then assayed. The concentration of labeled K^+, expressed as a fraction of the initial concentration, was then plotted against x/l, where x is the distance of the section from the cut end and l is the total length of the muscle (*9*) (Fig. 1).

The virtually straight line in Fig. 1A shows that before cutting and washing there is an even distribution of ^{42}K in the sartorius muscle except in the last two sections—at the extreme tibial and pelvic ends. From 13 June to 7 Decem-

ber 1972 we studied the diffusion of labeled K^+ at 25°C in a total of 72 muscles. As illustrated in Fig. 1, B to H, the results fall into two categories. The first category included 22 muscles. For this group (as illustrated in Fig. 1, F to H), virtually all the experimental points can be fitted to a theoretical curve described by a single value of $D_K t/l^2$, where D_K is the diffusion coefficient of labeled K^+, t is the duration of washing, and l is the length of the muscle (*10*). As a rule these data come from the healthiest muscles. The remaining 50 muscles fall into a different class, as illustrated in Fig. 1, B to E. In this group, points near the cut end sag under the theoretical curve, which fits the majority of points farther away from the cut end.

There are indications that this sagging of points near the cut end is the result of local cytoplasmic deterioration. For example, after very prolonged washing the sections of muscle near the cut end are usually somewhat opaque and swollen. One might alternatively suggest that the observed sagging is caused by technical errors, such as a leaky Vaseline seal. If this were the case, however, one would observe this sagging just as frequently in a study of the diffusion of labeled K^+

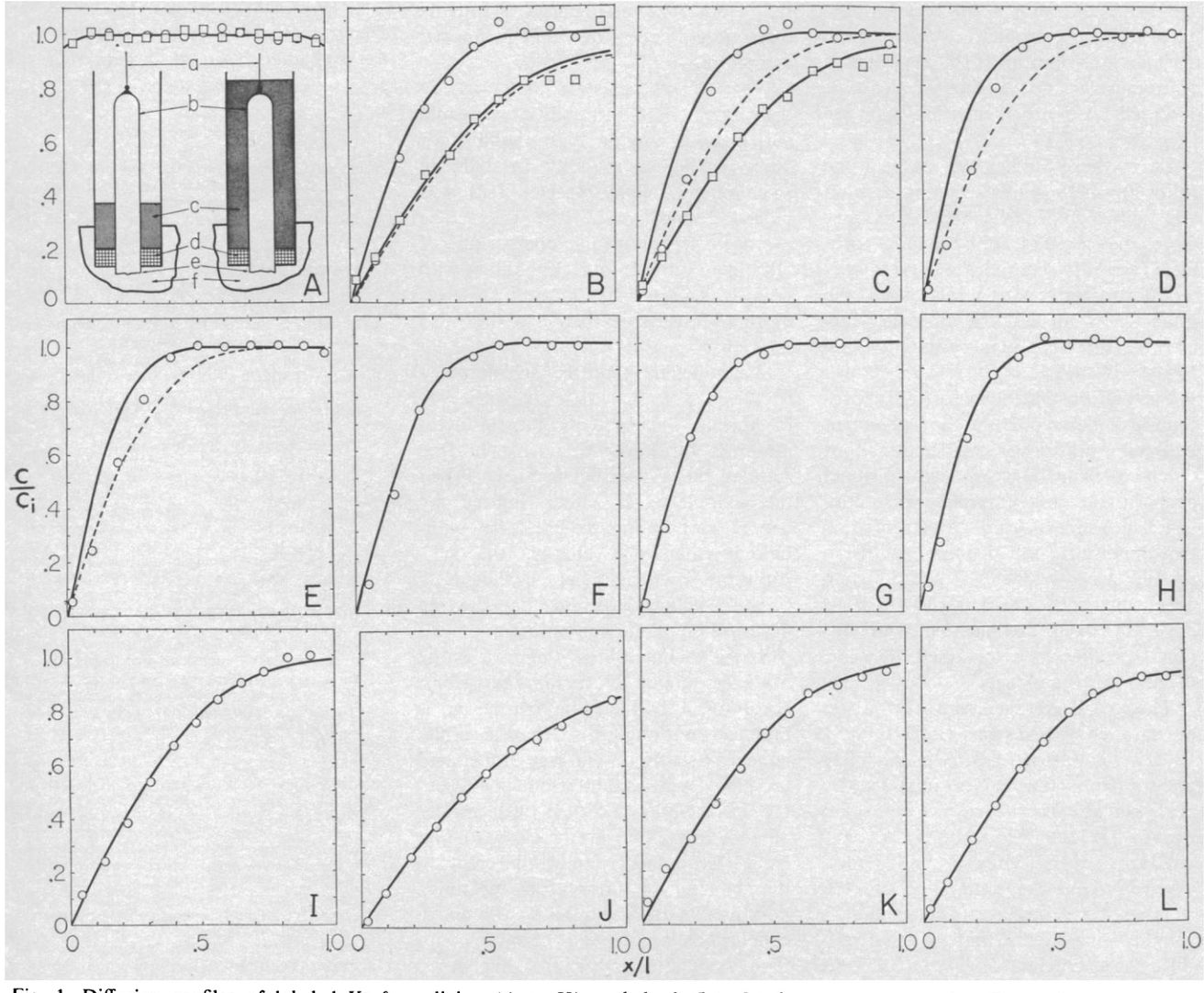

Fig. 1. Diffusion profiles of labeled K^+ from living (A to H) and dead (I to L) frog sartorius muscles. The ordinate represents the concentration (C) of labeled K^+ as a fraction of the initial concentration (C_i) in the muscle. The abscissa is x/l where x is the distance from the cut end and l is the total length of the muscle. Open circles represent ^{42}K, open squares represent THO. (A) The curve at the top shows the initial distribution of ^{42}K throughout the length of the muscle. The figures at the bottom illustrate two types of EMOC setups with varying levels of Vaseline: *a*, anchoring string; *b*, sartorius muscle; *c*, Vaseline; *d*, silicone rubber plug with close-fitting slit; *e*, cut end of muscle; *f*, washing solution. (F to L) The solid lines are theoretical diffusion profiles that fit all the experimental points on the the basis of a single value $D_K t/l^2$. (B to E) The solid lines going through most of the open circles are based on one $D_K t/l^2$ value, and the dotted lines going through the open circles near the cut edge are based on another value. The incubating solution for normal muscles contained 2.5 m*M* labeled K^+; that for muscles killed with IAA contained 5 m*M* labeled K^+ (to provide enough counts). The lengths of the muscles in centimeters; the duration of washing in hours; the initial concentrations of labeled K^+ in micromoles per gram, fresh weight; and the D_K values for ^{42}K for all or the majority of points in 10^{-6} square centimeters per second are: (B) 2.1, 12.6, 63.2, 2.94; (C) 2.1, 9.0, 84.9, 2.72; (D) 2.1, 8.5, 67.0, 2.61; (E) 2.0, 8.5, 64.0, 1.97; (F) 2.0, 12.0, 61.2, 2.22; (G) 2.1, 12.6, 67.8, 2.80; (H) 2.0, 13.0, 42.0, 1.39; (I) 2.6, 17.0, 5.1, 8.78; (J) 2.1, 21.0, 5.1, 9.35; (K) 2.15, 8.5, 5.0, 16.4; (L) 2.15, 8.4, 5.3, 19.6. For the sagging points in figures with disjunct profiles the values of D_K for ^{42}K in 10^{-6} square centimeters per second are: (B) 12.7; (C) 8.18; (D) 6.52; (E) 4.59. The values of the diffusion coefficient for THO are (B) 1.18×10^{-5} and (C) 1.43×10^{-5} cm²/sec.

in dead mucles as in the study of live muscles. On the other hand, if the disjunct diffusion profile arises from the separation of the muscle into healthy and deteriorated regions, each with a different D_K for K^+, one would anticipate no sagging in a uniformly dead muscle.

To choose between these interpretations, we studied the diffusion of labeled K^+ in sartorius muscles killed with iodoacetate (IAA), which were in rigor but kept at their natural lengths. The results show that the disjunct diffusion profile is due to cytoplasmic deterioration. In 19 sets of dead muscles, no sagging of the type observed in normal muscle was seen (Fig. 1, I to L).

From these studies we evolved the following method of extracting data on ^{42}K diffusion in healthy cytoplasm from the muscles with disjunct profiles: we fit theoretical curves only to the majority of points from the intact portion of the muscle and ignored the few sagging points (see Fig. 1, B to E).

Further justification for this procedure is derived from the agreement between diffusion coefficients thus obtained from muscles with disjunct profiles and from muscles with nondisjunct profiles: from 22 sets of data that showed no disjunct diffusion profile, we obtained a self-diffusion coefficient of $(2.70 \pm 0.13) \times 10^{-6}$ cm^2/sec; from 50 muscles with disjunct profiles, we obtained $(2.61 \pm 0.11) \times 10^{-6}$ cm^2/sec.

The gross average from all 72 sets of data combined was $(2.63 \pm 0.08) \times 10^{-6}$ cm^2/sec (*11*), which differs greatly from the values obtained by Hodgkin and Keynes (*2*) from squid axons or the value $(1.02 \times 10^{-5}$ $cm^2/sec)$ of Kushmerick and Podolsky (*3*) from the same type of cells as those we studied. Our value is roughly one-eighth that of the self-diffusion coefficient of ^{42}K in 0.1*N* KI solution $(2.005 \times 10^{-5}$ $cm^2/sec)$ (*12*) and rather close to the value of the self-diffusion coefficient of Na^+ in a sulfonate ion exchange resin, 1.95×10^{-6} cm^2/sec at 23°C (*13*).

Two sets of data (*14*) suffice to illustrate that the slow diffusion of K^+ in living muscle cannot be attributed to nonspecific mechanical barriers, which would have impartially slowed down the diffusion of tritiated water (THO) as well as K^+ in double-labeling experiments. In fact, in muscles where the diffusion coefficients for ^{42}K were only one-seventh of the value found in free diffusion, those of THO were more than one-half of the value in free diffusion (Fig. 1, B and C).

By fitting only the sagging points from the sets of data with disjunct profiles, we could also make a rough estimate of an apparent diffusion coefficient in this deteriorated region (Fig. 1, B to E). The value so obtained, $(7.60 \pm 0.37) \times 10^{-6}$ cm^2/sec (from 50 experiments), is roughly three times higher than that in normal cytoplasm.

Now if cytoplasmic deterioration truly increases K^+ mobility, we would anticipate a similar high mobility or an even higher mobility for ^{42}K in a completely dead muscle. This was indeed found to be the case. The average self-diffusion coefficient of ^{42}K from all 19 muscles killed with IAA is $1.47 \pm 0.10) \times 10^{-5}$ cm^2/sec, or three-fourths of that in a 0.1*N* KI solution.

We conclude that the self-diffusion coefficient of K^+ labeled with ^{42}K in normal healthy frog muscle cytoplasm is far lower than that in free solution in a specific manner. When the cytoplasm is either injured by nearby amputation or killed by withholding metabolic energy, the self-diffusion coefficient of labeled K^+ rises to values close to that of free diffusion in a Ringer solution.

These conclusions offer strong evidence against the membrane theory, according to which the cytoplasm is in effect nothing more than a dilute aqueous solution of free ions and proteins, with a diffusion coefficient for intracellular K^+ (as well as for other solutes) uniformly close to that in a free solution and indifferent to the effects of injury or metabolic poisons. On the other hand, the above conclusions are in full harmony with the association-induction hypothesis (*5*), according to which the living cytoplasm represents a system of closely associated proteins, water, and ions delicately balanced in a metastable state. In this metastable state, K^+ is selectively absorbed on β- and γ-carboxyl groups of cytoplasmic proteins with a free energy of adsorption equal to -3.82 kcal/mole (*15*) and hence a low diffusion coefficient. Water which is adsorbed in polarized multilayers, with far lower free energy, moves far more freely (*16*). The sustenance of the metastable state depends on the adsorption of adenosine triphosphate (ATP) on cardinal sites (*17*). When injury or iodoacetate arrests the continued production of ATP (and possibly the proper functioning of other cardinal adsorbents), the preference of the protein anionic sites for K^+ is lost (*5*) and the K^+ thus liberated moves with a diffusion coefficient close to that found in a free solution.

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L-Dopa in Legume Seeds: A Chemical Barrier to Insect Attack

Abstract. *Seeds of the genus* Mucuna *display both a remarkable immunity to attack by seed-eating insects and an unusually high concentration of free* L-*dopa. When seed powders or pure* L-*dopa were incorporated into artificial diets and fed to southern armyworm larvae, mortality increased, and abnormal pupae and adults resulted. At higher concentrations* L-*dopa acted as a feeding repellent.*

Mature seeds of the Central American genus *Mucuna* are conspicuously free from attack by small mammals and insects (*1*). Free L-dopa (3,4-dihydroxyphenylalanine), well known as a drug used for the treatment of Parkinson's disease in man, has been found in the embryos (including cotyledons) of six species of *Mucuna* at concentrations varying from 5.9 to 9.0 percent (*2*). In addition, these species contain varying concentrations of a cyclic imino acid (3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) which is assumed to be a derivative of L-dopa (*3*). We would expect that the metabolic expenditure required to synthesize and store such concentrations of an unusual free amino acid would be offset by some significant advantage conferred on the seeds by its presence. Bell and Janzen (*2*) have suggested that L-dopa serves a protective function in *Mucuna* seeds, discouraging attack by insects and small mammals. We confirm that L-dopa in low concentrations can produce toxic effects in at least one species of insect. At the higher concentrations found naturally in whole seeds of the genus *Mucuna*, this compound inhibits feeding completely.

The insect chosen for the bioassay of *Mucuna* seeds was the southern armyworm, *Prodenia eridania* Cramer (Noctuidae) (*4*). The caterpillar of this species is widely polyphagous with an exceptional ability to detoxify many foreign compounds (*5*) and, presumably, many toxic secondary chemicals encountered in its food plants (*6*). Demonstration of any repellent or toxic effects to an insect with such generalized feeding habits should be of broader interest than similar results with a more specialized insect. Since the armyworm is phytophagous, whole *Mucuna* seeds were ground and offered to larvae as a component of an artificial diet. The diet was a modified version of the one developed by Feeny (*7*). The fungicide methyl-*p*-hydroxybenzoate, which had been incorporated routinely into the diet, was eliminated, since analogs of that compound are inhibitors of tyrosinase activity (*8*). Twenty early fourth instar larvae were reared on each diet under constant conditions (temperature, 21°C during the day, 19°C at night; 30 to 40 percent relative humidity; and a 16-hour light and 8-dark photoperiod).

Table 1. Growth of *Prodenia eridania* larvae on artificial diets containing *Mucuna* seed powders (5 percent) or pure seed chemicals. Initial number of larvae on each diet was 20. Values are expressed as means ± standard error of the mean.

Diet	Mean initial weight (mg)	Mean pupal weight (mg)	Deformed pupae (No.)*	Normal pupae (No.)	Mean adult weight (mg)	Deformed adults (No.)*	Normal adults (No.)
Mucuna pruriens†	21.2 ± 1.0	370.8 ± 11.7	8	9	167.4 ± 6.7	0	15
M. mutisiana†	18.7 ± 0.7	329.7 ± 11.6	4	16	169.5 ± 7.6	1	14
M. holtoni†	10.8 ± .6	321.4 ± 11.3	1	19	163.3 ± 8.3	1	18
M. urens†	10.7 ± .6	309.1 ± 10.9	3	16	163.6 ± 9.6	3	0
Control (5% bean leaf powder)	18.8 ± 1.1	306.6 ± 9.4	0	20	154.7 ± 8.1	0	20
Bean leaf (5%) plus L-dopa (0.25%)	21.3 ± 0.9	328.8 ± 13.6	2	17	157.6 ± 10.0	0	17
M. pruriens (5%) plus L-dopa (0.25%)	19.1 ± .9	417.9 ± 14.0	9	2	176.0 ± 10.1	0	8
L-Dopa (5%)	10.9 ± .6	0‡	0	0	0	0	0
L-Dopa (5%)	62.9 ± 3.5	0‡	0	0	0	0	0
M. pruriens (5%)§	12.5 ± 1.9	335.7 ± 18.0	15	2		1	0
M. pruriens (5%)§	15.7 ± 0.9	347.2 ± 16.1	13	0	185.6 ± 17.9	2	0
Bean leaf (5%) plus L-dopa (0.25%)§	15.6 ± .8	290.7 ± 8.0	14	6	135.7 ± 8.9	3	7
Bean leaf (5%) plus cyclic imino acid (2.0%)‖	14.4 ± .2	281.1 ± 8.1	0	20	131.0 ± 9.2	0	20

* Pupae were deformed through failure of the pupal case to sclerotize over the ventral head, thoracic, and first three abdominal segments; adult deformity was manifested as malformed or stunted wings. † *Mucuna pruriens* seeds were collected on Isla Providencia, Colombia, 25 August 1969, and near Puntarenas, Puntarenas Province, Costa Rica, 11 March 1971; *M. mutisiana* seeds were collected on the Isla Providencia, Colombia, 24 August 1969. Seeds of *M. urens* and *M. holtoni* were gifts of Dr. W. H. Tallent, U.S. Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois. ‡ All larvae starved to death. §Methyl-*p*-hydroxybenzoate (0.1 percent) was added to the diet. ‖ 3-Carboxy-6,1-dihydroxy-1,2,3,4-tetrahydroisoquinoline, isolated from *M. mutisiana* seeds.