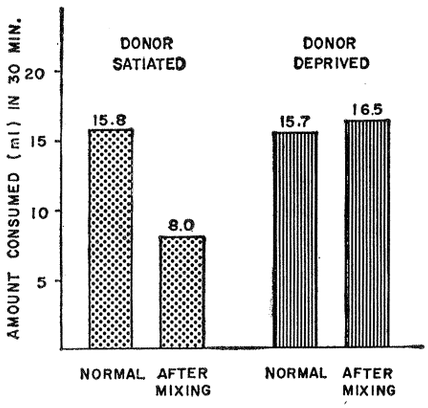

Fig. 1. Mean intake of food in 30 minutes by deprived animals. (Left) Mean intake on the day prior to transfusion (normal) compared with mean intake of the same animals immediately after mixing of their blood with that of a satiated donor (after mixing). (Right) Same comparison for animals whose blood was mixed with blood of donors deprived for 24 hours.

Only two of the 16 satiated rats did any drinking in the 30 minutes after transfusion (one drank 2.0 ml; the other drank 0.5 ml). We then investigated the possibility that our method of blood mixing caused debilitation which resulted in reduced intake of food. For 2 weeks, male albino Sprague-Dawley rats (14 pairs) were adapted to the milk diet and feeding schedules of the above-mentioned study. Twenty-four hours prior to blood mixing, all food was removed from the cages of the free-feeding animals. Blood was transferred 30 minutes prior to the usual feeding time of the animals on the deprivation schedule. Blood mixing in this experiment was thus between a pair of hungry rats rather than between a hungry and a satiated rat (Fig. 1). The difference in the intake before and after transfusion is not statistically significant ($t = 1.09$). The mean intake of the animals which had free access to food until 24 hours before blood mixing was 13.9 ml in the 30-minute period immediately after blood mixing.

Our two studies clearly indicate that the blood of an animal given free access to food contains a factor inhibiting food intake. We also investigated the possibility that a humoral factor might control food intake in animals fed only once a day for 30 minutes (*6*). In this experiment all rats were maintained for 2 weeks on a deprivation schedule with access to diluted condensed milk for 30 minutes in each 24 hours. Then, immediately before blood mixing, one member of each of ten pairs of rats was permitted to drink diluted milk for 30 minutes, by which time drinking had stopped. The blood of these animals was then mixed with that of the animals that had not been permitted to drink. Mixing in this case was between freshly satiated rats and those that had been hungry for 24 hours. Immediately after transfusion, each animal was given access to milk for 30 minutes. The mean 30-minute intake of the hungry animals on the 5 days before transfusion was 13.7 ml; this intake immediately after blood mixing with freshly satiated rats was practically identical (13.8 ml). The mean intake of the animals permitted to eat before blood mixing was 2.1 ml in the 30-minute period after blood mixing.

We found no evidence for the existence of humoral factors that increase food intake. When animals are permitted to eat for only 30 minutes a day, the volume ingested is probably limited by satiety of oral and gastric origin (*7*) rather than by humoral factors. However, our studies strongly suggest that humoral factors acting to limit food intake accumulate in animals with free access to food and that these factors disappear from the bloodstream during a 24-hour fast. Lack of evidence for a factor that increases food intake indicates that this intake is regulated by satiety rather than hunger factors. When animals are maintained on a free feeding schedule, they apparently start to eat when a blood factor falls below a critical level and not when a hunger factor builds above a threshold level.

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Strophanthidin-Sensitive Transport of Cesium and Sodium in Muscle Cells

Abstract. *Uptake of cesium-134 ions into muscle cells is reduced to very low values by the presence of 10^{-5}M strophanthidin in the Ringer solution. Cesium ions can induce extrusion of sodium from muscle cells in which the intracellular sodium content is elevated. The cesium-induced extra efflux of sodium-22 is inhibited by the external presence of 10^{-5}M strophanthidin. The coupling between inward movement of cesium and outward movement of sodium appears to be chemical in nature. The evidence suggests that cesium ions are transported into muscle cells by a system of sites or carriers that requires a source of metabolic energy for ion turnover to occur.*

The cesium ion crosses muscle- and nerve-cell membranes at rates much lower than those observed for the potassium ion, even though their aqueous mobilities and electrochemical behaviors are similar. Muscle cells, for example, undergo only a slight amount of swelling when placed in Ringer solutions containing elevated concentrations of CsCl, whereas, in similar solutions containing KCl, great and rapid swelling occurs (*1*). Also, cesium ions carry only small ionic currents across the membranes of giant nerve cells from the squid (*2*).

Though cesium movement is slow in frog skeletal muscle, the membrane specificity to potassium and cesium ions is similar enough for the two ions to show competitive effects during their entry into the cells (*1*). Rubidium ions compete with cesium ions for entry and it seems likely, therefore, that the mechanism of penetration is the same for both ions.

Adrian and Slayman (*3*) have demonstrated inhibition by the drug strophanthidin of entry of rubidium ions into sodium-enriched muscles. Sjodin (*1*) showed that rubidium ions can

Table 1. Effect of strophanthidin on uptake of cesium: the final cesium contents of members of pairs of muscles placed in 2.5-mM cesium Ringer solutions with and without $10^{-5}M$ strophanthidin (Str). Uptake leveled off rapidly in the presence of strophanthidin, so that the longer the experiment the greater the difference between experimental and control values. Cesium contents were determined from uptake of Cs^{134} and were checked by flame photometry by use of a cesium filter.

Uptake duration (hr)	Final Cs content of muscle (μmole/g)	
	Control	With Str
2.5	1.7	0.3
2.5	2.0	.7
4.0	1.1	.3
20.0	14.5	1.4
19.0	10.0	1.0
21.0	14.3	2.0
3.0	1.5	0.5
20.0	10.8	2.1
12.0	9.8	2.2

substitute for potassium ions in promoting outward transport of sodium, and Bolingbroke *et al.* (*4*) demonstrated increased inward transport of rubidium in sodium-enriched muscle cells. These findings indicate a coupling between inward movement of rubidium and outward movement of sodium under these conditions. Adrian and Slayman (*3*) present additional evidence indicating that the inward transport of rubidium ions is active in nature; that is, rubidium ions are "pumped" into muscle cells. Our purpose was to test the hypothesis (*1*) that cesium and rubidium ions have the same mode of entry into muscle cells.

First we determined the sensitivity of cesium influx to strophanthidin. The inward movement of cesium ions in the presence and absence of strophanthidin was measured by use of Cs^{134} as a tracer. The method for determining uptake has been described (*5*). Sartorius muscles from the frog *Rana pipiens*, tied to platinum frames at their normal resting lengths, were placed in radioactively labeled Ringer solution composed of [Na], 105 mM; [Ca], 2 mM; [Cs], 2.5 mM; and [tris], 1 mM (to buffer pH at 7.4). At regular intervals, muscles were removed from the radioactive solutions, rinsed for 2 seconds in unlabeled Ringer solution of identical composition, and assayed for radioactivity in the well of a γ-ray scintillation counter. Typical uptake curves for a pair of muscles from the same animal are presented in Fig. 1. The lower curve came from a muscle exposed to cesium Ringer solution containing also $10^{-5}M$ strophanthidin; the upper curve shows the uptake of cesium by the paired control muscle in the absence of strophanthidin. The presence of strophanthidin markedly inhibited uptake of Cs^{134} by all nine pairs of muscles studied (Table 1).

This behavior is to be expected if cesium influx is in some way coupled with the sodium efflux that is already known to be inhibited by strophanthidin (*6*). As a test for the presence of such an ionic coupling, muscles were brought to a state in which they produce a strophanthidin-sensitive, net, outward extrusion of sodium ions against an electrochemical gradient. It is well known that muscle cells whose sodium content has been elevated can actively extrude sodium ions in the presence of potassium ions in the external solution; under such conditions the sodium ions leaving the cells are replaced by potassium ions (*7*). It has been pointed out that rubidium ions can replace potassium ions in the promotion of sodium extrusion. To determine whether cesium ions also can substitute for potassium ions in the promotion of sodium extrusion, sartorius muscles were enriched in sodium by overnight immersion in Na^{22}-labeled, potassium-free Ringer solution at 4°C. The efflux of Na^{22} ions was then measured at 20°C in the presence of external potassium (5 mM) as a control, and also in the presence of external cesium at a concentration of 25 mM. The reason for selecting a cesium concentration of 25 mM was that entry of cesium into muscle cells is much lower than that of potassium, and the depolarization of muscle-cell membrane produced by 25-mM cesium very nearly equals that produced by 5-mM potassium (*8*). Because of the low penetration rate, CsCl is osmotically active in the bathing medium (*1*); therefore, the sodium concentration in the cesium Ringer solution used during efflux was lowered to 80 mM. To keep the energy barrier for sodium extrusion the same for all muscles during measurement of efflux, the sodium concentration was 80 mM in all efflux solutions. An appropriate amount of sucrose was used in the potassium-free and 5-mM potassium Ringer solutions to maintain osmotic balance.

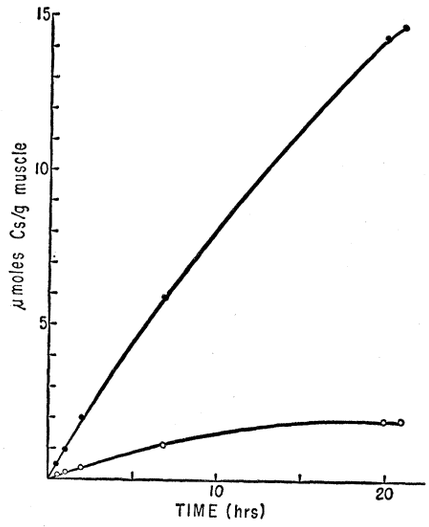

Fig. 1. Uptake of Cs^{134} ions by frog sartorius muscle cells in the presence and absence of $10^{-5}M$ strophanthidin in the Ringer fluid. Lower curve represents the presence of strophanthidin; upper curve (for the other of a pair of muscles) represents the absence of strophanthidin.

Both experimental and control members of pairs of muscles were placed in potassium-free solutions for the first 80 minutes of efflux in order to establish the normal base line for loss of Na^{22} when no net extrusion of sodium was taking place. Then the control muscle was placed in 5-mM potassium Ringer solution for 1 hour, while the experimental muscle was placed in 25-mM cesium Ringer solution for the same time. For the final hour of efflux, the compositions of the Ringer solutions for both muscles were unchanged except for the addition of $10^{-5}M$ strophanthidin.

A typical semilogarithmic plot of the efflux obtained from such an experiment is presented (Fig. 2). The results remarkably confirm the correctness of the earlier guess that cesium ions en-

Table 2. Rate constant for loss of Na^{22} in the presence of cesium ions, compared with rate constant measured in the presence of potassium ions; the effect of $10^{-5}M$ strophanthidin (Str) is included.

Muscle	Rate constant (hr^{-1}) in Ringer solution plus:				
	No K or Cs	5-mM K	5-mM K and Str	25-mM Cs	25-mM Cs and Str
Control	0.5	1.44	0.64		
Experimental	.5			1.38	0.49

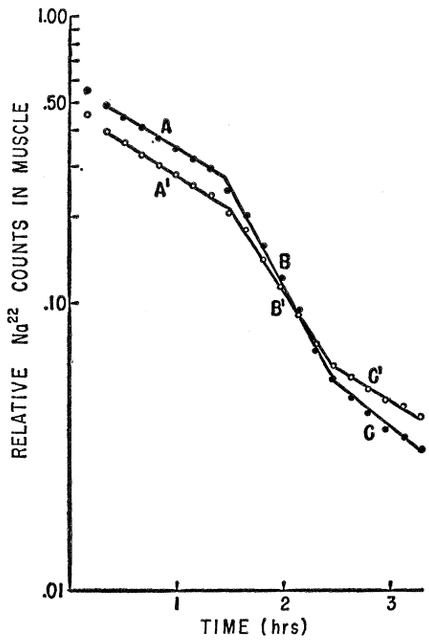

Fig. 2. Semilogarithmic plots of efflux of Na^{22} ions from sartorius muscle cells having elevated contents of sodium. Solid circles refer to a muscle from which the extrusion of sodium was stimulated by the presence of 5-m*M* potassium in the Ringer fluid (*B*). Open circles refer to the other of the pair of muscles from which extrusion of sodium was promoted by the presence of 5-m*M* potassium in the Ringer fluid, with no potassium ions present (*B'*). Curves *A* and *A'* were obtained in the absence of potassium and cesium ions from the bathing medium; *C* and *C'*, in the presence of $10^{-5}M$ strophanthidin (see text). The primes refer to the curve obtained from the experimental muscle exposed to cesium ions.

gage in strophanthidin-sensitive coupled exchange with sodium ions. It is evident that cesium ions at a concentration of 25 m*M* have a stimulating effect on the sodium-pumping rate that is very nearly equivalent to the effect of potassium ions at a concentration of 5 m*M*. Table 2 summarizes the rate constants, for loss of Na^{22}, obtained from the curves of Fig. 2.

These results lead to the conclusion that almost all the movement of cesium into muscle cells is by way of a mechanism that is more or less completely blocked by strophanthidin at a concentration of $10^{-5}M$. Furthermore, cesium ions can stimulate extrusion of sodium against an electrochemical gradient. One is tempted to surmise that cesium ions are actively transported into muscle cells although the data do not prove this point; movement of cesium was not against an electrochemical gradient in our experiments. It is possible that the sodium "pump" generates an electric-potential difference across the muscle-cell membrane (*9*), that pulls cesium ions into the cells. Opposing this possibility is the low permeability of the membrane to cesium ions (*1, 8*). The initial sodium-pumping rates observed when muscles were placed in the 25-m*M* cesium Ringer solution were around 30 μmoles of Na^+ per hour per gram of muscle. As this rate is several times the rate of inward movement of cesium ions normally observed, considerable hyperpolarization would be required for purely electrically controlled movement of cesium.

These findings suggest that the coupling between extrusion of sodium and inward movement of cesium is mainly chemical in nature rather than electrical. The amount of sodium efflux that is coupled to cesium influx must depend upon the internal concentration of sodium and the external concentration of cesium. Inasmuch as almost all the inward movement of cesium is by way of a mechanism that depends on metabolism, the purely passive membrane permeability to cesium ions is remarkably low—roughly equal to the membrane permeability to sodium ions.

The ability of cesium ions to substitute for potassium ions in the promotion of outward transport of sodium is about the same in squid giant axons as in muscle cells (*10*). This similarity suggests that the ionic transport mechanism may be the same in these two instances.

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Spruce Budworm Mortality as a Function of Aerial Spray Droplet Size

Abstract. *The size and number of aerial spray drops impinging on spruce budworm in its conifer forest habitat were determined by means of a new tracer method that uses fluorescent particles in a liquid spray. Examination of 1113 larvae affected by an experimental insecticide that had been applied to a 5000-acre (2024-ha) test area in Montana showed that 93 percent had not been contacted by any droplets larger than 50 μ in diameter. Small numbers of droplets 50 to 100 μ in diameter were found on 7 percent of the larvae, along with lethal numbers of smaller drops. No evidence was found that significant numbers of drops larger than 100 μ reached the target insects. Because about 95 percent of the spray applied to forests by current methods consist of droplets larger than 50 μ, the biologically effective portion of the drop spectrum is only a few percent. The data foreshadow a major potential reduction in insecticide requirements for the successful control of spruce budworm.*

The mountain forest is a formidable problem to the scientists who must control destructive forest insects in their natural habitat while minimizing adverse ecological changes. The typical mountain conifer forest is a highly complex, three-dimensional environment. Its rugged terrain with deep drainages, steep slopes, and high ridges fosters a complex meteorological situation. From the top of the trees to ground level, the conifer forest itself adds significant vertical dimensions to this environment and provides a habitat with both food and protection for insect pests.

In spite of these complexities, aerial spray techniques have been developed, largely by empirical methods, which successfully control many destructive insects. One of these is the spruce budworm, perhaps the most destructive forest defoliator in the United States and Canada. Until recently, it was controlled by aerial applications of DDT, in a broad-spectrum spray whose droplets ranged from about one or a few microns in diameter to as large as 300 to 400 μ. The persistence of DDT—which was most undesirable from the standpoint of hazard to beneficial insects and other nontarget or-