THE MOVEMENT OF POTASSIUM IONS

IN NORMAL AND DYSTROPHIC MOUSE MUSCLE

by

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ABSTRACT

The radioactive isotope K^{42} was used to measure the rate of potassium exchange in muscle from 129 strain mice. The results followed an unique course if plotted as K^{42} uptake versus (external potassium concentration • time)$^{\frac{1}{2}}$, and corresponded to the result predicted for K^{42} uptake mediated by an ion-exchange compartment in the muscle. Variations in external potassium concentration did not affect the uptake rate if plotted this way, but sodium ion exerted some effect on the rate. Dystrophic mouse tissue accumulated K^{42} more rapidly than did normal tissue, and the effect of varying the external potassium concentration did not alter this rate. The effects of sodium variation were more pronounced than in normal tissue.

Inulin space of muscle was measured *in vivo* as well as *in vitro*, to enable a correction for K^{42} in the extracellular space to be made. The inulin space was found to decrease with increasing muscle size, and this was thought to be related to the development of the muscle. Dystrophic muscle exhibited more of a dependance of inulin space on muscle size than did normal muscle. The suggestion was made that the dystrophic muscle membrane might be abnormally permeable to inulin.

Muscles were excised and assayed by flame photometry for sodium and potassium content. They were assayed when freshly excised, and also following incubation in a variant of Locke's solution. The muscle cations were stable for the first two hours of incubation, but after this time, intracellular

sodium rose and potassium fell. Fresh dystrophic mouse muscle had lower potassium and higher sodium content than normal fresh muscle. The cation changes following incubation resembled those found for normal muscle. The changes in intracellular cations were correlated with the K^{42} uptake results, and discussed in some detail.

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A. PURPOSES OF THE STUDY

The investigations reported in this thesis centre around two objectives:

to investigate and measure the rates of movement of potassium into mammalian skeletal muscle under varying environmental conditions, with the aid of the radioactive isotope K^{42}, and

to compare the rates of movement of the ion in normal and dystrophic mouse skeletal muscle.

The results were to be expressed as m. equiv. intracellular K moving per $(K_e \cdot t)^{\frac{1}{2}}$ unit. Thus, several problems required solving; the non-inulin space volume had to be determined, involving an estimation of the extracellular inulin space and a determination of the dry weight of the muscle; the normal concentration of K in fresh muscle tissue also had to be known. As the experiments would involve incubation in a modified Locke's solution for several hours, a knowledge of the intracellular ionic concentration changes due to incubation was necessary. Net alterations in K level during incubation could then be appropriately corrected.

The concentrations of sodium and potassium in some of the incubation fluids were altered, to see if differences in extracellular concentrations of the ions would affect the rate of K entry into the muscle fibre.

Concomitant observations of muscle resting potentials under similar experimental conditions have been investigated separately by Professor H. McLennan.

B. GENERAL ASPECTS OF BIOELECTRICITY

1. The Composition of Tissues

It is a characteristic of the excitable cell to exhibit a resting potential difference between its environment and the cell interior. It is also characteristic for protoplasm and tissue fluid to contain differing concentrations of certain ionic species, the most obvious examples being the alkali metal ions sodium and potassium (Na and K), which are present in high concentrations; this is illustrated in Table I. Other substances are also distributed unequally between the cell interior and environment; Table II, from Conway (1950), shows values for some substances found in frog and rat muscle.

To maintain the ionic balance of excised tissue, artificial fluids were devised to enable the tissue, when incubated in the fluid, to survive and function "normally". Sydney Ringer (e.g. (1883)) intensively studied the effect of ions on the frog heart. He observed that small quantities of KCl and $CaCl_2$ added to a 0.7% NaCl bathing solution would enable the heart to beat for hours; without these two salts, it stopped in a few minutes. Locke (1895) demonstrated that glucose had the same effect as addition of the salts. He also altered the tonicity, and used the fluid for perfusing mammalian heart, oxygenating and warming it to body temperature (Locke 1904). Many "physiological" solutions have been devised since then for specific tissues; Ringer-Tyrode for gut tissue, Ringer-Krebs-Hensleit for brain tissue, and Ringer-Conway for frog muscle are a few common ones. These fluids differ principally in the concentrations of their constituents, or have special substances added to them; they are all supposedly in osmotic equilibrium with the tissues for which they are designed.

TABLE I: K, Na, and RESTING POTENTIALS OF EXCITABLE TISSUES*. Ion values expressed as m.equiv. per litre of cell or plasma water. R.P. values in -mV. Calculated values from Nernst Equation.

TISSUE	K		Na		R.P.	
	CELL	ENVIRON	CELL	ENVIRON	CALC.	ACTUAL
FROG - Skel. muscle	124.0	2.2	3.6	104.0	98	92
RAT - Skel. muscle	152.0	4.7	3.0	150.0	87	74
CRAB - Muscle	146.0	12.9	54.0	513.0	61	72
CRAB - Nerve	112.0	12.1	54.0	468.0	60	82
SQUID - Giant axon	369.0	13.0	44.0	498.0	83	65

*Values from Shanes (1958), Ling and Gerard (1949), Hodgkin and Krynes (1955), Conway (1950), and Zierler (1959).

TABLE II: DISTRIBUTION OF SUBSTANCES IN FROG AND RAT MUSCLE. Values expressed as m.equiv. per litre fibre or plasma water. H^+ expressed as pH.

SUBSTANCE	FROG SKELETAL MUSCLE		RAT SKELETAL MUSCLE	
	FIBRE	PLASMA	FIBRE	PLASMA
SODIUM	3.6	104.0	3.0	150.0
POTASSIUM	124.0	2.2	152.0	6.4
CALCIUM	4.9	2.0	1.9	3.1
MAGNESIUM	14.0	1.2	16.1	1.5
CHLORIDE	1.5	74.3	5.0	119.0
BICARBONATE	12.4	25.4	16.0	24.3
PHOSPHATE	7.3	3.1	10.6	2.3
SULPHATE	0.4	1.9	-	-
AMINO ACIDS	8.8	6.9	14.7	3.2
H^+	Ca. 6.9	7.4	Ca. 6.2	7.4

2. Ionic Equilibria

Diffusion is the movement of particles from a region of higher concentration to one of a lower concentration, due to the random migrations of thermally agitated solute particles. In a fixed structure such as NaCl crystal, this movement does not occur; in the crystal lattice, each sodium ion is surrounded by six chlorides, fixed by electrostatic forces in a face-centered cube form. In solution, the ions dissociate from the crystal in equal numbers, and at any point in a homogenous solution, a situation similar to the lattice arrangement will tend to arise, the hydrated sodium ions surrounded by a cloud of hydrated chloride ions, and vice versa. In this solution, there will be no potential difference between any two points (Principle of Microscopic Electroneutrality), for the random movements of the ions cancel any transient imbalance. However, if these ion species are diffusing, the rate of diffusion will be proportional to the square root of their hydrated radii; a chloride ion, with a smaller radius than a sodium ion, will diffuse more rapidly; but as it moves, the rate will tend to be retarded by the attraction of the positive sodium ions left behind, and a small but measurable difference in distribution of electrical charges between various parts of the solution will arise. A region of high salt concentration will be more positive than a region of lower concentration, and a diffusion potential may be measured; this potential will run down as diffusion proceeds, and will disappear when diffusional equilibrium is attained.

To maintain a potential between two compartments, either there must be no net movement of the ions causing the potential, or there must be an indiffusible (impenetrable) ion in one of them. The Gibbs-Donnan equilibrium rule states that in a system where an impermeable

anion is on one side of a fixed membrane, and a solution permeable to the membrane (e.g. NaCl) bathes both sides of it, the ions will become distributed unequally in the two compartments, with more cation and less diffusible anion in the indiffusible anion compartment than in the other; two opposing tendencies will arise in this system: the movement of ions down chemical gradients and the maintenance of electric neutrality in the solutions. The indiffusible anion compartment will be slightly negative with respect to the other, thus cations will try to move in to it; this, however, is against the concentration gradient, and does not occur; also the movement would be retarded by the excess anion in that compartment. The distribution is complicated in living cells by the presence of more than one cation. The differences in ion concentrations of living cells and their surroundings (Tables I and II) are partially due to a Donnan system; there are unequal numbers of ions in the solutions, and the tendency to reach electric neutrality is opposed by the concentration potential across the membrane. If an electrode is placed on each side of the membrane in a Donnan system, a potential of magnitude

$$E = \frac{RT}{zF} \ln \frac{(C^+)_1}{(C^+)_2} \qquad \text{..... (Eq. 1)}$$

can be measured; R is the gas constant, C the cation concentration, z its valency and F is Faraday's constant.

The muscle membrane was formerly thought to be impermeable to Na and Cl; this concept would satisfactorily explain the resting potential as arising from passive distribution of K by the Donnan system, and account for the dependence of the magnitude of the potential on the external K concentration (K_e); the potential of Eq. 1 adapted for potas-

sium is

$$E = \frac{RT}{F} \ln \frac{(K^+)_i}{(K^+)_o} \quad \text{..... (Eq. 2)}$$

The resting potential is linearly related to the log K_e for most values of K_e, but for muscle the slope of plotted experimental observations is not the same as the slope of the Nernst equation values. Boyle & Conway (1941) showed that the membrane is permeable to chloride ions; Steinbach (1940) showed chemically and Heppel (1940) with radioisotopes that frog muscle was permeable to Na. In accordance with these observations, McLennan (1961), working with mouse muscle, found that whereas K_e had the greatest influence on the resting potential, it was also affected by the Na ion ratio; his data were best fitted by the equation

$$E = \frac{RT}{F} \ln \frac{K_i}{K_e} + \frac{0.005\ Na_i}{0.005\ Na_o} \quad \text{..... (Eq. 3)}$$

3. Maintenance of Ionic Gradients

The distribution of muscle K (or Na) during steady state conditions can be explained electrochemically; the resting potential of 60-80 mV will influence the K ions by attracting them; the ions will flow "passively" from a region of high electrochemical potential to a lower one, even though the chemical gradient be opposing the movement; the K ions may move against a 25:1 ratio, and still be moving "passively". This is distinctly different from diffusion, for the latter occurs down a concentration gradient, not "uphill" against a gradient. The term "passive transport" has been coined to describe this phenomenon of ion movement by electric forces against a diffusion gradient. This passive transport does not require metabolic energy

and should not be stopped by metabolic inhibitors.

The Nernst electrochemical potential equation consists of two terms, an osmotic, and an electric, which are opposed; for in transferring an ion against the concentration gradient, osmotic work is done on the system, while electric forces concomitantly try to push the ion back down the gradient. If the two terms are equal, work is zero, and no transfer occurs; the osmotic term RT n Ki/K_e is equal to the potential term zFE.

Sodium ions do not follow this Nernst relationship; how, then, does one account for the Na ion distribution? A mechanism, termed "active transport" was postulated for maintaining the distribution (Dean 1941); a simple definition would be "transport against an electrochemical gradient, using energy derived from cellular metabolism"; consequently, the sodium ion was extruded as fast as it penetrated the cell, keeping the intracellular Na concentration low, and providing a functional impermeability to the ion. In distinction to passive transport, energy was required for this process; a wealth of studies (e.g. references page 60 of Ussing (1960)) showed a dependance of the continued extrusion on a continuous metabolic supply of energy; if Na extrusion could thus be maintained, K ions would tend to equilibrate with the electrochemical gradient of the tissue, i.e. be distributed by a Donnan system.

This theory is able to account for most of the phenomena observed in excitable tissues. However, it has been suggested (e.g. Ling (1953)) that active transport is an unnecessary hypothesis, and that a series of fixed sites that bind K and Na (the binding requiring continuous metabolic energy) would exhibit the properties experimentally observed.

C. TISSUE COMPARTMENTS

In Part B, it was seen that intracellular ion concentrations differ from extracellular concentrations. Tissue fluid ion concentrations in turn differ from those in plasma; thus, it would be misleading to draw off a sample of blood, analyse the plasma, and represent this plasma value as being equivalent to the tissue fluid concentration of the ion, for the plasma sample is not a true representation of the interstitial fluid, i.e., the fluid bathing the cells. The tissue fluid is essentially an ultrafiltrate of plasma, although it contains some protein. A Gibbs-Donnan equilibrium is established between the plasma and tissue fluids; since the plasma protein concentration is greater than the tissue fluid protein concentration, there will be slightly less Na and K, and slightly more Cl in the interstitial fluid than in the plasma; the ratio tissue fluid K/plasma K is theoretically 0.90-0.95; some experimental values reported were as low as 0.75 (Manery 1954); if these latter plasma samples were used to determine extracellular K concentrations, they could have been 25% in error.

Caution must also be used when estimating extracellular Ca or Mg ion; these ions may be partially bound to plasma proteins, for their distribution ratios are much lower than the K or Na values.

Tissue fluid is scant in muscular tissue; it is difficult to sample, for there are no normal accumulations of it; it was estimated that in a muscle belly with an extracellular space (ECS) of 10%, the tissue fluid would be only a 15 micron film covering the surface of the cells. Maurer (1938) punctured frog muscles and drew off small quantities of fluid that he regarded as normal interstitial fluid; his Cl analyses corresponded closely to the calculated values reported by

Gamble (1950). Edema fluid has been analysed (Manery 1954); the ionic concentrations depended upon the amount of protein in the fluid, and corresponded to tissue fluid in ion content only if the quantity of protein in the two fluids was the same.

Accurate intracellular concentrations could not be calculated unless the ECS was known; Na and Cl were considered to be exclusively extracellular ions, and since they appeared not to enter the tissue cells, they were often used as indicators for ECS estimations. The basis for this was that if Na and Cl were found only in the extracellular fluid, then the amount of Cl (or Na) in tissue and plasma samples could be used to calculate the ECS of the tissue. However, muscle cells have been shown to contain both these ions and the possibility of their cellular uptake was suggested to account for the higher values calculated for these spaces. Cl and Na spaces are now used less often for estimating ECS than in the past. Thiocyanate (SCN), a negative ion similar in mobility to chloride (but not normally found in the body), has also been used to determine the ECS of tissues; values for this space were 4% too large according to Pappius and Elliott (1956), for this amount was taken up by the tissue cells.

And "ideal" indicator for ECS determinations (either tissue or whole-body ECS) would be a substance that was not harmful to the animal or its tissues, was distributed evenly in the extracellular fluid, did not penetrate into or adsorb onto the cell, and was readily assayed. The disaccharides maltose and sucrose (MW 360 and 342) and the polysaccharide inulin (MW 991) satisfied these criteria; with the disaccharides, there was the possibility of metabolic utilization, whereas inulin was not metabolized, but excreted from the body unaltered. Radioiodinated serum albumin (RISA) has also been used for ECS studies

(e.g. Tasker, Simon, Johnstone, Shankly and Shaw (1959)); results using this tracer corresponded closely to inulin space estimations. The values for other space estimations varied according to the indicator used; for amphibian sartorii, the spaces measured with Na, Cl, CNS, sucrose, inulin, and RISA decreased in that order; it was suggested that the higher values obtained may have been due to the tracer entering the cells (Tasker, et al,1959).

The volume of muscle ECS must be known to calculate the muscle fibre or non-ECS volume, and ideally it should be determined for each muscle used. This is often impractical, and many investigators have excised paired muscles such as sartorius, analysed one muscle for ions, and assayed the other for ECS. There is usually a variation between the ECS values of the pair, but the value for the companion muscle is a better estimation of the ECS than are group averages. Tasker et al (1959), showed a significant variation of the average ECS in sartorii from different batches of toads; they also found a seasonal variation in ECS and tissue ion content. The authors concluded that applying group ECS values to individual muscles was not a valid procedure because of the large variations among and within groups of muscles.

Each muscle seems to have a characteristic ECS value, which varies with site, age, weight, and condition. McLennan (1956) reported rat diaphragm ECS to be 25%, gastrocnemius 12%, and extensor digitorum longus to be 15%. Tasker et al (1959) found a negative correlation between ECS and muscle weight in excised toad sartorii; this finding was confirmed by Burr and McLennan (1960) for mouse muscle. The latter authors assayed dystrophic tissue as well, and found the ECS to be greater than in normal muscle.

D. ELECTROLYTES AND TISSUES

1. Nerve Tissue

Individual mammalian nerve fibres are too small for convenient study; consequently, many investigators of cation movements in nerve have used giant nerve fibres - the squid and cuttlefish giant axons that are 0.2-1.0 mm. diameter. These nerve fibres exhibited a resting potential of 40-80 mv (inside negative), apparently maintained by an active transport of Na and K; when studied *in vitro* the axons were liable to lose K and gain Na. Hodgkin (1957), Shanes (1955) (1958), and Keynes (1951) wrote review articles on the bioelectric and ionic phenomena observed in nervous tissue. Ion movements associated with nerve activity will not be considered in this thesis.

If nerve is exposed to suitable low-K solutions, it loses K and gains Na; in high-K media, the reverse occurs. The distribution of K between the fibre and its environment seemed to be a Donnan distribution, and it was proposed that Na was pumped out and K equilibrated passively. On the other hand, Hodgkin and Keynes (1955a) showed that there was an apparent active transport of K inwards in giant axons. The inhibitors of Na active transport also reduced the influx of K from sea-water. This drop in K influx equalled the change in Na efflux, suggesting a coupled exchange of the two ions; however, some Na extrusion persisted in K-free solution, and the active Na flux exceeded the active K flux. In these cases, the excess Na must be accompanied by a negative ion, or a cation must enter the cell to balance the electrical loss.

The resting potential did not change when K and Na fluxes decreased after poisoning the nerve (1955a); apparently the active transport *per se* does not affect the potential. The persisting fluxes

may have been due to passive diffusion of the ions; this passive movement did not vary as expected when the resting potential was artificially altered; the flux change was much greater than predicted (Hodgkin & Keynes 1955), and always occurred down the K electrochemical gradient. Hodgkin and Keynes (1955) proposed a "long-pore" membrane model that adequately accounted for the observed phenomena. Excess K ions moving out may have diminished the K influx by competing for sites in the membrane.

2. Muscle Tissue

Approximately half the weight of the human body is skeletal muscle; the individual muscles are composed of groups of cells 1 to 40 mm long and 10 to 40 micra in diameter; simultaneous contraction of the fibres produces a violent shortening of the muscle.

Each fibre is a complete cell, multinucleate, with a membrane enclosing it. In the space between the fibres is tissue fluid, the milieu interier of the body. A potential difference of 60-80 mv (inside negative) exists across the membrane of these cells, due mainly to differing concentrations of K inside the cell and in the tissue fluid; most animal cells, including muscle fibres, are high in K and low in Na (see Table I). Recent publications by Harris (1960), Conway (1957), Ussing (1960), and Harris and Sjodin (1961) discuss K in muscle tissue.

The permeability of muscle to K was first demonstrated in 1916 by Meigs and Atwood, who found that in high KCl solutions, muscles would swell and take up KCl. In 1939, Heppel showed that muscle cells were also permeable to Na, which could replace some of the K in rat muscle; he confirmed this a year later using radioactive Na (Heppel 1940).

Excised frog muscles soaked in ordinary Ringer solution lost K and gained Na; this was prevented if the K concentration in the bathing fluid was higher than normal. Soaking sartorii in K-free fluid reversibly depleted the intracellular K (the K_i). This was accompanied by a reversible increase in Na_i. It was further demonstrated (Creese 1952) that the addition of bicarbonate to the normal medium decreased K loss. In high-KCl solutions, the fibres swelled and gained KCl. These solutions resulted in the replacement of some of the Na_i by K; however, the resting potential fell due to the lowered K_i/K_e ratio. Chloride therefore entered the cell more readily, and K plus an osmotic equivalent of water moved with it, to maintain electrical and osmotic neutrality; consequently, the cells swelled. If non-penetrating anions such as methyl-sulphate were used, the swelling was markedly reduced.

Dean (1941) proposed the "Na pump" theory to explain the maintenance of Na and K gradients; Keynes (1954) suggested that K was the exchange partner for the pump (resting potential must be kept constant), for a reduction of Na efflux occurred in K-free bathing fluids, and an increase in K-rich fluids. Steinbach (1952) showed an optimum K_e for Na extrusion in Na-loaded muscle; he suggested that K diffusion in the muscle was the limiting factor in the exchange of Na for K.

The low K_e of interstitial fluid could affect K exchange with muscle fibres, because a small rise in K_e could lead to an appreciable back diffusion into the fibre; this factor could lead to incorrect interpretation of tracer flux calculations. Harris and Burn (1949) found the fibre-interspace exchange to be about equal in rate to the interspace-soaking solution, if the muscle fibres impede

the movements of the ions. McLennan (1956) (1956a) (1957), working with mammalian muscle discussed the problem at some length, and concluded that in high phosphate solutions the interspace K diffusion rate was one-twentieth of the free solution diffusion values. With similar assumptions regarding the interspace K diffusion rate, Harris and Burn calculated the K ion flux across frog muscle membrane to be 1.1×10^{-6} m. equiv./cm^2/min, close to McLennan's value of 1.4 for rat muscle. The latter found that K^{42} efflux from equilibrated muscles could be resolved into two exponentials with different time constants; there appeared to be an inexchangeable fraction of muscle K (more at low temperature), but an increased K_e would enable all K to exchange. The presence of phosphate in the bathing solution appeared to prevent K loss, but impeded K diffusion; McLennan suggested the phosphate might have formed a complex that could bind K, and the slow dissociation of the complex resulted in an abnormally low apparent diffusion rate for K. Creese (1960) showed that deeper diaphragm fibres exchanged less K than more superficial ones; the turnover of total muscle K, at 38°C was apparently complete, but individual cells exchanged faster than the total muscle (Creese, Neil and Stephenson 1956).

Frog muscle studies using K^{42} tracer showed a rapid phase of movement of an amount of K, too large to be simply that of the extracellular space, and less than the total fibre K. The amount of K in this fast portion seemed to vary with the K_e of the bathing solution (Carey and Conway (1954)). The authors concluded that some of the K and most of the fibre Na was in a "special region", one that had unique ion exchange properties. Na that replaced K in tissue appeared to differ from "normal" Na: the efflux into non-electrolyte solutions was slower, High K_e solutions increased the efflux more than usual, and the

temperature dependance was greater (Conway & Carey 1956). Possibly this Na had replaced K in this "special region"; if only a limited number of sites to bind cations were available, competition for them might have retarded K or Na movement into or out of the cell.

E. INTERPRETATION OF RESULTS: MUSCLE MODELS

The "classical" concept of a muscle cell involved a protoplasmic mass enclosed by a thin, resistive membrane, one with no capacity to store ions, and through which ions passed when entering or leaving the cell; the resistance supposedly varied for each ionic species, as some ion species could enter more easily than others; the membrane was considered as the rate-limiting step for ion movement into or out of the cell. It was assumed that extracellular diffusion would maintain the tissue fluid ionic composition constant, and that intracellular diffusion was more rapid than the rate of movement through the membrane into the cell; consequently, the intracellular ions became homogenously distributed, and new ions entering the cell would readily diffuse and equilibrate within it. The characteristics of ion uptake therefore depended upon the rate of internal mixing and the resistance of the membrane, assuming that the membrane contained no ions; thus the uptake should have followed first-order kinetic relations, i.e., have a simple exponential time course. But it was necessary to use two or three exponential terms to interpret experimental observations of ion movements instead of a single one; various "fractions" of muscle K were assigned to each exponential, but the total K in the fractions did not always equal the total muscle K. If muscles were incubated in radioactive K solution, and if full exchange had not been reached, there appeared to be a portion of the muscle K that did not undergo exchange;

however, the amount of exchangeable K increased if the K_e of the bathing solution was above normal. Radioactive K also failed to become uniformly mixed with frog muscle K after limited exposure to the tracer (Harris & Steinbach 1956); the specific activities of successive extracts of these muscles diminished rapidly; the inhomogeneity of the K seemed to be intracellular, and not due to a greater degree of exchange of superficial than deep fibres. The membrane theory inferred that the tracer efflux from loaded muscles would be independent of the time of loading, but this result has not been confirmed; muscles exposed for a short time appeared to have more K that would exchange rapidly than others incubated for a longer time (Harris 1953). Apparently this thin resistive membrane model did not adequately interpret the kinetics of ion movements; Ling, Shaw et al, and Harris have each presented hypotheses in an attempt to explain their observations more completely.

Ling (1952) (1955) (1960) proposed a "fixed-charge" hypothesis to explain selective K accumulation by muscle cells. The hypothesis maintained that free moving ions (such as K and Na) tended to approach fixed anions (e.g. protein chains) as closely as their charge would allow; since hydrated K is smaller than hydrated Na, it moved closer to the fixed anion; this represented a lower energy state for K than for Na, and the K would be held in a more stable manner. Since a system will tend to attain its lowest energy state, the model proposed would take up K rather than Na from a solution containing both ions. Ling further stated that this K was not chemically "bound", but could detach from the anion by acquiring enough thermal energy, or by replacement by another ion of the same species. Ions with a greater "absorption energy" would replace a "bound" cation (absorption energy varies as 1/hydrated radius); but Rb (with a smaller hydrated radius

than K) was found to compete for these fixed sites, instead of replacing the already bound K. The anionic sites were maintained by energy from cellular metabolism; poisoning the muscle led to K loss and Na gain, but poisoning of an "active transport" system was not necessary to explain this shift; a lack of ion binding gave the same result.

The theory could not adequately explain cation shifts in cooled muscle; neither did it account for Na replacing K intracellularly, nor the sequence of relative cation permeability of the muscle.

Shaw and his collaborators exhaustively investigated the relations of Na, K and Cl of toad sartorii (Shaw, Simon & Johnstone 1957) (Simon, Johnstone, Shankly & Shaw 1959) (Frater, Simon & Shaw 1959) (Simon, Shaw, Bennett & Muller 1957); they found that muscle K did not exchange as expected when K_e was altered, but that Na and Cl movements were independent of K movements or concentrations. The muscles did not swell in hypertonic KCl, but did in high NaCl solutions; also, there was no replacement of Na by K in low Na solutions. To account for these findings, the authors postulated a "three-phase theory", consisting of extracellular, free intracellular, and ordered phases. It was assumed that ions accumulated by the cell were adsorbed to the ordered phase, and that other species were excluded from it. The free intracellular phase was in diffusional equilibrium with the extracellular phase; the apparent cellular concentration gradient was due to ion exclusion from the ordered phase. No energy was thus necessary to maintain the cell in this manner.

Harris (1953) (1957) postulated a permeation-diffusion model to account for tissue K exchange; this differed in several respects from

the models presented above. Ion accumulation was regarded as a two-stage process: exchange between ions in the tissue fluid with others in an outer region of the cell, and subsequent diffusion from this outer region into the cell interior. This second step would be balanced by diffusion from the internal region to the outer one; consequently, there would be no net change in the K concentration of the cell. The model was thus a three compartment one, with the compartment between intra- and extra-cellular fluids having ion exchange properties; K movement in this compartment was slow, due to binding by fixed anionic charges. Harris and Sjodin (1961) have shown that there are two types of K exchange possible in this model: (1) when the outer-layer ions exchange with those in the solution more rapidly than they exchange with internal ions, and (2) when the internal diffusion is so rapid that the rate of passage through the outer region, rather than equilibration within it, determines the rate of exchange. Condition (2) was the "classical" resistive membrane that gave rise to first-order kinetics of K movement; condition (1) followed ordinary diffusion laws.

This model can satisfactorily account for the kinetic phenomena observed in K exchange experiments. The model can be used to distinguish between K--K exchange and net cellular gain of K, whether the latter is with an anion or in exchange for Na; the K--K exchange seems to rely on thermal agitation and diffusion, while the net movement may be associated with expenditure of metabolic energy. The Harris model facilitates an explanation of the phenomena observed by Harris and Steinbach (1956) regarding K movement following incomplete exchange with tracer K. The effect on K uptake of adding other cations to the incubation media may be due to a competition for sites in this interme-

diate compartment; gain or loss of K_i will also affect the rate of K* uptake, especially if added ions alter the K_i. Metabolic poisons would tend to weaken the binding of the cations in the special region, and lead to a loss of K and gain of Na by the cells. Temperature would affect the uptake rate, by increasing the thermal agitation of the particles, thus speeding up the exchange process; increased temperature would give an increased rate of K* uptake.

Since the permeation-diffusion model appears to follow ordinary diffusion laws, Harris and Sjodin could show that K equilibration is independent of the intracellular diffusivity (D) if r^2/D is less than unity (r is the radius of a cylinder, and a constant dependant upon K_e); the ratio is usually ca. 0.5 for muscle cells; the authors further showed that plotting exchange against $\cdot t$ (or $K_e \cdot t$) will approximate all curves to one. Any K--K exchange will fall along this curve, but if the muscle is gaining or losing K the values will be above or below the exchange curve. If the K uptake is plotted against $(K_e \cdot t)^{\frac{1}{2}}$, much of the exchange will follow a linear relationship.

The experimental results for K exchange presented in this thesis will be interpreted with the aid of the Harris permeation-diffusion muscle model, for it is felt that this model can best aid in the explanation and interpretation of the kinetic data recorded.

F. MUSCULAR DYSTROPHY: HUMAN AND MURINE

Until the nineteenth century, medical practitioners generally believed that all muscular disorders, weaknesses, and atrophies were of neurogenic origin, consequent upon interruption or damage of the nerve supply. In 1848, G. B. Duchenne, a French neurologist,

described various muscle disorders (Duchenne 1848) and introduced the term "myopathy", meaning a muscular weakness not due to a demonstratable pathological alteration in the nervous system. The modern term progressive muscular dystrophy is applied to those myopathies characterised by an hereditary pattern of occurrence, symmetry of muscle wasting, and gradual involvement of the muscle groups.

In 1951, Ann Michelson of Bar Harbour, Maine, discovered a myopathy in her colony of strain 129 mice (reported in Michelson, Russell & Harmon 1955); it was subsequently shown (Stevens, Russell & Southard 1957) to be similar to the human muscular dystrophies.

1. Electrophysiological Changes in Dystrophic Mouse Muscle

Dystrophic mice have been used to study electrical alterations in muscle. Conrad and Glaser (1959) reported micro-electrode studies showing no change in resting potentials in dystrophy, but reported an increased excitability of the dystrophic muscle to electrical stimulation, as well as an increase in Na conductance. This latter observation was confirmed by McLennan (1961), who also suggested that the similarity between the potentials from the two types of muscle was because resting potentials could only be recorded from sound fibres, and not from degenerate ones. Sandow and Brust (1958) reported dystrophic muscles to be weaker (1/10 to 1/5 the strength), the "active state" duration to be 1/3 shorter, and the relaxation period three times longer than for the muscles of normal littermates.

2. Histology of Dystrophy

Adams et al (1954) reviewed the histology of dystrophy; great variation in fibre size and the large proportion of fat and fibre cells were striking features of dystrophic muscle sections. Fibres were found randomly scattered in their bundles, with swollen

and atrophied fibres mixed. The hypertrophy observed clinically is evidently due to fatty infiltration of the bundles; the fibre nuclei are swollen and more numerous, and vacuolation and granulation are typical intrafibre changes. Tissue culture (Geiger & Garvin 1957) has shown that the cells develop hypertrophy and begin to degenerate after 3 to 4 weeks of growth.

The 129 strain mice show similar microscopic changes. Michelson et al (1955) could see no pathological alteration in any nervous tissue examined, but muscle fibre preparations revealed changes similar to those found in human muscle.

3. Electrolyte Studies

Few studies have been done on the electrolyte levels associated with human muscular dystrophy. Danowski (1955) reported slightly elevated calcium and phosphorous levels and decreased Cl levels in serum samples of children with dystrophy; other indices such as serum Na, K, protein, and NPN were essentially normal. Dowben and Holley (1959) described erythrocyte electrolyte levels in dystrophic patients:

Sodium:	Normals	13.1 m. equiv./Kg	
	Dystrophics	15.5	"
Potassium:	Normals	87.2	"
	Dystrophics	91.0	"

This elevated K level is in contrast to the usual lowered muscle K levels associated with dystrophy; it may indicate a reciprocal relationship between muscle and plasma potassium, with the possibility of the normal ratio being altered by any changes in muscle membrane permeability.

Williams (1957) used neutron activation analysis to assay for muscle Na and K, and found that the Na level was elevated (normal 113 m. equiv./Kg dry weight, dystrophic 191 m. equiv.), and that the K level was lowered (normal 376 m. equiv., dystrophic 181 m. equiv.), confirming the results reported by Horvath, Berg, Cummings & Shy (1955).

Studies on dystrophic mice revealed electrolyte variations similar to those found in human dystrophic subjects. Potassium concentrations in thigh muscle of the animals were sub-normal: normal K was 100 m. equiv./Kg fresh muscle, whereas dystrophic K was 76 m. equiv./Kg fresh muscle. Sodium determinations indicated an increase of ca. 43% in the sodium values, from a normal average of 46 m. equiv./Kg fresh muscle to 66 m. equiv./Kg; the deficit in muscle K of 24 m. equiv. is almost balanced by the 20 m. equiv. increase in muscle Na. The authors suggested that this may be due to replacement of intracellular space by extracellular space, with the resultant increase in Na and decrease in K levels, or to the leak of K from the cell, with compensatory gain of Na. Unfortunately the authors did not take into account the problem of extracellular space; it is difficult to draw conclusions about intracellular electrolyte levels from their data.

Young, Young, and Edelman (1959) analysed skeletal and cardiac muscle from dystrophic mice for Na, K and lipid content. They reported a 19% decrease in dystrophic skeletal muscle Na, and no difference in cardiac muscle K between the normal and dystrophic

animals; cardiac Na values were not reported. Again, no allowance for extracellular space was mentioned.

SECTION II

METHODS

A. Experimental Animals

B. Incubation solutions

1. Extracellular space experiments

a. Inulin solutions
b. Thiocyanate solutions

2. Radioactive Isotope experiments

C. Dissection technique

D. Chemical methods

1. Extracellular space estimations

2. Dry weight determinations

3. Non-radioactive incubation experiments

E. Radioactive Isotope techniques

A. EXPERIMENTAL ANIMALS

The animals used in this investigation were pure inbred mice of the Bar Harbour 129 strain, some of which develop an inheritable muscle weakness similar to human progressive muscular dystrophy. They were obtained from the Roscoe B. Jackson Laboratory, Bar Harbour, Maine, and from the colony maintained by Dr. James R. Miller of the Department of Neurological Research, University of British Columbia. Purebred normal mice, with no pedigree history of dystrophy, and littermates of afflicted animals (those which did not develop muscular weakness) provided control animals.

The dystrophic animals were 2½ to 8 weeks old, of varying body weights, and in varying stages of disability due to the dystrophy. The controls were used only if in perfect condition, and ranged from about 2 weeks to several months of age. The weight increase with age is predictable in the normal animals; the dystrophics, however, do not grow as regularly nor as quickly; they lag behind the normal animals by about 35-50% in their development; a three-week old normal mouse may weigh 15-20 gm., while a dystrophic littermate may be only 8-9 gm. in weight. At maturity the normal animals weigh 25-35 gm., depending on sex, while the dystrophics are only 15-18 gm. in weight.

The animals were kept in the departmental animal room for several days before being used. During this time the dystrophics were kept separately from their normal littermates, and were maintained on powdered commercial chow and water, both *ad libitum*.

The chow and water were placed in special low feeding containers for ease of reach by the dystrophics. The normal animals were treated similarly, with the exception that no special containers were provided, and the food was in pellet form.

B. INCUBATION SOLUTIONS

Locke's solution, as mentioned earlier, is an artificial extracellular fluid. In the majority of the experiments performed, this solution, or variants of it, was used for the *in vitro* incubation of skeletal muscles. For convenience, stock isotonic solutions (0.154 M. for salts dissociating into two ions, 0.11 M. for those hielding three) were prepared. Table III lists the stock solutions used.

From these constituents the experimental solutions of desired ionic composition could readily be prepared, and were freshly made up before each experiment. The final ionic concentrations in the various solutions used are shown in Table IV.

TABLE III

ISOTONIC STOCK COMPONENT SOLUTIONS

NaCl	90.0	gm/litre*	Choline Cl	21.49 gm/litre
KCl	11.47	gm/litre	$KHCO_3$	15.41 gm/litre
$NaHCO_3$	12.49	gm/litre	KH_2PO_4	19.94 gm/litre
Na_2HPO_4	21.25	gm/litre	$CaCl_2$	16.18 gm/litre
NaH_2PO_4	15.62	gm/litre	$MgCl_2$	22.34 gm/litre

*10 times normal strength.

TABLE IV: COMPOSITION OF EXPERIMENTAL BATHING SOLUTIONS. Final ionic concentrations of constituent substances expressed in m.equiv. per litre. 3 gm. of glucose per litre was added in all cases, and the solutions equilibrated with 95% O_2-5% CO_2 gas mixture.

SOLUTION	Na	K	Cl	HCO_3	Ca	Mg	Choline	Phosphate
1	144	6	134	24	2	1	-	-
2	150	-	134	24	2	1	-	-
3	138	12	134	24	2	1	-	-
4	265	6	254	24	2	1	-	-
5	18	6	134	24	2	1	126	-
6	144	6	7	-	2	1	-	151

1. Extracellular Space Experiments

(a) Inulin Space Estimations: The normal Solution 1 was used for the in vitro incubation of excised muscles for inulin space estimations, with dried B. D. H. Inulin added to 1% (w/v) concentration. Inulin was relatively insoluble at room temperature, so the appropriate amount was weighed out accurately and dissolved in hot 0.154 M NaCl solution. After the inulin-saline had cooled slightly, the other constituents of the Solution 1 were added.

For the in vivo injection of inulin into nephrectomised mice, a concentrated solution was needed. A 25% (w/v) solution was prepared in hot 0.9% saline. This amount of inulin dissolved readily in the hot NaCl, but on cooling to room temperature solidified completely. Gentle reheating resulted first in a milky fluid, which upon further heating cleared completely.

A standard solution for the colourimetric estimation of muscle inulin was needed; the Dilute Inulin Standard was 1.0 ml. of the inulin-containing bathing fluid diluted in a volumetric flask to 100 ml. This provided a reference standard of 100 microgm. inulin/ml. The reagents for analysis were prepared as follows:

Resorcinol: 125 mgm. resorcinol dissolved in 100 ml. of alcohol;

30% HCl: 80 ml. of concentrated HCl were added to 20 ml. of water;

Trichloroacetic Acid: 12 gm. of TCA was dissolved in 100 ml. of demineralised water.

(b) Thiocyanate Space Estimations: Estimations of thiocyanate space were done in vitro as a check for the inulin space determinations. The thiocyanate bathing solution was prepared with a CNS^- concentration of 48 m. equiv./l. replacing chloride in the normal bathing solution.

A dilute standard reference solution for the colourimetric estimation of muscle thiocyanate was prepared by diluting 1.0 ml. of the bathing solution to 100 ml., giving a CNS^- concentration of 10 microgm. CNS^- per ml. The 12% TCA reagent used in the CNS^- estimation is the same as for the inulin; ferric nitrate reagent was prepared by dissolving 5.0 gm. of $Fe(NO_3)_3 \cdot 9H_2O$ in 2.5 ml. of concentrated nitric acid, and this was made up to 100 ml. with distilled water.

2. Radioactive Isotope Solutions

There were two problems in preparing the K^{42} incubation solutions; first, the specific activity of the stock 0.154 M. $K^{42}Cl$ solution was usually not too high, and second, the required concentrations of potassium ions in the various bathing solutions was only about 1/25 that of the sodium concentrations; thus, most of the KCl composing the bathing solutions had to be radioactive, or else the specific activity of the fluid would be too low to be useful.

Each shipment of the powdered $K^{42}{}_2CO_3$ arrived by air from Amersham, England, encapsuled in two nestled screw-top aluminum containers, sealed inside a thick lead castle. The screw-top containers were opened with tongs and pliers, and the container and its contents (6.65 gm. of $K^{42}{}_2CO_3$, powdered) were carefully lowered into a large beaker of water. The powder was washed out of the

container, and dissolved in the water. Indicator was added, and the $K^{42}{}_2CO_3$ converted to $K^{42}Cl$ with 1.0N HCl. This solution was then diluted to 0.154 M and used as a stock solution for making up the experimental incubation fluids.

The fluids were prepared with the $K^{42}Cl$ added as the last ingredient: 48 ml. of KCl-free non-active solution of the desired type was placed in a small glass bottle; to this was added 2.0 of the isotonic $K^{42}Cl$, completing the ionic requirements.

C. DISSECTION TECHNIQUE

The m. gastrocnemius of the 129 strain mice has been used almost exclusively in these experiments.

The mice were sacrificed by cervical fracture of the spine. The skin of the thigh was cut completely around with a small pair of sharp scissors and peeled down the leg and over the heel, exposing the muscles, tendons and fasciae. The skinned leg was then gently washed free of hair cuttings, etc., with a cotton pledget soaked with 37°C. Solution 1. A small pair of forceps was carefully inserted between the tendon of Achilles and the tibia, and moved back and forth to create a small patent space. Another pair of forceps was then used to strip away the fascial coverings of the gastrocnemius and the nearby muscles, starting from this space. The popliteal nerves were severed in the popliteal space, close to the body of the gastrocnemius; the muscle was then freed by blunt dissection from those bordering it, the tendon of Achilles severed near the calcaneous, and the entire muscle lifted upwards by pulling on the freed tendon. The soleus muscle was then separated from the gastrocnemius, and the leg was cut through completely just above the knee; the preparation was placed on the stage of a

dissecting microscope, on a warm, moistened paper towel. Great care was exercised during the entire procedure to avoid tearing, cutting, stretching or otherwise damaging the muscle; it was moistened frequently with 37°C. Solution 1 to prevent drying of the tissues.

Using fine forceps and a sharp scalpel, excess fat and nervous tissue was stripped and cut from the preparation. The tendon (Achilles) was trimmed free of fat and residual shreds of soleus muscle, and the excess length of tendon was carefully cut off. However, it was not cut off too short so as to damage any muscle fibres, and was left long enough so there was sufficient free tendon to accomodate a firm grasp by forceps, to enable it to be handled easily. Now the knee joint was carefully trimmed and scraped free of all muscle insertions except the gastrocnemius, the preparation bathed in more warmed fluid, and then the tendinous ends of the gastrocnemius gently freed from the femur, with a minimum amount of damage to the muscle. The completely dissected muscle was then weighed immediately on a Roller-Smith Precision Torsion Balance, and transferred immediately to the test tube containing the bathing fluid. Fow a few of the inulin space studies, m. peroneus longus was similarly excised by loosening and cutting the tendinous ends.

Small pieces of thin coloured thread were tied around the tendon of each gastrocnemius for identification purposes while being incubated. It was determined that this thread did not appreciably affect the radioactive counting.

D. CHEMICAL METHODS

1. Extracellular space estimations

The polysaccharide inulin has been used in this study to

estimate the volume of extracellular space in muscle. Muscles, dissected and weighed as above, were incubated for 4 hours in a 1% inulin bathing solution. At the end of the incubation period, the muscles were rinsed briefly in demineralised water, blotted, and weighed rapidly; they were then placed in small clean mortars, 1.0 ml. of 12% TCA and some acid-washed sand added, and ground with a pestle. The mixture was placed in a graduated centrifuge tube, the mortar rinsed three times with 1.0 ml. portions of demineralised water, and the whole mixture centrifuged.

For estimation of the muscle uptake of inulin in vivo, bilateral ligation of the renal pedicle was carried out under ether anaesthesia, and 0.3 ml. of 25% inulin solution injected under the skin of the back. After a variable period of time ($\frac{1}{2}$ to $3\frac{1}{2}$ hours), the animals were killed by exsanguination from the heart, and the gastrocnemii and/or peroneii longii routinely excised, rinsed, blotted, weighed, and further treated as described above. The blood sample taken from each animal was placed in a tube containing a small known amount of heparin solution, centrifuged, and 0.1 ml. aliquots of the plasma treated with TCA and assayed for inulin.

Aliquots of the muscle supernatant solutions were taken and analysed by the method of Hubbard and Loomis (1942). A standard inulin solution was prepared from the incubation solution, and portions of this were further diluted to give varying concentrations of inulin for use as standards for colourimetric analysis. Five standard tubes were prepared, with 1.0, 0.75, 0.50, 0.25, and 0.0 ml. of dilute inulin standard made up to 1.0 ml. with deminera-

lised water. One ml. of each of the muscle homogenate supernatant solutions were pipetted into test tubes also. To each tube was added 0.25 ml. 12% TCA, 1.0 ml. resorcinol, and 3.0 ml. of 30% HCL. This mixture was shaken well, heated in an 80°C. water bath for 8 minutes, and then placed to cool for 30 minutes in ice water. The contents of all the tubes were diluted to 10 ml., mixed well by inversion, and colourimetric values read at 510 millimicrons in a Klett-summerson Photoelectric Colourimeter.

As a check on the extracellular spaces estimated by the inulin method, estimations of thiocyanate space were done on some normal mouse muscles, to see if there was a significant difference between the apparent inulin spaces and the apparent thiocyanate spaces. The m. gastrocnemii were routinely excised, weighed, and incubated in vitro in the thiocyanate bathing solution for a period of two hours. The muscles were then ground and centrifuged, and assayed for thiocyanate by the method of Crandall and Anderson (1934). Standards were prepared by making 2.0, 1.0, 0.50, and 0.25 ml. portions of dilute thiocyanate standard (1.0 ml. bathing solution diluted to 100.0 ml. with water) up to 5.0 ml. with demineralised water. The unknown solutions were prepared by adding 4.0 ml. of water to 1.0 ml. of the supernatant solution. To every test tube 2.0 ml. of ferric nitrate reagent was added, and the mixture shaken well. The resulting colour was read immediately at 510 millimicrons in a Klett Summerson Colourimeter. The thiocyanate content of the muscles could then be calculated with reference to the standard solutions.

2. Dry Weight Determinations

For the determination of percentage water of representa-

tive muscles, the following procedure was employed:

(a) Small metal planchets were washed, dried, heated for 2 hours at 110°C., and cooled overnight in a dessicator. These were then weighed on a Sartorius Selecta balance, reheated for one hour, cooled and weighed to constant weight. The planchets were stored in the dessicator until used.

(b) Each muscle was excised in the routine way, placed on the planchet, and rapidly weighed on the same balance.

(c) When a number of muscles were ready, they were placed in a 110°C. constant temperature oven, dried for one hour, cooled in a dessicator, and weighed carefully. They were then reheated, cooled and weighed to constant weight.

3. Non-Radioactive Incubation Experiments

The mice were sacrificed, and the gastrocnemii excised as previously described; the muscles were then immediately weighed, and transferred to the selected bathing solution. The bathing fluid was contained in large glass test tubes suspended in a constant temperature (33°C.) water bath. The bathing solution was oxygenated and circulated in the test tubes with a 95%O_2-5%CO_2 gas mixture.

After a variable time of soaking, the muscles were retrieved from the bottom of the tubes, immersed briefly (less than 2 seconds) in distilled water to remove any adhering drops of bathing solution, blotted lightly on dry filter paper, transferred to acid rinsed boro-silicate test tubes, and digested with 4 drops of concentrated nitric acid. The tubes were kept tightly corked until ready for analysis.

The samples were heated slightly to ensure the complete digestion of the muscles. The nitric acid solutions were diluted with a few ml. of demineralised water, and transferred into graduated "Pyrex" cylinders; an additional few ml. were added to the digestion tube, and these rinsings added to the cylinder. The solution was diluted to 5.0 ml. with water, and filtered through Whatman #40 filter paper in small plastic funnels into test tubes. Two 1.0 ml. aliquots of the filtrate were placed in separate test tubes; to one was added demineralised water to give the amount of dilution necessary to bring the concentration of sodium into a range suitable for analysis (usually 2 to 10 ml.); to the other 1.0 ml. aliquot 1.0 ml. of 0.25 M NaCl and the same amount of water for the K determination were added. The amounts of Na and K in these solutions were determined with the aid of an Evans Electroselenium Flame Photometer. All analytical results are expressed in terms of milliequivalents of the appropriate ion per kilogram fresh weight of muscle.

E. RADIOACTIVE ISOTOPE TECHNIQUES

Radioisotope tracer experiments formed the major part of the experimental work done in this investigation, with the soaking experiments, extracellular space determinations, and dry weight analyses all ancillary to the isotope flux studies. The isotope K^{42} was used as a tracer; whenever the radioactive solutions were to be handled, gloves were worn to protect the hands from contact, and all work was done over an enamel tray lined with tinfoil and absorbent paper. After each experiment or each diluting operation involving the radioisotope storage bottles, the

entire area was thoroughly monitored with an end-window portable Geiger counter. At all times, extreme care was used to limit any contamination by the active solution of equipment used (such as glassware) to a bare minimum.

The main disadvantage of using the K^{42} was its short half-life. Every 24 hours 74% of it disintegrated, and after 4 days, the level of activity would be down to 0.40% of the original value. Thus the experiments had to be done in the first three or four days after receipt of a shipment of the K^{42}. There was no disposal problem, for the radioactive solutions were kept for 3 weeks after the termination of the run of experiments, and then treated as being non-radioactive.

The solutions were prepared in the usual non-active form, and the 2.0 ml. portion of K^{42} stock solution added to the 48 ml. of the bathing fluid to be used. This active solution was the medium for the influx studies; it was placed in a "Pyrex" test tube in a 33 C. water bath, and mixed and oxygenated with a $95\%O_2$-$5\%CO_2$ gas mixture. The muscles were routinely excised, rapidly weighed, and placed in the solution to incubate; after approximately 10, 30, 60, 90, 120, and 150 minutes of incubation the muscles were removed from the solution with a curved glass rod, and placed for 1.0 minutes in a beaker containing 200 ml. of non-active solution of the same composition as the incubation medium. This short soaking period effectively reduced the extracellular K^{42} to ca. 50% of its previous level (McLennan 1955), thus lessening the error in the counting of the fibre K. The muscles were then blotted briefly on filter paper, and arranged on a plastic counting

tray in a standard reproducible position. The tray and muscles were then placed under a Geiger-Muller end-window counter and counted for three one-minute intervals. The impulses from the counter were transmitted to an electronic scaler and mechanical recorder. The scaler and counter were Nuclear Chicago equipment, the scaler a 161A model, and the counter a D-34 model, with a thin mica window. At the end of the experiment, the muscles were weighed and placed in boro-silicate test tubes with 0.1 ml. of concentrated nitric acid, to digest. To this digest was then added 0.9 ml. of demineralised water, the tube shaken, and the mixture poured into a metal counting planchet. The tube was rinsed with 1.0 ml. of the water, and this was added to the planchet. The planchet plus diluted digest was set in a sample holder tray, and placed under the Geiger counter to be assayed for radioactivity. After the counting period was finished, the planchet was removed from the sample holder, and the digested muscle mixture poured back into the boro-silicate test tube; the planchet was rinsed twice with 2.0 ml. portions of demineralised water, these added to the test tube, and the tube tightly corked until used for flame analysis. A 0.1 ml. aliquot of the bathing solution was placed in a metal planchet, diluted with 1.9 ml. water, and similarly assayed for radioactivity. Thus, a relationship between the amount of K^{42} in the muscle and that in the bathing solution would be established. After 2 to 3 weeks, when the radiation was at a low level, the muscles were assayed for total sodium and potassium by flame photometry.

SECTION III

RESULTS

A. Extracellular Space Estimations

1. Muscle Inulin Space: Normal Strain 129 mice

2. Muscle Inulin Space: Dystrophic Strain 129 Mice

3. Muscle Inulin Space: Swiss Albino Mice

4. Muscle Thiocyanate Space: Normal Strain 129 Mice

B. Dry Weight and Non-Inulin Space Water Estimations

1. Dry Weight Determinations

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C. Chemical Analyses

1. Fresh Muscle

2. Incubated Muscle: Solution 1

3. Incubated Muscle: Summary of Results of incubation in solutions 2 to 6

4. Conculsions

D. Results of K^{42} Uptake Studies

1. Normal Mouse Skeletal Muscle

   a) Effects of Varying K_e
   b) Effects of Varying Na_e
   c) Effects of Varying K_e and Na_e

2. Dystrophic Mouse Skeletal Muscle

   a) Effects of Varying K_e
   b) Effects of Varying Na_e

3. Summary of K^{42} Results

A. EXTRACELLULAR SPACE ESTIMATIONS

Intracellular ion concentrations cannot be estimated without making adequate allowance for the ion content of the extracellular fluid. To make this correction, the volume of the extracellular space (ECS) and the concentration of the ion in the extracellular fluid must be known. The calculated amount of ion present in the ECS can then be subtracted from the total muscle content to give the intracellular value. Muscles from normal and dystrophic animals were assayed for ECS as described on page 28 but the averaged results from groups of muscles were found to be variable from group to group; small muscles seemed to have larger ECS values than larger ones, and muscles from the dystrophic animals seemed to have larger ECS values than comparable muscles from normal animals. When the percent ECS was plotted against fresh muscle weight, the graphs resembled Fig. I, i.e., there was a negative correlation between muscle weight and magnitude of the ECS.

1. Muscle Inulin Space: Normal Strain 129 Mice

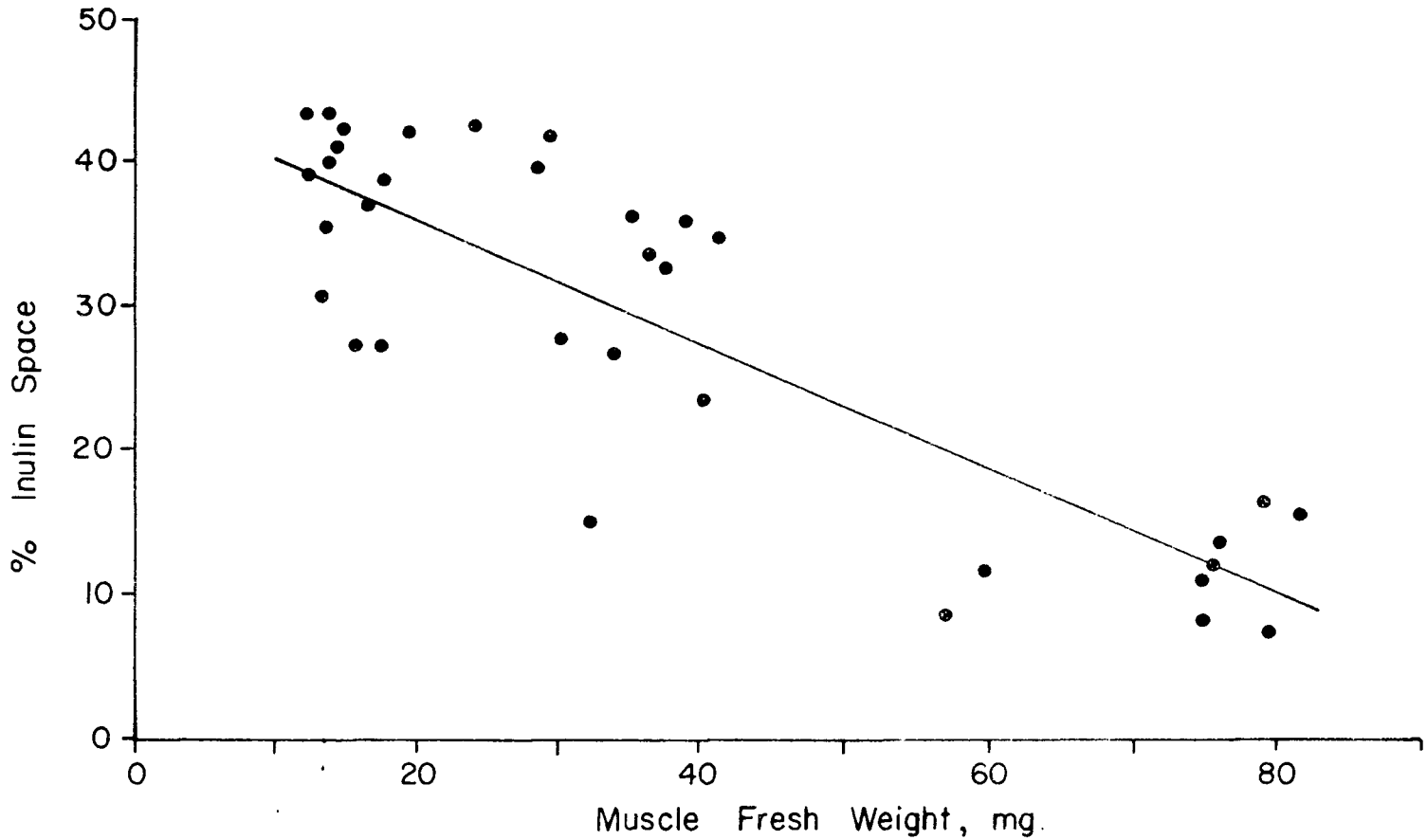
Gastrocnemii from normal strain 129 mice were soaked for a standard time of four hours in an inulin-containing solution; the muscles were analysed for inulin content, the raw data corrected for muscle swelling during incubation, and the results divided by the muscle weights to give the amount of inulin per gm. of muscle; this was expressed as

$$\frac{\text{mg. inulin per gm. fresh muscle}}{\text{mg. inulin per ml. soaking solution}} \times 100\%$$

Fig. I is a graph obtained when the inulin spaces of 34 muscles were plotted against the fresh muscle weights. On inspec-

Fig. I. Inulin Space of normal Mouse Muscle:

In Vitro Method.



tion, there was an obvious relationship between the size of the muscle and its inulin space, with the space decreasing with increasing muscle size. The calculated regression line (as drawn in Fig. I) is $y = 44.7 - 0.43x$ (S.E. $\pm$ 1.1).

However, it was considered that excised muscles incubated in an artificial fluid might not have the same inulin space as muscles in situ. In order to examine this possibility, normal strain 129 mice were nephrectomised and injected with inulin solution subcutaneously; after a variable time, gastrocnemii and/or peronei longi were excised and analysed. (Peroneus longus was a small muscle, with an ECS similar to the gastrocnemius, and it provided a useful check on the values previously found for the gastrocnemii.) Plasma samples were taken and assayed for inulin content; each plasma value was used as the divisor for muscles from the same animal, in place of the soaking solution value. Results were calculated as described above, and plotted similarly.

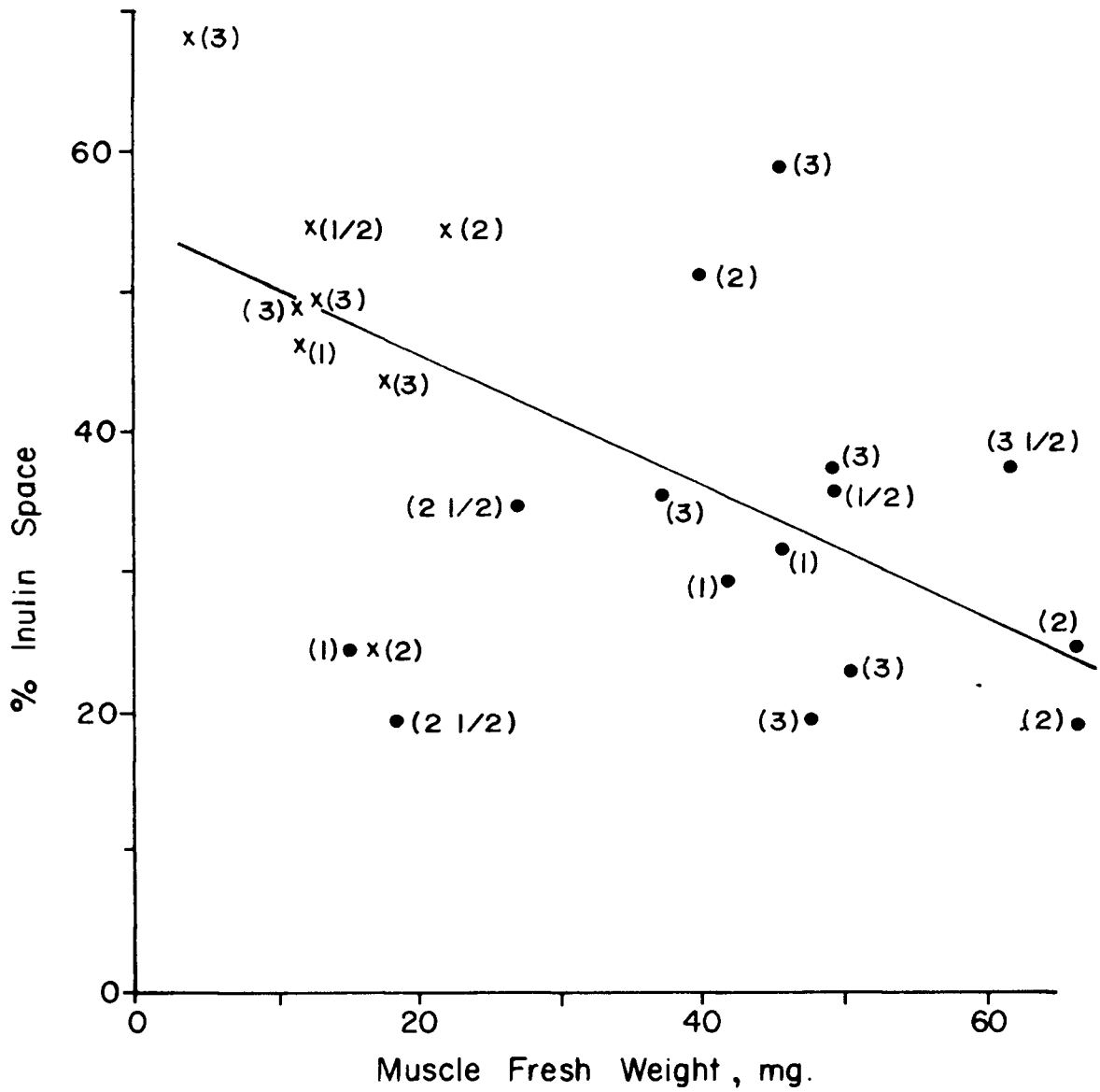
Fig. II illustrates the results obtained; values from both the gastrocnemius and the peroneus longus muscles are plotted; the calculated regression line of the 23 points is $y = 54.0 - 0.472x$ (S.E. $\pm$ 2.8). The lines in Figs. I and II do not differ significantly. This result indicates that incubated muscles have inulin spaces slightly less than but not significantly different from those of fresh muscles; at least part of the swelling noted during incubation appears to occur in a region of the muscle that is not available to inulin.

2. Muscle Inulin Space: Dystrophic Strain 129 Mice

There was only a limited number of dystrophic mice available for this investigation, and in the light of the previous results

Fig. II. Inulin Space of normal Mouse Muscle : Injection Method.
• Gastrocnemii
x Peronei Longi

Figures in parentheses show the number of hours between injection and sacrifice of the animals.



with normal mice, it was decided to pool the results obtained on dystrophic tissue by the incubation and injection methods. Since the peronei longi were too small to excise intact, only the gastrocnemii of these dystrophic animals were used; Fig. III is a graph of the dystrophic muscle inulin spaces plotted against the muscle weight. The negative correlation described for normal muscles is also observed in these 17 dystrophic muscles, but there is a greater dependance of the inulin space on muscle weight. The calculated regression line is $y = 94.6 - 1.65x$ (S.E.$\pm$ 2.8). It should be noted that some of the smallest muscles had inulin spaces as high as 90%; this may have been due to a permeability of the muscle membrane to inulin. If this was the case, and the inulin could penetrate into the degenerating muscle cells it would account for the high inulin space values observed.

3. Muscle Inulin Space: Swiss Albino Mice

Inherited muscular dystrophy is unknown in Swiss albino mice. Some analyses of gastrocnemii from Swiss mice were done to eliminate the possibility that the previously observed results were a peculiarity of the 129 strain. Twenty-three muscles were excised, soaked, and analysed in the usual way. A relationship between muscle weight and inulin space similar to that observed in the strain 129 muscles was noted (see Fig. IV). The calculated regression line in Fig. IV is $y = 39.8 - 0.249x$ (S.E.$\pm$ 1.2). This line is not significantly different from that of either Fig. I or Fig. II.

4. Muscle Thiocyanate Space: Normal Strain 129 Mice

Some thiocyanate (SCN) estimations were done on normal strain 129 mice, as a check on the inulin space results. Twenty-three muscles were excised and incubated as usual, and analysed for

Fig. III. Inuline Space of Dystrophic Mouse Muscle : In Vitro Method.

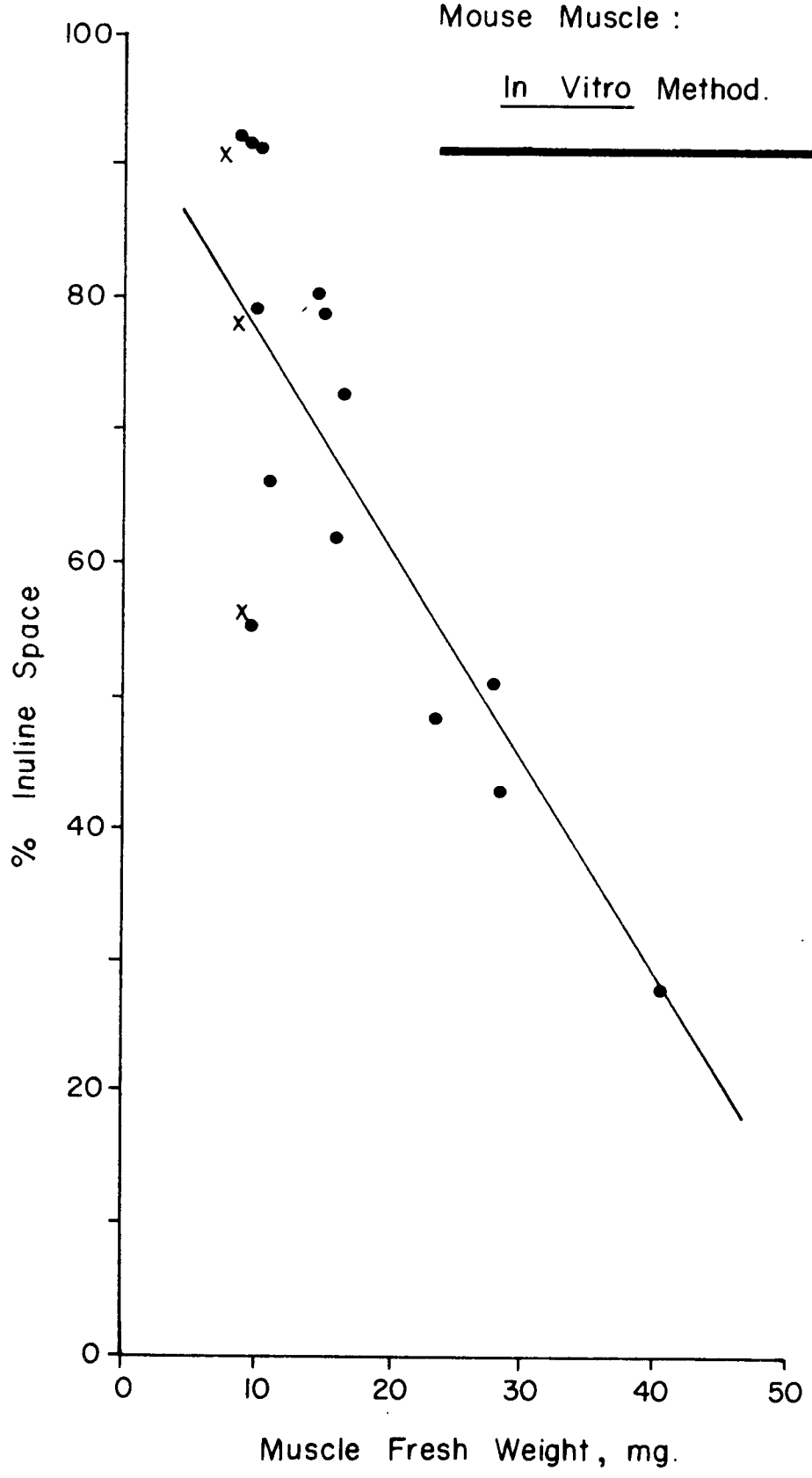
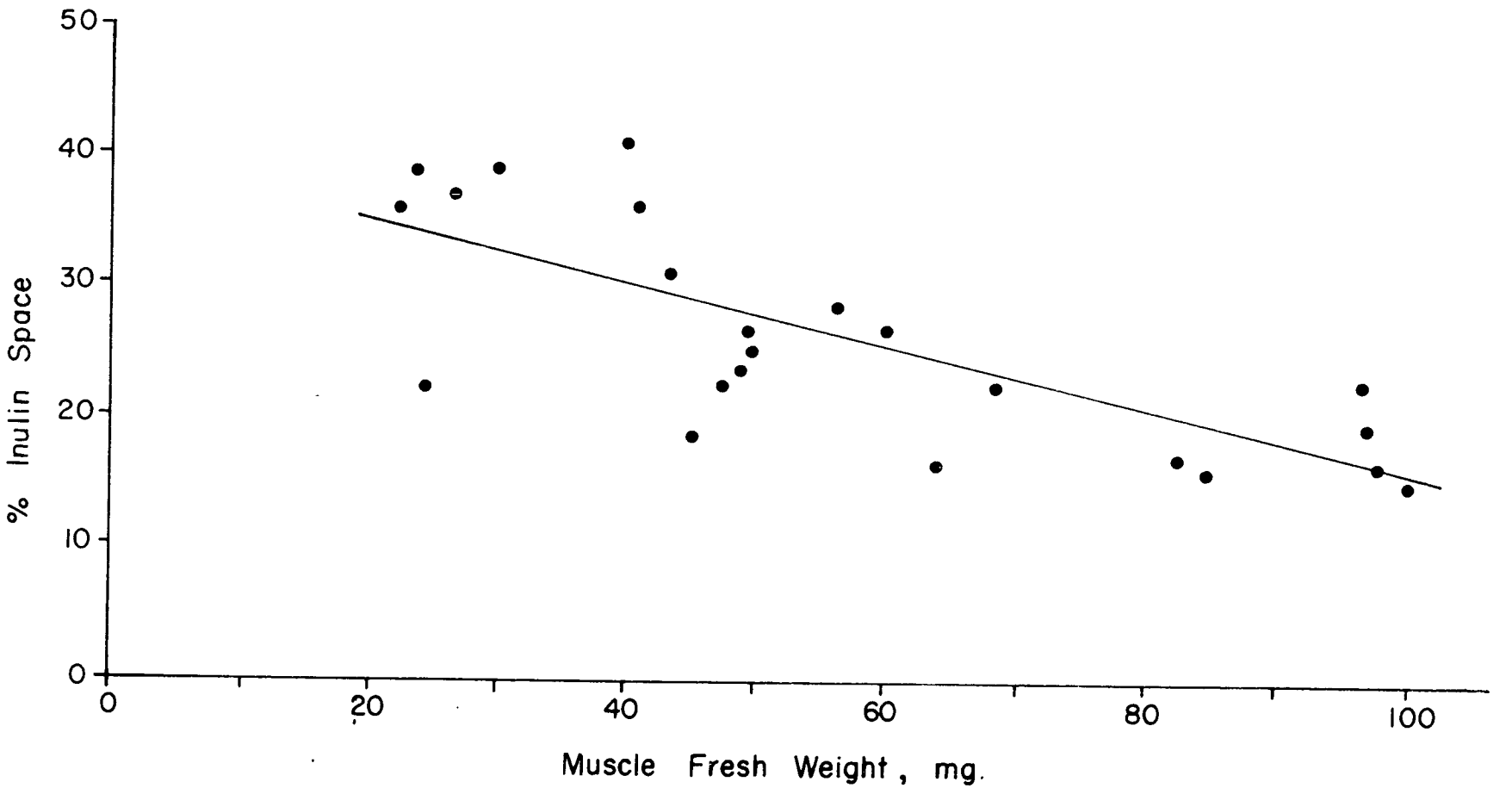


Fig. IV. Inulin Space of Swiss Albino Mouse Muscle: In Vitro Method.



SCN. These results are illustrated in Fig. V; the calculated regression line drawn is $y = 35.7 - 0.196x$ (S.E.$\pm$ 1.2), and the slope of the line is, in this case, not significantly different from zero, but also is not significantly different from those of Figures I, II, or IV. However, there appeared to be a trend for the SCN space to decrease with increasing muscle weight.

B. DRY WEIGHT AND NON-INULIN SPACE WATER ESTIMATIONS

1. Dry Weight Determinations

Results of the Na and K analyses were to be expressed as intracellular ion per kilogram fresh tissue weight, for which purpose a knowledge of the total water content and the volume of the ECS of the tissue was necessary (see previous section). Muscles were excised, weighed and treated as described on page 33; a series of muscles was also incubated for several hours before the dry weight assay; Table VI is a tabulation of the average percentage weight loss, expressed as

$$\frac{\text{weight loss}}{\text{fresh weight}} \times 100\%$$

None of the averages listed in Table VI are significantly different from one another. The fresh and incubated muscles seem to have the same total water content of 76%; also, no significant difference in water content between normal and dystrophic tissues was apparent.

2. Non-inulin Space Water Estimations

The non-inulin space water volumeof a tissue is that volume of water not accessible to diffusible inulin, either after injection of inulin into the nephrectomised animal, or following incubation of an excised muscle in an inulin-containing solution. The available inulin space, usually considered as being identical to the ECS, was determined as described on page 28. The non-inulin space water was

Fig. V. Thiocynate Space of normal Mouse Muscle : In Vitro Method.

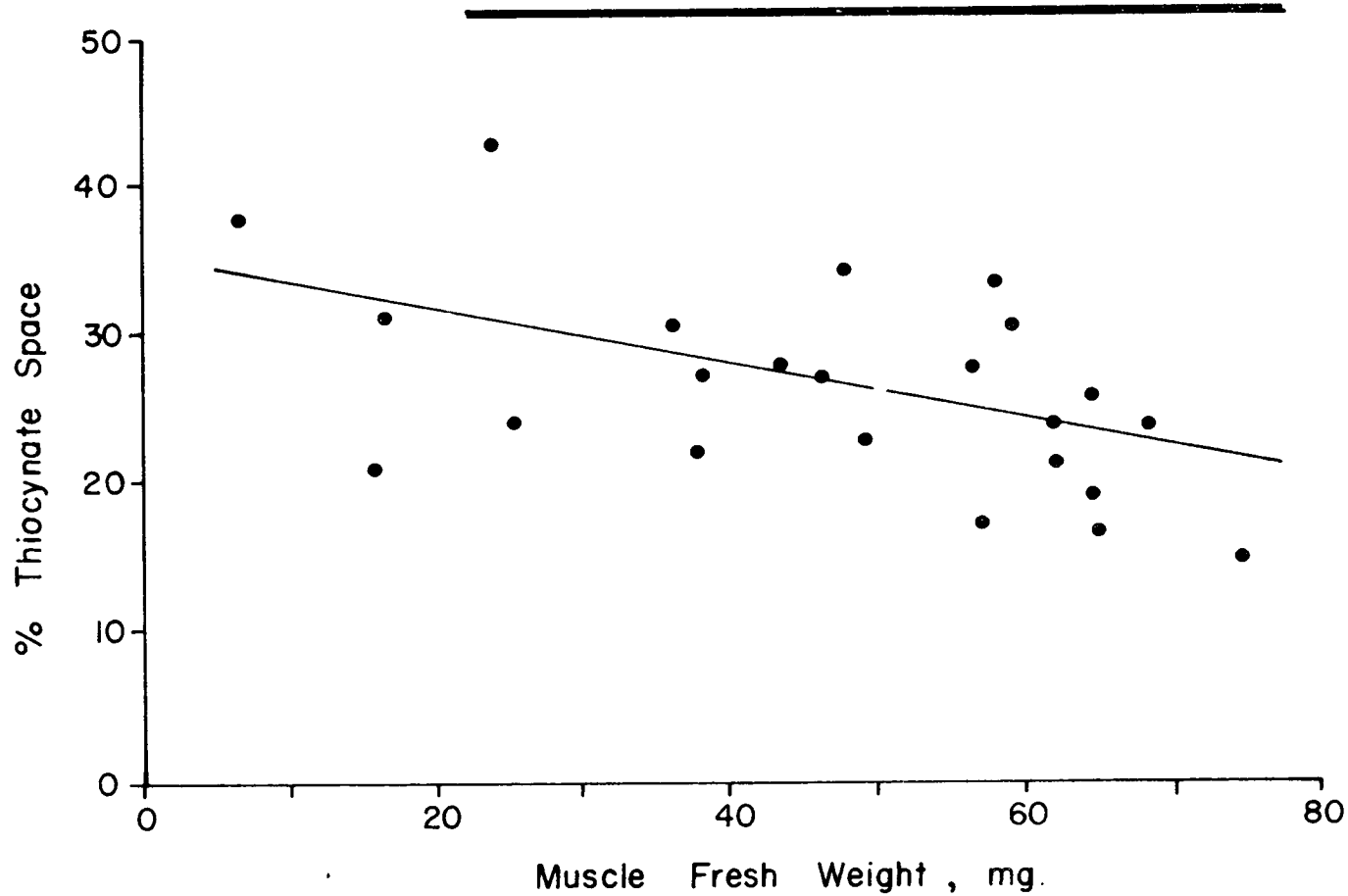


TABLE VI: WEIGHT LOSS OF FRESH AND INCUBATED MUSCLE.

INCUBATION SOLUTION	HOURS INCUB.	MUSCLE TYPE *	PERCENTAGE WEIGHT LOSS
FRESH	-	N	75.6
		D	77.2
1	2	N	75.9
	4	N	75.4
		D	76.8**
2	4	N	76.8

* N = Normal muscle, D = Dystrophic muscle.

** All standard deviations are less than 1.1, except this average, which is 3.8.

calculated from a knowledge of this ECS value, the dry muscle weight, and the fresh weight, and was estimated as being

$$\frac{\text{fresh muscle weight - ECS - dry weight}}{\text{fresh weight}} \times 100\%$$

this was expressed as a percentage of the fresh muscle weight. Table VII contains the average non-inulin space water values obtained for fresh and incubated muscles. The normal muscle values increased greatly with incubation, in both normal and K-free incubation solutions; however, the fresh dystrophic muscle value was not significatnly different from the soaked muscle value. The dystrophic muscle non-inulin space water was less than half the normal muscle value for fresh tissue; following incubation for 4 hours, the normal value rose from 47% (fresh) to 65% of the fresh tissue weight, while the dystrophic value remained unchanged at 21%. This increase in non-inulin space water in normal tissue may reflect a swelling or increase of volume in an area of the muscle not available to inulin. The decrease observed in inulin space with incubation, and the constancy of the total tissue water, suggests that during incubation the muscle fibres swell by taking up water from the ECS. The increase in cellular water might be more than has been measured here; if the muscle membrane were distended by this extra water, it might be more permeable to the inulin molecule; inulin could enter the fibre, and the apparent inulin space would be larger than the "true" space. Zierler (1957) reported that normal rat muscle was permeable to the enzyme aldolase (MW147,000); he also reported that dystrophic mouse muscle had a higher efflux of aldolase than did normal muscle (Zierler 1958). It seems possible that if normal muscle were per-

TABLE VII: PERCENTAGE NON-INULIN SPACE WATER OF FRESH AND INCUBATED MUSCLE*.

INCUBATION SOLUTION	HOURS INCUB.	MUSCLE TYPE**	PERCENTAGE NON-INULIN WATER	S.D.
FRESH	-	N	46.8	4.6
		D	20.9	5.9
1	2	N	58.4	8.0
	4	N	65.3	1.8
		D	21.6	6.5
2	4	N	64.2	3.0

* Expressed as percentage fresh tissue weight.

** N = Normal muscle, D = Drystrophic tissue.

meable to aldolase, it could likewise be permeable to the much smaller inulin molecule (MW991).

C. CHEMICAL ANALYSES

Na and K analyses of skeletal muscle will not yield useful data about the intracellular ion concentrations unless a correction for the ion content of the ECS is made. Also, if incubated muscles swell, there are likely to be ions from the incubation media in the water of swelling. Correction of muscle analysis data for these factors should yield the intracellular ion per unit weight of tissue, per litre of intracellular water, per gram of fat-free dried solids, or in other ways, providing the parameters can be calculated. The analytical results in this thesis will be expressed as m. equiv. non-inulin space ion per Kg. fresh tissue. This is probably comparable to the intracellular ion content in fresh muscle, but with incubated muscle one must account for the apparent increase in non-inulin space due to swelling, and the intracellular ion content and non-inulin space ion content may not be similar.

Analyses were performed on fresh and incubated normal and dystrophic muscle tissue to determine the intracellular (non-inulin space) Na and K concentrations. The muscles were treated as described on page 34, the raw data corrected for inulin space and water of swelling ion content, and this result divided by the appropriate tissue weight. The calculated results are expressed as m. equiv. ion/Kg fresh tissue; the standard deviations of the values are also given.

Tabulated results of the Na and K analyses appear in Table VIII.

TABLE VIII: Na, K CONTENTS OF FRESH AND INCUBATED MOUSE MUSCLE. Results expressed as m.equiv. non-inulin space ion per Kg. of fresh tissue.

INCUBATION SOLUTION	HOURS INCUB.	MUSCLE TYPE	Na	K	S.D. Na	S.D. K
FRESH	-	N	30.0	89.9	5.2	5.2
		D	58.2	68.2	11.4	11.1
1	2	N	31.6	90.6	7.8	2.6
		D	77.4	60.0	4.7	4.2
	4	N	41.3	57.6	19.1	4.0
		D	77.1	63.4	13.3	8.5
2	2	N	34.6	72.1	8.8	6.6
		D	78.2	47.3	11.2	6.6
	4	N	69.5	37.4	12.0	8.9
		D	95.1	39.7	23.8	3.5

(CONTINUED OVERLEAF)

(CONTINUED FROM PREVIOUS PAGE)

INCUBATION SOLUTION	HOURS INCUB.	MUSCLE TYPE	Na	K	S.D. Na	S.D. K
3	2	N	43.0	90.6	10.6	11.9
		D	73.5	80.4	9.7	7.4
	4	N	41.2	62.3	10.9	6.3
		D	82.4	91.8	9.2	7.6
4	2	N	110.1	60.8	8.2	6.6
	4	N	134.0	41.2	16.4	9.2
		D	126.1	62.4	4.5	4.3
5	4	N	14.1	27.2	2.5	3.2
		D	17.7	40.6	2.0	2.5
6	4	N	45.9	35.5	10.2	1.6

1. Fresh Muscle

Normal mouse muscle contained 30.0 m. equiv. Na/Kg, while the dystrophic muscle had 58.2 m. equiv. Na/Kg. The normal muscle K was 89.9 m. equiv./Kg, but the dystrophic K was only 68.2 m. equiv./Kg. Both dystrophic values for the intracellular ions (Na_i and K_i) are significantly different from the normal muscle values ($p < 0.001$); this gain of Na and loss of K in dystrophic tissue might be caused by a leakage or altered permeability of the muscle membrane, resulting in an inability to retain or exclude certain ion species; if the Na and K ions were to diffuse down their concentration gradients across such a leaky membrane, the cellular K would be expected to fall, and the cellular Na to rise. This "membrane leak" would be one explanation for the observed alterations in the cellular ion contents.

2. Incubated Muscle: Solution 1.

Incubation of excised normal muscle in Locke's solution (Solution 1, Table II) did not significantly alter the non-inulin space Na or K content from that calculated for fresh muscle. However, the Na content of dystrophic tissue rose 33% to 77.4 m. equiv. Na/Kg, and the K dropped 12% to 60.0 m. equiv./Kg; both these changes are significant ($p < 0.01$).

After 4 hours soaking, normal tissue Na had risen to 41.3 m. equiv./Kg, and K had dropped to 57.6 m. equiv./Kg; the dystrophic tissue contents were 77.1 m. equiv. Na/Kg and 63.4 m. equiv. K/Kg.

The excised muscle tissue can be incubated for 2 hours in Locke's solution, and the intracellular (non-inulin space) ion contents will not have changed. The ion levels are not stable for a

longer period, however, for after 4 hours soaking there are significant alterations in the intracellular Na and K. Apparently the changes in ion content that occur are the same in both types of tissue, that is, the Na rises and the K falls. The dystrophic muscle seems to undergo these changes more rapidly than the normal muscle; this may be a reflection of the larger ECS of the dystrophic tissue, or a consequence of an abnormal permeability of the dystrophic fibre membrane.

3. Incubated Muscle: Summary of Results of Incubation in Solutions 2 to 6.

The data for ion content following 2 hours and 4 hours of incubation in the altered media are to be found in Table VIII; these data will be summarised and briefly compared in this section.

The tendency of Na to rise and K to fall, as mentioned above, was noted as occurring generally in most muscles incubated in the altered media; the change was especially marked following 4 hours' incubation.

Muscles incubated in altered K_e solutions gained Na; the gain was less in 12 mM K_e than in 0 mM K_e solution; the dystrophic muscles seemed to gain Na more rapidly, and the gain appeared to occur earlier than in the normal tissue. Incubation of normal muscle in the altered K media led to a decrease in muscle K; the loss was greater in the K-free solution, and less in the 12 mM K_e solution, than observed in the "reference" Solution 1. Dystrophic tissue K rose in the 12 mM. K_e solution, but rapidly fell in the K-free medium. During the first 2 hours of incubation, the K_i value for normal muscle in the 12 mM K_e was constant, but in the K-free medium it fell somewhat; after 4 hours soaking, normal muscle K was significantly lower than the fresh value.

Solution 4 contained 288 mM Na_e, and was hypertonic; as expected, both types of muscle gained Na during incubation; normal muscle K had dropped significatnly after 2 hours of soaking, and after 4 hours, both normal and dystrophic tissue K was significantly lower than the fresh tissue value. Soaking excised muscle in 18 mM Na_e lowered Na and K in both types of tissue, a greater drop in Na occurring in the dystrophic tissue than in the normal, but less of a decrease in K_i.

Solution 6 was a phosphate buffered solution; it was used in an attempt to reduce the swelling that occurred during incubation in the bicarbonated-buffered media; perhaps a reduction in the amount of swelling would aid the cellular retention of Na and K. However, the swelling during incubation still occurred, and to almost the same degree as in Solution 1. The tissue Na and K levels also changed during the 4 hours incubation, the changes being greater in this solution than in Solution 1. On the basis of these results, it was decided not to use this Solution 6, or variants of it, for incubation purposes.

4. Conculsions

It was apparent from the foregoing results that muscle K levels were relatively stable for at least 2 hours of incubation, at least in most soaking media. This stability of K_i is a necessary condition if one wishes to interpret radioactive K (K*) data in the light of the Harris diffusion-permeation hypothesis, for in order to calculate and plot a normal K exchange relationship (K* uptake against time) the K_i must be constant, or the data will not fall along the predicted line. It was decided to limit the interpretation of K* exchange studies to the data obtained during the first 2 hours of incubation in the radioactive media, for correc-

tions could not be made for K_i changes that occurred after this time. Consequently, only the uptake of K* could be followed; efflux studies would necessitate a previous loading of the tissue with radioactive K by non-incubation methods, and this was not attempted. If the exchange follows diffusion principles, the efflux graph should be similar in form to the influx graph.

D. RESULTS OF K^{42} UPTAKE STUDIES

The radioactive isotope K^{42} was used as a tracer in the experiments reported in this section; the studies were done to determine the rate at which radioactive K (K*) in solution exchanged with intracellular K, and whether alterations in the ionic composition of the incubation solutions would affect the exchange rate. The methods of incubation, radioactive assay, and chemical analysis were described on page 35. In this section, exchange means the movement of an ion occurring in the absence of a net change in tissue ion content (the exchange of K, as followed by the uptake of K^{42}, will be the only exchange process reported); the Harris diffusion-permeation hypothesis states that the exchange of tissue K should follow a diffusion curve if the K* uptake is plotted against the expression (K_e x time); if the square root of this expression is used, the resulting curve should be a single straight line for most of its extent. All K* uptake points should fall along this line, and be independant of the external K concentration providing the intracellular K level is constant. It was thought that this method of expressing K* uptake results might provide evidence to support or repudiate the Harris hypothesis; if the "diffusion-permeation" concept (see Introduction, page 16) is an accurate representation of the conditions existing in skeletal

muscle tissue, these data may help to elucidate the kinetics of ion movements in muscle.

Preliminary experiments showed that tissue K levels remained fairly constant under most conditions for the first 2 hours of incubation (Table VIII and Burr and McLennan (1961)), and were comparable to fresh tissue values. Data from the K^{42} exchange experiments were plotted as m. equiv. K* per Kg tissue (ordinate) against $(K_e \cdot t)^{\frac{1}{2}}$ (abscissa), a correction having been applied for the amount of K* remaining in the ECS, assuming 50% removal during the timed 1.0 minute wash (McLennan 1955). The method of plotting is illustrated in Fig. VI; the slope of the calculated line is expressed as m. equiv./Kg/$(mM \cdot min)^{\frac{1}{2}}$. Comparison of the slopes of the calculated regression lines enabled an estimation of the relative rates of K exchange under the various experimental conditions to be made.

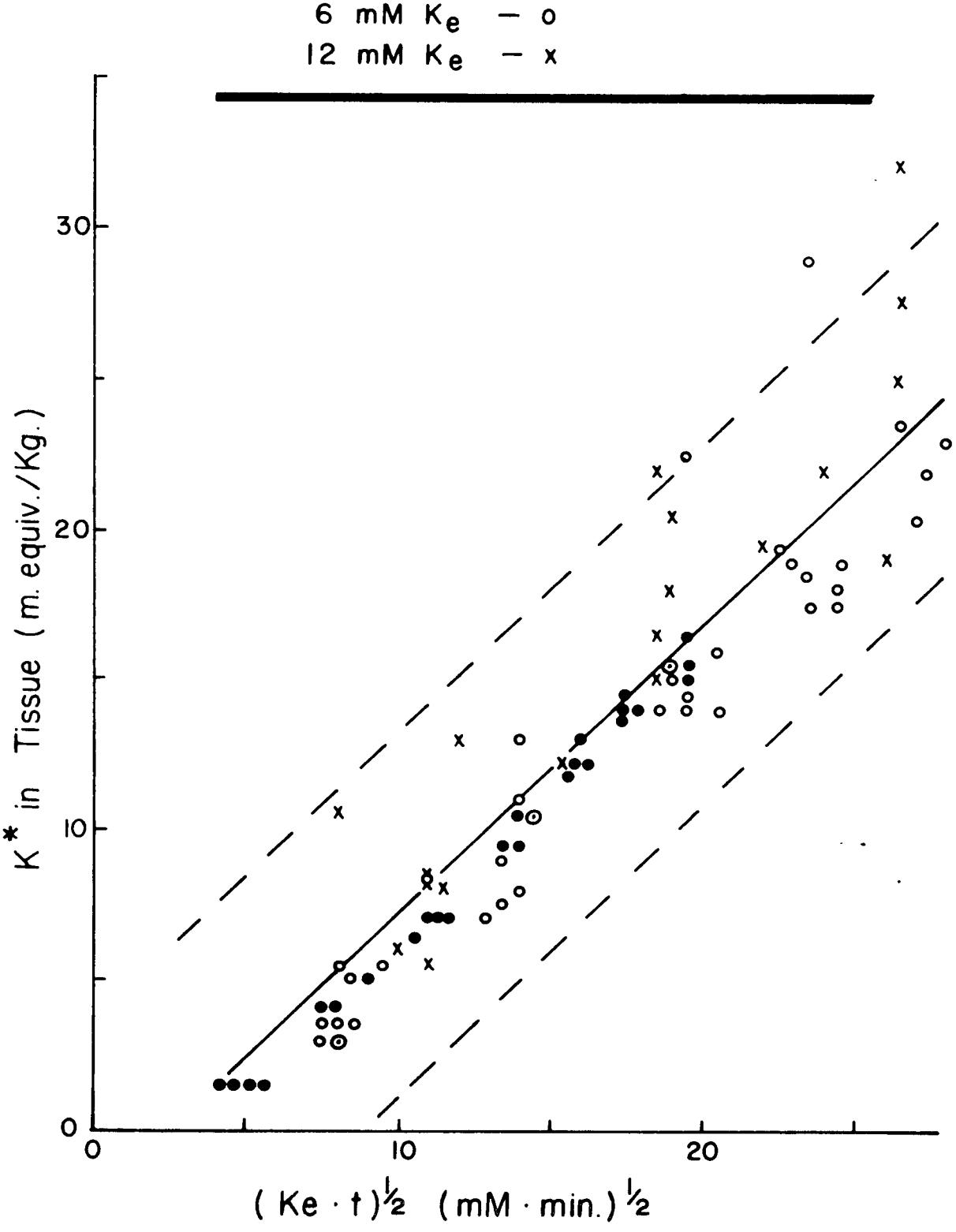
1. Normal Mouse Skeletal Muscle

(a) Effects of varying K_e.

The rise in K* will represent exchange only if the K_i remains constant; if the tissue is gaining K, the net increase in tissue radioactivity will be the sum of the exchange and the net gain. Fig. VI shows the results from 19 experiments, with data from muscles incubated in 2 mM, 6 mM, and 12 mM K_e solutions all plotted on the same graph; it is apparent by inspection that the points cluster around a straight line, and the calculated regression line drawn in the Figure has a slope of 0.98 m. equiv./Kg/$(mM \cdot min)^{\frac{1}{2}}$ (S.E.$\pm$ 0.03). Muscle K exchange observed in these incubation media compares favourably to that predicted by the Harris diffusion-permeation model, in which the rate controlling step is equilibration with-

Fig. VI. Uptake of K^{42} by normal Mouse Muscle: Normal and altered K_e Incubation Media.

2 mM K_e — ●
6 mM K_e — o
12 mM K_e — x



in an adsorbed layer of K. With this "normal" exchangeability established, it was possible to measure K* uptake under conditions leading to a net increase of tissue K.

Incubation of muscle in high-K media (24 mM, 48 mM K_e) resulted in rapid uptake of K* by the tissue, and led to a net increase in K_i. Fig. VII is a graph of the K* uptake of normal muscle tissue in the high-K media; results from 4 experiments were plotted; there were not enough points to calculate a meaningful regression line, so the solid lines in the Figure are freehand estimations of the slopes of the 24 mM (lower line) and the 48 mM solution (upper line) uptake curves; the slopes of the linear portions of the curves are 2.65 and 3.11 m. equiv./Kg/$(mM \cdot min)^{\frac{1}{2}}$ respectively. The dashed line is the regression line from Fig. VI. Both high-K lines are obviously different from the normal line. The net gain in K_i can be calculated from the K^{42} data; Table IX compares the chemical analysis data and the calculated K^{42} data for the net K gain. K gain by analysis in 24 mM K_e was 85 m. equiv./Kg fresh weight; the K gain from the K^{42} data was calculated as 80 m. equiv./Kg; comparable values for 48 mM medium were 120 and 126 m. equiv./Kg. If the K analysis values for each K* uptake reading were calculated, and the net gain subtracted at each K* point, one would predict that the corrected points would fall along a straight line, one which would correspond to the normal solution K* uptake line.

From the foregoing data it seems that K exchange in high-K solutions is similar to that observed in the normal K_e media, if appropriate correction is made for the net K gain; the K exchange seems to be a diffusional phenomenon, but it is obscured in the

Fig. VII Uptake of K^{42} by normal Mouse Muscle: High – K Incubation Media.

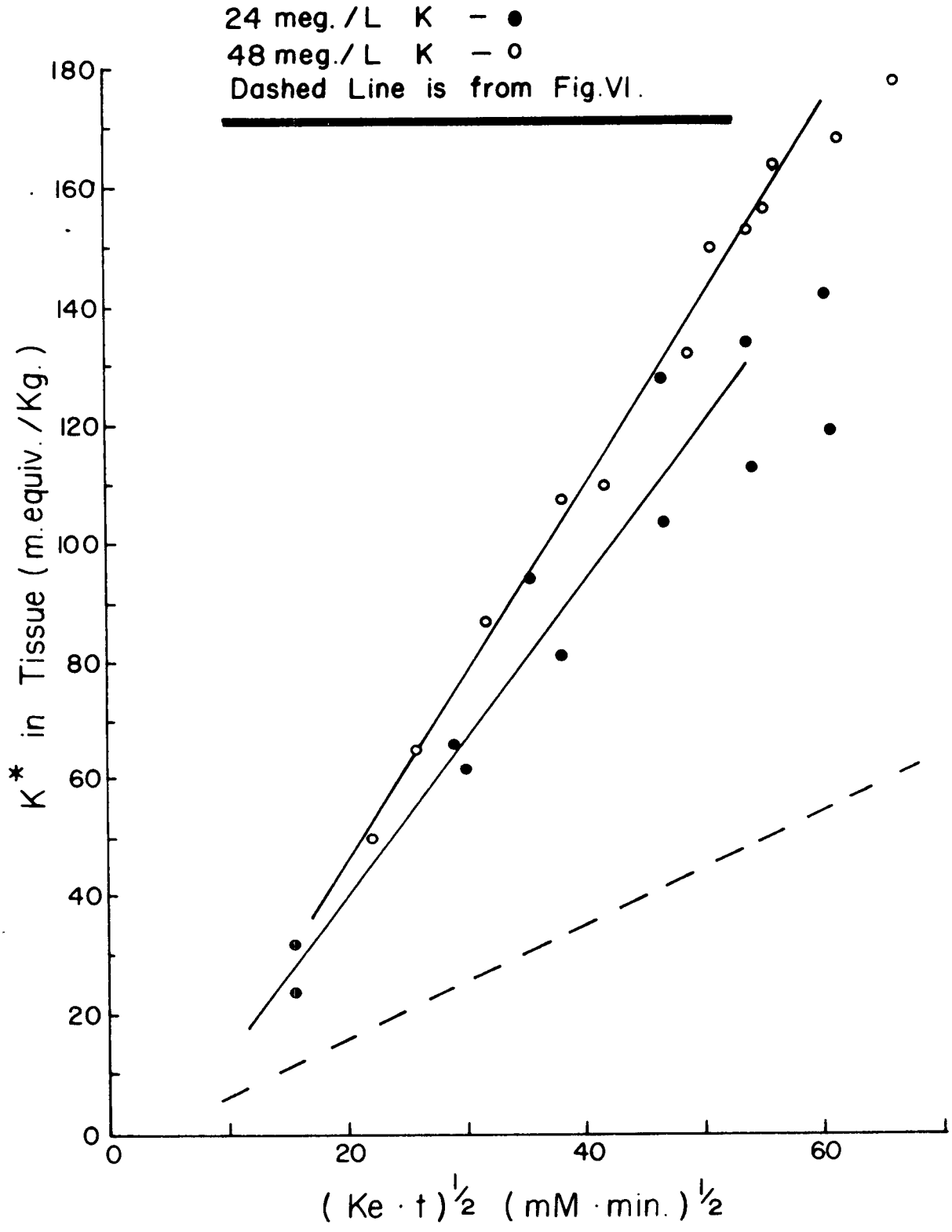


TABLE IX: EXCHANGE AND NET GAIN OF K IN NORMAL MUSCLE : HIGH -K_e INCUBATION MEDIA.
Values in m.equiv./Kg. fresh tissue.

K_e	1 NET GAIN BY ANALYSIS (1)	2 K^* AFTER 2 HOURS (FIG. VII) (2)	3 K^* EXCHANGE AT 2 HOURS (FIG. VI) (3)	4 DIFFERENCE COLUMN (2) -(3) (4)
24	85	127	47	80
48	120	194	68	126

high-K media by the large uptake of K* due to net tissue K gain, both in exchange for Na_i and as uptake of KCl.

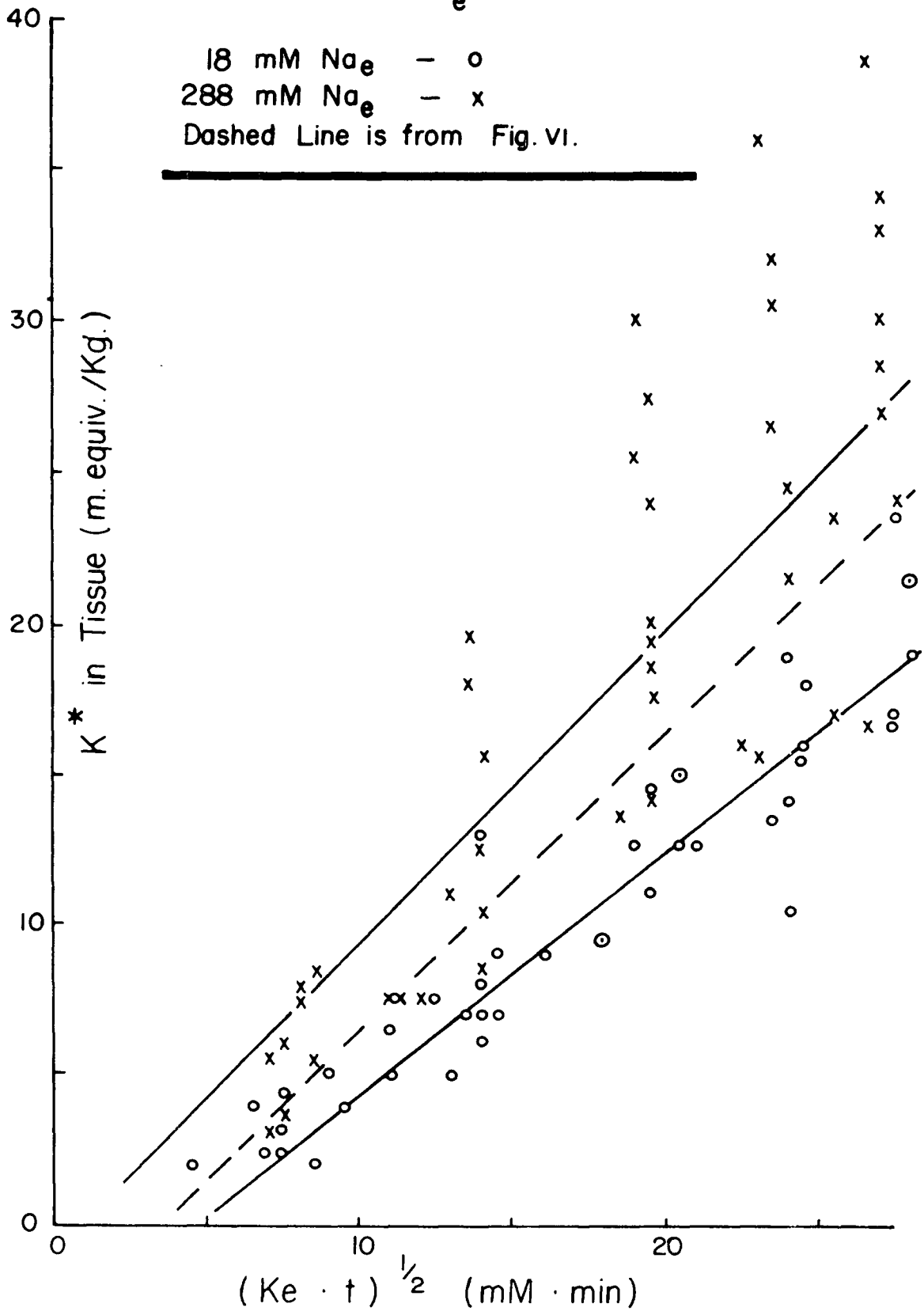
(b) Effects of varying Na_e.

The interdependance of Na and K ions in muscle has been repeatedly demonstrated, and changes in the external concentration of one ion can affect the movement of the other (see Introduction). Muscles were incubated in two altered Na_e solutions, one with 18 mM Na_e, the other with 288 mM Na_e. The latter solution was hypertonic. If K* uptake is plotted against the appropriate $(K_e \cdot t)^{\frac{1}{2}}$ values, the resultant points fall along two lines, as illustrated in Fig. VIII. The dashed line is from Fig. VI; the solid line above it is the regression line for 8 experiments measuring K uptake in 288 mM Na_e medium; the slope is 1.02 m. equiv./Kg/$(mM \cdot min)^{\frac{1}{2}}$ (S.E.$\pm$ 0.08). The low-Na regression line is significant at the 1% level if the uptakes in normal (144 mM) Na and low (18 mM) Na are compared. The tendency for exchange to be slightly increased in high-Na incubation media is not significant. The tissues were losing K in both media, but appeared, on the basis of the 4 hour incubation values, to be losing it more rapidly in the 18 mM Na_e solution. The unknown interaction between K leaving the muscle and the K* from the ECS attempting to enter and exchange with the K_i might affect the net uptake of K*, and alter the slope of the uptake curve; the K* uptake appears to be reduced in the 18 mM Na_e solution, and to be slightly raised in the 288 mM solution; the reduction may have been due to this interaction, while the gain in 288 mM medium could have been influenced by the large rise in Na_i observed even after 2 hours incubation in this fluid.

(c) Effects of varying K_e and Na_e

A few experiments were performed using combinations of

Fig. VIII Uptake of K^{42} by Mouse Muscle : Altered Na_e Incubation Media.



increased and decreased Na_e and K_e levels. The number of experiments was such that statistical evaluation would be inconclusive; only the general tendencies illustrated in Fig. IX will be reported. (Normal K_e = 6 mM, normal Na_e = 144 mM.).

In 12 mM K-288 mM Na solution, the line of points seems to be slightly above the "normal" regression line, and approximates the upper solid line in Fig. VIII.

One experiment in 2 mM K_e - 18 mM Na_e medium showed the K exchange to be below the "normal" line, and to approximate the lower line in Fig. VIII.

Incubation in 12 mM K_e - 18 mM Na_e solution resulted in the plotted points lying between the normal regression line and the lower line in Fig. VIII.

Chloride-free medium (sulphate replacing Cl) was used for two experiments. With 6 mM K_e the uptake line was slightly below the normal regression line, but with 12 mM K_e approximated it.

These results correspond to those reported earlier, that is, slight alterations of K_e did not seem to affect the rate of K exchange significantly; altering Na_e as well as K_e should have produced a curve similar to those seen previously for altered Na_e alone; 2 mM or 12 mM K_e should not affect the uptake appreciably. The experiments with Cl-free media yielded an uptake curve resembling the normal one, indicating that chloride may not exert a significant effect on K exchange in muscle. Harris and Sjodin (1961) stated that K exchange was somewhat reduced in Cl-free media; the reduction noted here corresponds to this finding, and may have been due to a decreased net uptake of K due to a lack of swelling by the muscle in the Cl-free media.

Fig. IX. Uptake of K^{42} by normal Mouse Muscle : Altered Na_e and K_e Incubation Media.

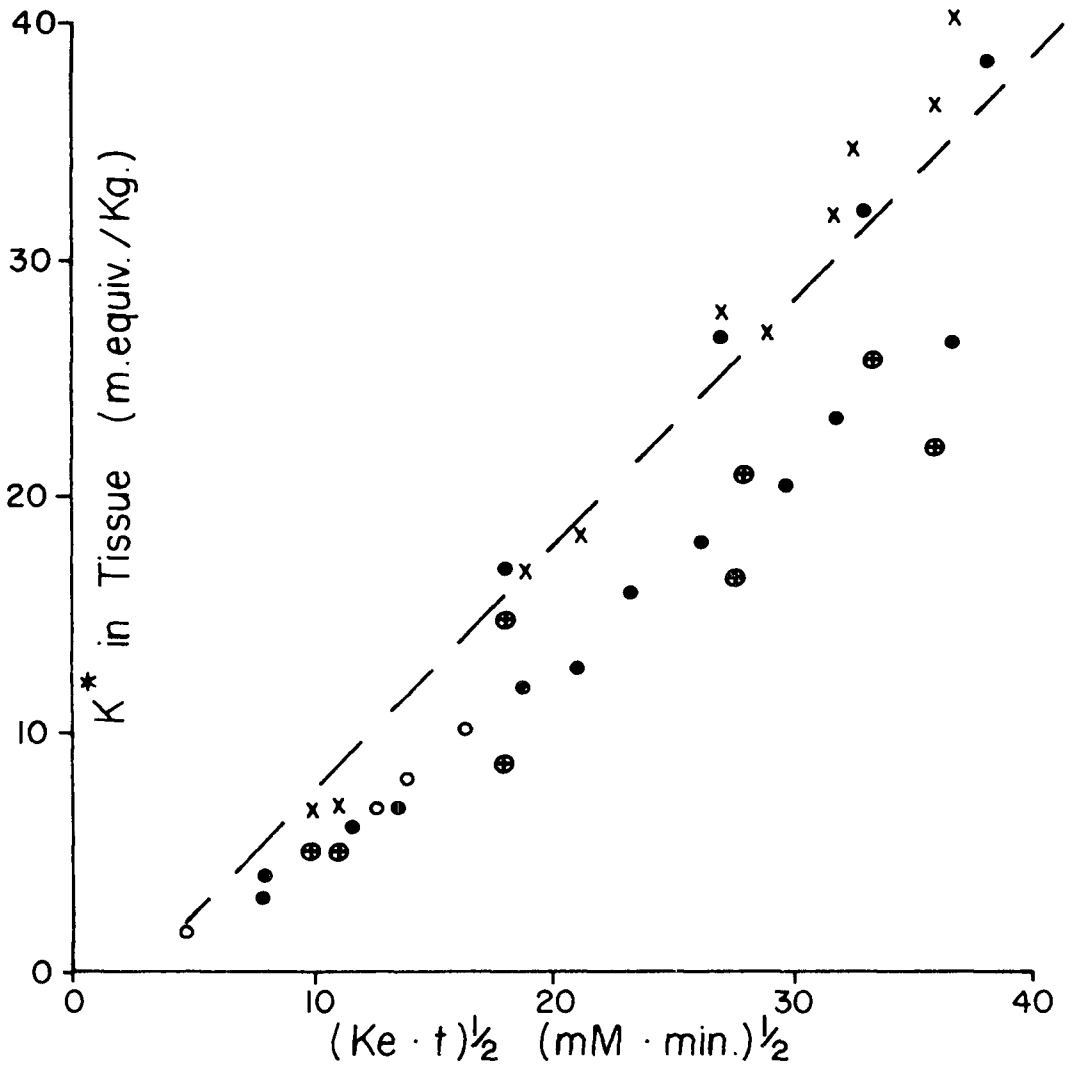
x 12 mM K_e – 288 mM Na_e

o 2 mM K_e – 18 mM Na_e

⊕ 12 mM K_e – 18 mM Na_e

• Chloride — free medium

Dashed line is normal uptake slope



2. Dystrophic Mouse Skeletal Muscle

(a) Effects of Varying K_e

Thirteen experiments were performed with muscles from dystrophic animals. Muscles were incubated in 2 mM, 6 mM and 12 mM K_e incubation media, and the K* uptake plotted; Fig. X shows the experimental values calculated, the dystrophic regression line (solid), and the normal tissue regression line (dashed). Again, the points fall along a single line when plotted against $(K_e \cdot t)^{\frac{1}{2}}$; the slope of the line is 1.33 m. equiv./Kg/(mM • min)$^{\frac{1}{2}}$ (S.E.$^{\pm}$ 0.06), as compared to 0.98 for the normal tissue. The slopes of the lines are significantly different ($p < 0.01$).

(b) Effects of varying Na_e

A few experiments have been performed using altered Na_e media; the results for 18 mM and 288 mM Na_e media are plotted in Fig. XI; it can be seen that K* uptake tends to be somewhat more rapid in the 288 mM medium, for the slope of the line is 1.80 m. equiv./Kg/(mM • min)$^{\frac{1}{2}}$ (S.E.$^{\pm}$ 0.04) as compared with 1.33 for dystrophic tissue in normal K solutions. The difference between this slope and the normal slope is significant ($p < 0.01$). The 18 mM Na medium exhibits a definitely slow K* uptake, for the slope of this line is 0.81 (S.E.$^{\pm}$ 0.04), considerably less than the 6 mM dystrophic tissue line; the difference between the two lines is significant ($p < 0.01$).

3. Summary of K^{42} Results

Uptake of K* from incubation media with 2 to 12 mM K^e seemed to follow a unique course when the results were plotted as K* uptake against $(K_e \cdot t)^{\frac{1}{2}}$, as described by Harris and Sjodin (1961). The uptake rate for normal muscle in these solutions was 0.98 m. equiv.K*/Kg/(mM • min)$^{\frac{1}{2}}$; the comparable rate for dystrophic

Fig. X: Uptake of K^{42} by Dystrophic Mouse Muscle: Normal and altered K_e Incubation Media.

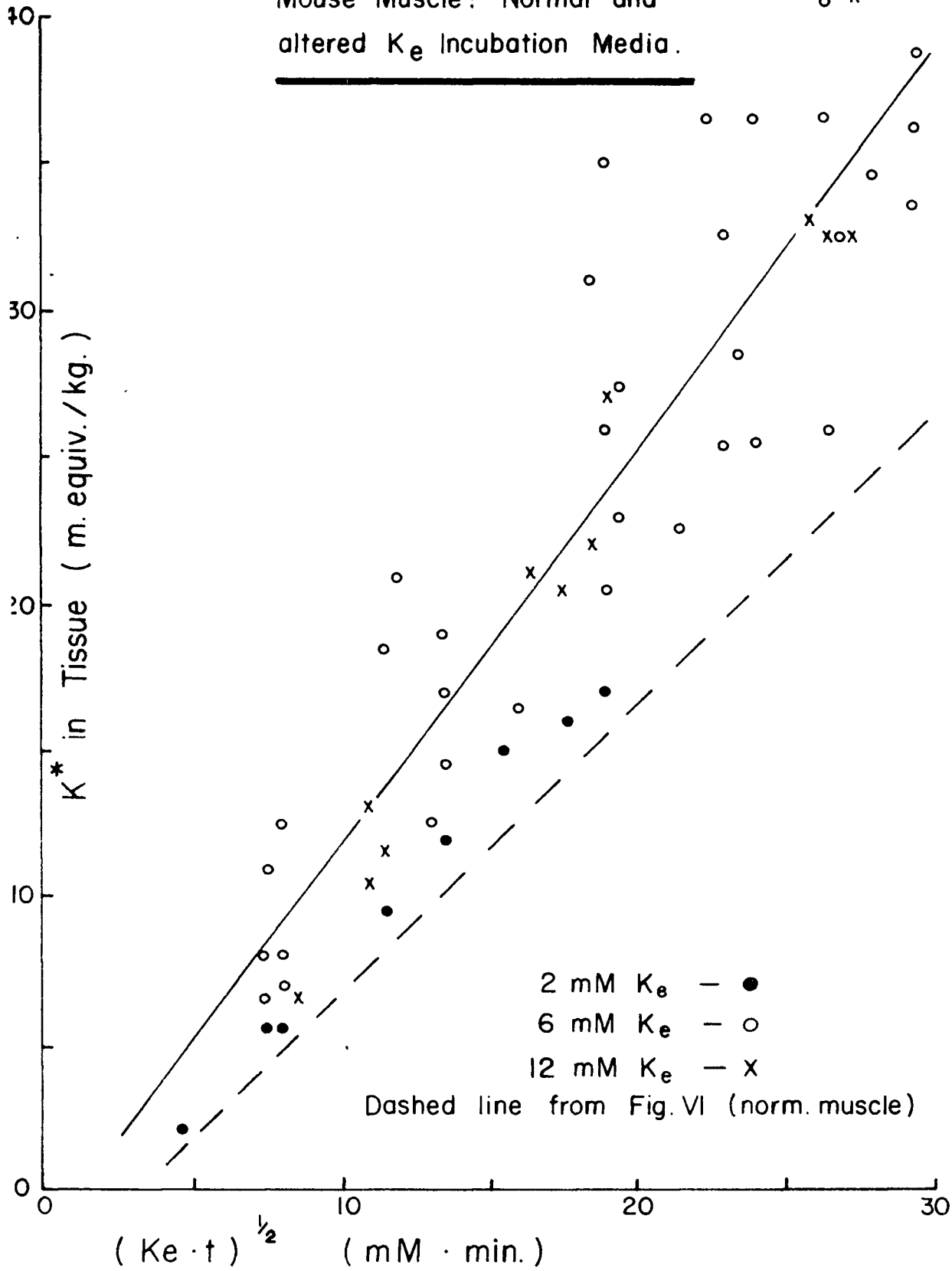
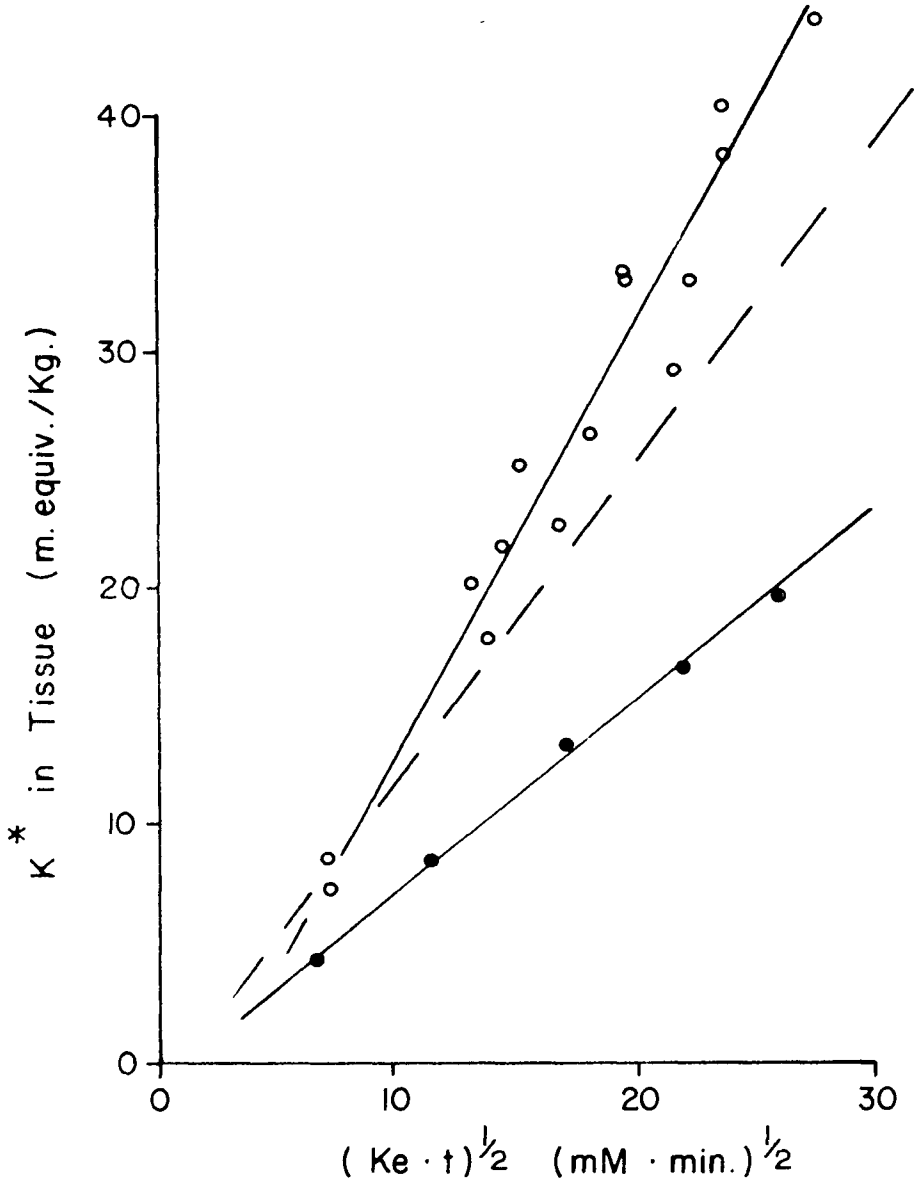


Fig. XI. Uptake of K^{42} by Dystrophic Mouse Muscle: Altered Na_e Incubation Media.

● 18 mM Na_e
o 288 mM Na_e

Dashed line is dystrophic regression line from figure X



muscle was 1.33, one-third more than for normal tissue.

In high-K_e media, normal muscle accumulated K; this was reflected in a very high uptake rate of 2.65 m. equiv./Kg/ $(mM \cdot min)^{\frac{1}{2}}$ for 24 mM K_e and 3.11 for 48 mM K_e incubation media.

Doubling Na led to an increase in the rate of uptake in both normal and dystrophic tissue, from 0.98 m. equiv./Kg/ $(mM \cdot min)^{\frac{1}{2}}$ to 1.02 for the former, and from 1.33 to 1.80 for the latter. Incubation in 18 mM Na_e slowed uptake to 0.80 in normal muscle, and to 0.81 in dystrophic muscle. The effect noted in 18 mM Na_e medium may be due to interaction between K being lost from the cell during incubation (see Table VIII) and the K* entering the cell. Harris and Sjodin (1960) found that altering Na_e (replacing any deficit with sucrose) did not appreciably affect the rate of K* uptake; a slight tendency for enhanced or decreased uptake (as compared with normal uptake) might be due to cation interaction in those cases where the muscles are gaining or losing K. This faster rate of K* uptake in dystrophic tissue is not due to any net gain of KCl by the cells, but may be an abnormal permeability of the cell membrane, resulting in a rapid uptake of K. There has been a report of increased permeability of dystrophic tissue to aldolase (Zierler 1958); possibly the capacitative membrane or region of the dystrophic cell is changed in some way, enabling the K in solution to equilibrate within this resistive layer more rapidly than usual, thus facilitating its diffusion into the interior of the cell. The mechanism by which equilibration within this region occurs does not seem to have been affected by the dystrophic change, for the uptake curve is still a straight line, even though the rate of uptake is increased.

SECTION IV

DISCUSSION

A. Tissue Compartments

B. Chemical Analyses: Fresh and Incubated Muscle

C. K^{42} Uptake Studies

1. K^{42} Uptake of Normal Muscle

(a) "Normal" K_e Solutions

(b) The Effect of High K_e Media

(c) The Effect of Low K_e Media

(d) The Effect of Na_e

2. K^{42} Uptake of Dystrophic Muscle

A. TISSUE COMPARTMENTS

The ECS volume of muscle appears to depend on the size of the muscle, and to vary according to its condition and possibly its location in the body. This latter factor may be only a reflection of muscle size, for whole muscles vary in size depending on their location and function. On the basis of the inulin space results, one would predict that the extensor digitorum longus muscle, a naturally small muscle, would have a larger ECS value than the gastrocnemius, and this has been reported (McLennan 1956). The size dependence of ECS on muscle size observed in the strain 129 mice could have been a peculiarity of the strain; since the dystrophics exhibited the dependency to a more marked degree than the normal animals, the normal animals might have carried a mild subclinical muscle defect. However, ECS assay on muscles from Swiss mice yielded results similar to those found for the strain 129 mice; the same dependency of ECS to muscle size was noted, and it was concluded that this relation would probably be found for most muscle tissue; Tasker *et al* (1959) reported finding a similar negative correlation between size and extracellular volume for toad skeletal muscle.

This dependency may be a function of the development of the muscle; it is believed that the number of fibres in a skeletal muscle is fixed at birth (Ham 1957) and that growth of a muscle in size is due to an increased volume of individual fibres, and not to the generation of new fibres. Hines (1952) showed that there was a decrease in the relative vascular volume of a muscle with age, but the effect of this change on the ECS seems small compared to the differences observed in the present study. If the muscle

fibres were to expand by growth, they would first encroach on the ECS surrounding them, and a limited growth of 5 to 10% might occur without an increase in the volume of the muscle; this factor may account for a large amount of the variation found in muscles of the same weight (see Figures I and II). The expansion might initially occur in the myofibrils of the muscle fibre, for these structures could hypertrophy at the expense of the sarcoplasm; only when the hypertrophy was such that the sarcoplasm volume was minimal would the whole fibre enlarge. This myofibrillar (and subsequent fibre) growth would be gradually occurring, as the muscle is developing in situ. The amount of tendon or tough fibrous tissue in the muscle would probably affect the ECS, for the ECS of tendon is much larger than that of muscle (Manery and Hastings 1939). As the muscle increased in length, the relative amount of tendon might not increase in proportion to muscle size, and the total muscle ECS would be less if compared to developmentally earlier ECS values. These factors would also be related to the "packing" of the muscle fibres which seems to occur during development; the fibres attempt to occupy the least volume compatible with maximum s trength and performance, and only the minimum amount of connective tissue, blood vessels, et cetera is present in the muscle belly.

The muscle inulin space appeared to be less following incubation, for although the slopes of the regression lines for the in vivo and injection methods were essentially the same, the injection ECS values tended to be greater than the incubation values for the same size of muscle. It also appeared that the non-inulin space increased during incubation; the muscles swelled during incubation

and although a correction was deducted for this swelling, a portion of the ECS seems to have been added to the non-inulin (non-ECS) space. It may be that during incubation in an artificial medium, the muscle sarcolemma becomes permeable to inulin, which penetrates into the sarcoplasm, and occupies the region inside the sarcolemma that is not occupied by myofibrils. Equilibration of this region (which apparently contains the sarcoplasmic reticulum) may take several hours, resulting in the slow increase in non-inulin space water previously noted. Zierler (1957, 1958) reported a leakage of the enzyme aldolase (MW 147,000) from normal rat and mouse muscle; Cotlove (1954) showed that prolonged intravenous administration of inulin or sucrose into rats would eventually lead to the muscle ECS indicated by these substances equalling the space occupied by chloride ion, an ion that is largely extracellular. These findings suggest that the muscle membrane may be permeable to inulin, although this does not seem to be the case for incubation up to 4 hours' duration (McLennan 1956). Muscles from dystrophic mice seem to have abnormally permeable membranes and Zierler (1958) observed a noticeably greater leakage of aldolase from dystrophic tissue. It is possible that the abnormally large inulin space values for dystrophic tissue reported in this thesis are due to a penetration of the degenerating muscle membrane by the inulin tracer. The ECS of a small normal muscle would be fairly high, but small dystrophic muscles have inulin spaces almost twice as large as their normal counterparts. Either the sarcolemma of the fibres has broken down, leaving the protoplasm and myofibrils exposed to the extracellular fluid, or the membrane permeability has been altered sufficiently to permit entry of inulin

into the fibre. The smallest dystrophic muscles had inulin spaces of ca. 90%; this would indicate few functional muscle fibres if the inulin permeable ones were completely degenerated, yet visible twitches could easily be observed upon electrical stimulation of the muscle, and resting and action potentials could be recorded without difficulty (Burr and McLennan, unpublished observation). Thus it seems likely that the change in the dystrophic tissue is in the nature of an alteration in permeability of the fibre sarcolemma; this may be followed in terminal degenerative stages of the fibre by complete degeneration of the membrane, effectively adding the volume of the cell to the extra cellular space.

Percentage water loss averaged 76% for normal muscle and 77% for dystrophic muscle (see Table VI); these two values were not significantly different. The normal muscle water loss was the same whether the muscles were fresh, incubated in Solution 1, or incubated in Solution 2. It seems that incubation did not affect the total muscle water content; however, when the non-inulin space water was calculated for these muscles (see Table VII), it was found (for normal muscle) to rise from 47% of the fresh weight for non-incubated tissue, to 58% after 2 hours incubation, and to 65% after 4 hours incubation. One must conclude that the non-inulin space water increases at the expense of the extracellular water. The total muscle water was also constant in muscles of varying size; if the ECS progressively decreased with increasing size, the non-inulin space water must have progressively increased along with the muscle size.

The ECS results were obtained from a large number of muscles; the dry weight and calculated non-inulin space water

figures were from a small number of muscles; one feels that a more thorough investigation of the problem is necessary before any further conclusions regarding the variability of the non-inulin space water with muscle weight or incubation time can be justifiably made.

B. CHEMICAL ANALYSES: FRESH AND INCUBATED MUSCLE

A summary of the K and Na analyses is found in Table VIII (page 51); approximately 375 analyses for K and Na were done on both types of muscle. The expected distribution of ions was observed in fresh normal muscle, for the K_i was high and the Na_i low; the figures of 89.9 m. equiv./Kg and 30.0 m. equiv./Kg respectively compare with 100 m. equiv./Kg and 46 m. equiv./Kg as reported by Baker, Blahd and Hart (1958), and 121.0 m. equiv./Kg and 36.5 m. equiv./Kg as reported by Williams et al (1957). These reported values have not been corrected for ECS ion content, and thus are not strictly comparable to the figures from Table VIII. The ECS correction for Na is especially important, because the Na_e is a large value. Conway (1950) found that if one assumed the intracellular sodium to be in two fractions, one bound firmly to cellular constituents, and the other free to exchange rapidly with the extracellular fluid. He assumed further that the "true" intracellular Na was the bound portion and was only 2-3 m. equiv./Kg instead of 15-20 m. equiv./Kg as usually reported. His data were based on kinetic studies of Na* efflux from muscle for a discontinuity in the efflux curve indicated two discreet fractions of ion that differed in the time taken to leave the muscle fibre. The correction made in this thesis was the subtraction of the appropriate ion content of the 4 hour inulin space water, with no attempt having

been made to correct for the "fast fraction" of non-inulin space Na.

Muscle tissue from dystrophic mice was found to contain 68.2 m. equiv.K/Kg and 58.2 m. equiv. Na/Kg (Table VIII). These higher Na_i and lower K_i levels were similar to that observed by Baker et al (1958), who reported that K_i was 76 m. equiv./Kg and Na_i 66.0 m. equiv./Kg. The percentage rise in Na_i or fall in K_i is the same in both these instances. Williams et al (1957) found the dystrophic K_i to drop to 56.7 m. equiv./Kg, nearly half the normal figure, and Na_i to rise 60% to 60.0 m. equiv./Kg. Young et al (1959) found similar changes in ion levels in their normal and dystrophic analyses, with dystrophic K_i 18% greater than the normal muscle values. Analyses of muscle biopsies from human patients with muscular dystrophy (Horvath, Berg, Cummings and Shy 1955) showed Na levels to be increased and K levels to be decreased as compared to cation levels in biopsy samples from normal individuals. Blaxter (1952) reported similar alteration of electrolytes in muscle tissue from dystrophic calves.

It is apparent that the Na and K differences between normal and dystrophic tissue are not limited to mouse muscle, but are rather a manifestation of the dystrophic change in general. The abnormal permeability of the muscle membrane, noted above for inulin, might itself account for the lowered K_i in dystrophic tissue, either directly or by leakage of intracellular protein. Since a large part of the K_i is held in a Gibbs-Donnan equilibrium, a lower intracellular protein would be expected to reduce this effect, and thus to make K_i more nearly equal to K_e. There is the possibility also that this lowered protein content could be due to an abnormal cellular metabolism. Thus, for example, Milman (1954) reported

that glycogen synthesis was abnormal in dystrophic animals; Rosenkrantz and Laferte (1960) observed that dehydrogenase activity in dystrophic mouse muscle was greater than normal, and Weinstock, Epstein, and Milhorat (1958) found that cytochrome oxidase activity was increased in dystrophic mouse tissue. Other enzyme systems appear to be affected as well; Kruh, Dreyfus, Schapira and Gey (1960), using C^{14}-labelled glycine, reported a faster protein turnover rate for dystrophic mouse tissue. Zymaris, Saifer, and Volk (1960) found a faster turnover rate of acid-soluble nucleotides in dystrophic muscle and Rabinowitz (1960) reported more rapid lipogenesis in dystrophic mice than in their normal littermates. From the purely ionic analyses performed on mouse muscle for this thesis, it is not possible to support either the increased membrane permeability hypothesis of the altered cellular metabolism hypothesis as being the cause of the abnormal Na_i and K_i levels found in dystrophic mouse muscle. The inulin space data seems to support the former view, but it is difficult to differentiate adequately between the two conditions.

Incubation of muscle tissue generally resulted in K loss and Na gain; even the 12 mM K solution did not maintain K_i constant during 4 hours of incubation. It is believed (Harris 1960) that correct incubation fluid ionic concentration and correct isotonicity are not the only conditions that must be provided to maintain cellular stability in vitro; adequate oxygen supply, suitable temperature, and adequate substrates for metabolism are also needed. These requirements are especially important in mammalian muscle, which has a high rate of cellular metabolism, and which relies heavily on continuous oxidative metabolism for its energy supply. Creese, D'Silva, and Northover (1958), observed

that the addition of colloids to the incubation media aided K and Na retention in excised mammalian muscles; recently Creese and Northover (1961) reported that crude human serum globulin would maintain rat diaphragm cations at the normal level during 2 hours of incubation.

Baetjer (1935) showed that a 20% reduction of blood supply to muscle in situ would cause a marked K loss and Na gain. The solutions that were used for incubation media in the present study were constantly oxygenated by a 95% O_2 - 5% CO_2 gas mixture. The CO_2 that dissolved in the medium formed a buffer pair with HCO_3, and maintained the pH at ca. 7.4; the bicarbonate medium was found to maintain cellular electrolytes better than a phosphate-buffered medium (Solution 6). Creese (1960) showed that rat diaphragms lost K rapidly if incubated in hypoxic media; Hill (1928) showed that thick tissues (more than 1.95 mm.) would become anoxic during incubation because of the limitation of the rate of oxygen diffusion. If any anoxia occurred in the incubated muscles in the present study, the rapid K loss and Na gain from the anoxic fibres could not be differentiated from a slight K loss in all fibres; however, for the purpose of measuring K^{42} uptake, the media used appeared to be adequate, for the intracellular K was stable for 2 hours in the normal and 12 mM K_e solutions. All the muscles incubated for 4 hours lost K; those incubated in 12 mM K did not lose as much as tissue in 6 mM K. Muscles in the K-free solution lost the most K, although in the low Na medium, much K as well as Na was lost. Choline had been used to replace the deficit of Na in the latter solution; one would expect a loss of Na to occur under these conditions, accompanied by an initial gain of K to

compensate for early Na loss; the continued Na loss may have altered the metabolism or the ionic balance of the cell to the extent that there was interference with K binding. Renkin (1961) showed that choline entered frog sartorii at a rate comparable to that of Na ion, but was lost more slowly; Keynes and Swan (1959) observed the same effect when Li was substituted for Na, but Li can substitute for Na in maintenance of the resting potential, whereas choline cannot. The replacement of Na by choline intracellularly leads to a drop in the resting potential (Keynes and Swan 1959); this could be one cause of the excessive K loss noted in the choline incubation medium. It is not known whether mammalian muscle fibres are permeable to choline but frog muscle is apparently permeable to both Li and choline, and mammalian muscle may be expected to show similar characteristics.

C. K^{42} UPTAKE

"Exchange" of K has been defined on page 56. Measurement of the rate of exchange by following K^{42} uptake, will only give a true picture if the K_i is constant, or if a known correction for K_i rise or fall can be calculated. The Harris permeation-diffusion hypothesis, described briefly on page 18, is used as a model to aid the interpretation of the kinetic data which have been presented in this thesis. Under this model exchange of an ion involves two processes; first, an exchange with ions adsorbed near the cell surface, and second, the diffusion of ions inwards from the adsorbed layer at the same time as an outward diffusion of an equal number of internal ions takes place. The ions moving outward will compete for sites in the adsorbed region with those ions entering the region from the extracellular space. Thus the adsorbed region can act as

an ion exchanger, with chemically different ions such as K, Na, and Rb being interchanged, or ions of the same species being exchanged, such as the exchange occurring between labelled K ions from the ECS and unlabelled K ions from the cell. The equilibration of the adsorbed layer is the rate controlling process.

If the adsorption region of the cell accumulates ions at a rate that is proportional to the applied concentration, it can be shown (Harris and Sjodin 1961) that when the amount of exchange is plotted against (applied concentration x time)$^{\frac{1}{2}}$, all experimental results will fall along a single curve (at constant temperature).

1. K^{42} Uptake of Normal Muscle

(a) "Normal" K_e Solutions

McLennan (1955) working with mammalian muscle, and Harris and Steinbach (1956) using frog muscle, could not fit their kinetic data to a single exponential function of time, as is required by the "classical" concept of a thin resistive membrane separating two ionic reservoirs. The results presented earlier in this thesis do not fit this single exponential curve either, but when plotted in accordance with the Harris hypothesis, concur with his results for K-K* exchange in frog muscle. It is useful to consider the muscle cell as possessing "regions" that have a definite K capacity, and a low diffusivity for ions equilibrating within the cell. Ion movement through the adsorbed ion region will involve a series of exponential terms to describe it, for each exchange of outer layer tracer ions for non-tracer ions of an inner region will delay the equilibration of the outer adsorption layer. There can be interference to diffusion in this K region, and a cell losing K would maintain a low K* concentration within its outer region, and impede

equilibration with the rest of the cell. Interaction between species of ions could also occur in this region, and influence the K* flux by competition for adsorption sites.

In Fig. VI, the uptake of K^{42} follows a single straight line (slope 0.98 m. equiv./Kg(mM • min)$^{\frac{1}{2}}$) for muscles incubated in 2 mM, 6 mM, and 12 mM K_e solutions. It was determined (page 51, and Burr and McLennan 1961) that in these solutions the K_i remained constant for the first two hours of incubation; measurement of K* uptake would therefore give a true measure of the normal exchange process, without the necessity of correcting for any net K gain or loss. The uptake curve is linear for most of its length, and even at the beginning, during the initial equilibration of the ECS with the bathing solution, which is unquestionably a diffusion process, there is no difference between the K uptake curves in the various K_e media. The K^{42} uptake characteristics also preclude the necessity of invoking non-exchangeable fractions of muscle K, for the increase in K_e does not lead to a greater degree of K being available for exchange or to a faster rate of exchange. This is in contradistinction to the "classical" interpretation of potassium kinetics (see Introduction pages 16 to 18 for a brief account of the "classical" view).

A similar curve would be expected if K diffusion in the ECS were the rate-limiting step. The timed 1.0 minute wash given to the muscles after removal from the K* soaking solution reduced the extracellular K* by 50%; this is presumably regained in the first minute following immersion in the incubation fluid, for Carey and Conway (1954) observed that the wash does not affect the K* uptake kinetics. Harris (1957) thought that diffusion in the ECS would be slower than intracellular diffusion; a comparison of his

results for intracellular diffusivity (1954) and those of McLennan (1955) lead on to the same conclusion. Hodgkin and Horowicz (1959) studied K uptake of single muscle fibres and observed uptake curves similar to those found for whole muscle. Harris and Burn (1949) calculated that frog sartorius would take only 15% longer to equilibrate than a single fibre; Harris and Sjodin (1961) showed that the Na diffusivity was even closer to the free solution value. The latter authors also compared data from Hodgkin and Horowicz (1959) with their figures for whole muscle K exchange and concluded that turnover in the larger single fibres is about the same as the mean exchange in a whole muscle. It appears that the slight hindrance imposed by extracellular diffusion is compensated by the presence of the smaller fibres so as to make the result similar to that holding for a large single fibre.

(b) The Effect of High K_e Media

Incubation of muscles in high KCl media leads to a net gain of K_i (Boyle and Conway 1941). The gain occurs fairly rapidly and depends on the amount of K in the medium. A swelling of the muscle is associated with the gain from media containing chloride, but this is eliminated if non-penetrating methyl-sulphate is used instead. Harris and Sjodin (1961) found that K* uptake was more rapid in Cl media than methylsulphate; the Cl may facilitate K entry by accompanying it into the cell, and no electrical imbalance is incurred.

Fig. VII shows the results of incubation in 24 mM and 48 mM K_e media. The slopes of the lines are markedly steeper than the normal exchange line, for net gain of K (as well as exchange) has occurred. The net gain and the exchange can be separated only if net gain were known for each K* uptake point. Table X showed

the differences at the end of 2 hours soaking; the net gain K values as estimated by K^{42} data and non-radioactive incubation data are similar. This would indicate that the $(K_e \cdot t)^{\frac{1}{2}}$ method of plotting sensitively reflects cellular ion levels; upon inspection of an uptake curve, and comparison with a normal curve, one could accurately predict whether net K gain had occurred or not.

(c) The effect of Low K_e Media

When muscle is exposed to a K-free medium, it loses cellular K rapidly. The uptake curve, in this case, would not be as steep as the normal curve, for K is being lost, and the outward ionic movement will tend to retard the rate of adsorption of K* onto the cell. Experiments in low K_e media are best done on K-loaded cells. Harris and Sjodin (1961) reported that the K* uptake from loaded muscles was initially less than normal, and their graph of K* uptake showed a concavity during the first hour of soaking, but the remainder of the curve had essentially the same slope as a normal uptake curve. They attributed the long concavity at the beginning to the K loss occurring; when the excess K had left the muscle, the exchange curve approached the usual slope.

(d) The Effect of Na_e

The movement of muscle K appears to be affected by alterations in sodium concentration of incubation media (Steinbach 1950). Harris and Sjodin (1961) found that K uptake was less when Na_e was lowered, but considered that this was due to a loss of K from the tissue. Keynes and Swan (1959) showed that there

is not complete independance of the Na and K fluxes, for alterations in K_e will affect Na fluxes (see also Keynes 1954). McLennan (1957) noted (in rat muscle) that incubation in low-Na_e media resulted in less K uptake as compared to normal Na_e solutions. The parallelism observed between Na and K movements suggests that their movements are linked in some way. But the movement is not always a rigid 1:1 exchange of Na for K or vice versa. McLennan (1957) found the ratio to be closer to 2:1 (Na:K); however, Hodgkin and Keynes (1955) found the ratio in squid axon to be 1:1.

The results of K^{42} uptake in altered Na media are shown in Fig. VIII. The regression line of the 288 mM Na_e medium points is not significantly different from the normal line, but the 18 mM Na_e line is significant at the 1% level. In 288 mM Na_e, the muscles were rapidly gaining Na, but were also losing some K. This doubling of the incubation medium Na_e did not affect K uptake by mouse muscle; the same phenomenon was observed in frog muscle by Harris and Sjodin (1961).

The 18 mM Na_e solution caused a large fall in intracellular K. The slope of the uptake curve is less than the normal curve, and this can be attributed to the interaction between the K and Na being lost from the muscle, and the K* attempting to enter. The rate of buildup of K* on the surface layer, or the exchange from this region to the cell interior, may be hampered by the large amount of cation leaving the cell. Again, this concurs with the results reported by Harris and Sjodin (1961).

2. K^{42} Uptake of Dystrophic Muscle

Uptake of K^{42} by dystrophic tissue during incubation in 2 mM, 6 mM, and 12 mM K_e media is plotted in Fig. X. The slope of the line is much greater for the dystrophic tissue than the normal; evidently the entry of K^{42} into the muscle fibres occurs more rapidly than usual. This may be a reflection of an abnormal permeability of the muscle membrane, as postulated by Burr and McLennan (1960). With the Harris model of the muscle membrane, the increased rate of exchange appears to be due to one of two things: a facilitated increase in the equilibration of the ion exchange layer, or an increase in the K diffusivity of the internal cellular compartment. These possibilities may be related to the higher rate of metabolism in dystrophic tissue, to an early dystrophic change in the sarcoplasmic reticulum (Grant 1960) causing a decrease in the amount of material with ion-exchanger properties, or to an alteration in the structure or functioning of the ion exchange layer. Harris and Sjodin (1961a) took electron photomicrographs of normal and incubated frog muscle, and inferred from the results that part of the ion-exchange compartment might be situated in the cytoplasmic reticulum. The earliest change occurring during the development of muscular dystrophy in mice has been found by Grant (1960) to be vacuolation of the reticulum close to the I band; the vacuolation progresses until it involves the entire reticulum. This may effectively reduce the size of the ion exchange compartment, and lead to a more rapid equilibration of the muscle interior with the external environment.

There does not seem to be a qualitative change in the mode of operation of the ion adsorption region, for the uptake

curves from dystrophic muscle show no indications of differing from those for normal muscle, except that the slope is steeper.

The effects of altering the Na_e of the incubation media for dystrophic muscles were essentially the same as noted for normal muscles, except the effects were more marked.

The high ECS values found earlier are not sufficient to explain these dystrophic findings, unless the membrane permeability is grossly different. The differences in metabolism of dystrophic tissue mentioned previously may be of some importance in interpreting these exaggerated phenomena found in the dystrophic muscles. If the ion exchange compartment were smaller than usual, it might not function as effectively as it does in the normal muscle; there could be a leak of K ions from the interior of the muscle into the ECS, thus reducing the K_i to the value reported in Table VIII. This could also account for the minor differences in resting potentials observed by Conrad and Glaser (1959) and McLennan (1961).

There is no reliable method of testing the disability of the dystrophic mice, either physiologically or biochemically. If a suitable method for estimating the degree of affliction would be devised, it might be easier to interpret the observations regarding the dystrophic muscle; if the nature of the dystrophic change were known, or if one could describe more accurately the properties of the ion exchange compartment, phenomena concerned with uptake of ions by mouse tissue, both normal and dystrophic, could be more accurately and properly described.

SECTION V

CONCLUSIONS AND SUMMARY

A. Conclusions

B. Summary

A. CONCLUSIONS

1. There is a negative correlation between extracellular volume and muscle size in strain 129 mice. Mice afflicted with hereditary muscular dystrophy show the dependency more strongly than normal mice. The inulin molecule may be able to penetrate the membrane.

2. Normal mouse muscle (fresh or incubated) loses 76% of its water during heating and dessication. During the incubation, the non-inulin space water appears to increase from 47% to 65% of the muscle volume. This latter observation needs further study before conclusions can be drawn.

3. Fresh normal mouse muscle contains 30.0 m. equiv. Na_i/Kg tissue, and 89.9 m. equiv. K_i/Kg. Dystrophic muscle has 58.2 m. equiv. Na_i/Kg and 68.2 m. equiv. K_i/Kg. Intracellular ion concentrations are stable during the first 2 hours of incubation in artificial media; prolonged incubation results in Na_i rising and K_i falling. Varying the concentrations of Na and K in the incubation media influences the changes in intracellular Na and K. The changes appear to occur more rapidly in dystrophic tissue.

4. K^{42} is taken up by normal mouse muscle in much the same way as it is in frog muscle (Harris 1957, Harris and Sjodin 1961). The uptake appears to be an exchange of K* in solution for unlabelled K in the muscle fibres, and occurs at the rate of 0.98 m. equiv. K*/Kg tissue per $(K_e \cdot t)^{\frac{1}{2}}$ unit. The process appears to be governed by the laws of diffusion, and the rate of

uptake to be independent of the K_e and of the time of incubation in the medium. Doubling the Na_e does not affect the uptake rate, but reducing Na_e to 18 mM slows the uptake noticeably.

Dystrophic mouse muscle exchanges K* for K faster than normal muscle; the rate is 1.33 m. equiv. K*/Kg per $(K_e \cdot t)^{\frac{1}{2}}$ unit. Varying the K_e or the length of incubation does not affect the rate of uptake, but altering the Na_e has more of an effect on the dystrophic muscle than on the normal muscle.

5. K exchange in muscle may be mediated by a compartment in the fibres that has ion-exchange properties. This compartment would result in the K* uptake curve following the general pattern of a diffusion curve, with the rate being independent of the time of exposure. The dystrophic change appears to be either a change in membrane permeability or a reduction of the ion-exchange compartment. These two possibilities cannot be adequately differentiated by the methods used in this thesis.

B. SUMMARY

1. Muscle from 129 strain mice was assayed for ECS following *in vivo* administration of inulin or soaking of the muscle in an inulin solution. The ECS was found to decrease with increasing muscle size, and to be linear. The results obtained on 129 strain mice were confirmed on Swiss albino mice, so the tendency is not a peculiarity of the 129 strain.

The ECS of muscle from dystrophic strain 129 mice shows a greater dependency on muscle size than muscle from normal animals. The possibility of an increased permeability of the

muscle is suggested.

2. Muscles were excised from 129 strain mice and dried. Normal muscle lost 76% of the fresh weight, and dystrophic muscle 77% of the fresh weight, either fresh or following incubation in an artificial medium. A tendency for the non-inulin space water to increase during incubation was noted.

3. Na and K analyses were done on normal and dystrophic tissue (fresh and after incubation). Results for fresh normal mouse muscle were Na_i = 30.0 m. equiv./Kg tissue and K_i = 89.9 m. equiv./Kg. Fresh dystrophic tissue had Na_i = 58.2 m. equiv./Kg and K_i = 68.2 m. equiv./Kg.

Incubation of excised muscles in a modified Locke's solution resulted in a rise in Na_i and a fall in K_i after 4 hours' soaking. During the first 2 hours of incubation, the ion levels were comparable to fresh tissue values.

4. The radioactive isotope K^{42} was used to follow the K exchange of muscles during incubation in variants of Locke's solution. K^{42} uptake followed an unique course when plotted as described by Harris and Sjodin (1961). The results are interpreted in the light of the Harris diffusion-permeation hypothesis. The effect of varying the Na and K concentrations of the soaking solution on the uptake of K^{42} is considered in some detail. Results of uptake studies on dystrophic mouse muscle are presented and the differences between normal and dystrophic tissue discussed.

SECTION VI

BIBLIOGRAPHY

Adams, R.D., Denny-Brown, D., and Pearson, C.M. (1954). Diseases of muscle; a study in pathology. Paul B. Hoeber, New York.

Baetjer, A.M. (1935). The diffusion of potassium from resting skeletal muscles following a reduction in the blood supply. Amer. J. Physiol. 112, 139.

Baker, N., Blahd, W.H., Hart, P. (1958). Concentrations of Na and K in skeletal muscle of mice with a hereditary myopathy (Dystrophia Muscularis). Amer. J. Physiol. 193, 530.

Blaxter, K.L. (1952). Composition of the tissues of normal and dystrophic calves. Brit. J. Nutr. 6, 144.

Boyle, P.J. & Conway, E.J. (1941). Potassium accumulation and associated changes. J. Physiol. 100, 1.

Burr, L.H. & McLennan, H. (1960.) The apparent extracellular space of mammalian skeletal muscle. Can. J. Biochem. Physiol. 38, 829.

Burr, L.H. & McLennan, H. (1961). The transport of potassium ions in normal and dystrophic mouse muscle. J. Physiol. 158, in the Press.

Carey, M.J. & Conway, E.J. (1954). Comparison of various media for immersing frog sartorii at room temperature and evidence for the regional distribution of fibre Na. J. Physiol. 125, 232.

Conrad, J.T., & Glaser, G.H. (1959). A study of membrane resting and action potentials of dystrophic mammalian muscle. Trans. Am. Neur. Assoc. 1959, 170.

Conway, E.J. (1950). Calculation of the idiomolar value and its electrostatic equivalent in normal mammalian skeletal muscle. Irish J. Med. Sci. Series 6. 293, 216.

Conway, E.J. (1957). Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. Physiol. Rev. 37, 84.

Conway, E.J. & Carey, M.J. (1956). Active secretion of sodium from isolated Na-rich skeletal muscle. Nature, London 178, 644.

Cosmos, E. & Harris, E.J. (1961). In vitro studies of the gain and exchange of calcium in frog skeletal muscle. J. Gen. Physiol. 44, 1121.

Cotlove, E. (1954). Mechanism and extent of distribution of inulin and sucrose in chloride space of tissue. Amer. J. Physiol. 176, 396.

Crandall, L.A. Jr., & Anderson, M.X. (1934). Estimation of the state of hydration of the body by the amount of water available for the solution of sodium thiocyanate. Am. J. Digest. Dis. Nutr. 1, 126.

Creese, R. (1952). Bicarbonate ion and muscle potassium. Biochem. J. 50, XVIII.

Creese, R. (1960). Potassium in different layers of isolated diaphragm. J. Physiol. 154, 133.

Creese, R., D'Silva, J.L., Northover, J. (1958). The effect of insulin on sodium in mammalian muscle. Nature 181, 1278.

Creese, R., Neil, M., and Stephenson, G. (1956). Effect of cell variation on potassium exchange of muscle. Trans. Faraday Soc. 52, 1022.

Danowski, T.S. (1955). Electrolyte and endocrine studies in muscular dystrophy. Amer. J. Phys. Med. 34, 281.

Dean, R.B. (1941). Theories of electrolyte equilibrium in muscle. Biol. Symp. 3, 341.

Duchenne, G.B. (1848). De l'electrisation localisée. J.B. Ballière et Fils, Paris.

Frater, R., Simon, S.E. & Shaw, F.H. (1959). Muscle: a three phase system. The partition of divalent ions across the membrane. J. Gen. Physiol. 43, 81.

Gamble, J.L. (1954). Chemical anatomy, physiology, and pathology of extracellular fluid. Harvard University Press. Cambridge, Mass.

Geiger, R.S., & Garvin, J.S. (1957). Pattern of regeneration of muscle from progressive muscular dystrophy patients cultured *in vitro* as compared with normal human skeletal muscle. J. Neuropath. Exp. Neurol. 16, 532.

Grant, A.E. (1960). Fine structure pathology in dystrophic muscles from strain 129 mice. Graduation thesis for M.D. degree, University of British Columbia.

Harris, E.J. (1953). The exchange of frog muscle potassium. J. Physiol. 120, 246.

Harris, E.J. (1957). Permeation and diffusion of K ions in frog muscle. J. Gen. Physiol. 41, 169.

Harris, E.J. (1960). Transport and accumulation in biological systems. 2nd Edition. Butterworths Publications, London, Ch. 7.

Harris, E.J. & Burn, G.P. (1949). The transfer of sodium and potassium ions between muscle and the surrounding medium. Trans. Faraday Soc. 45, 508.

Harris, E.J. & Sjodin, R.A. (1961). Kinetics of exchange and net movement of frog muscle potassium. J. Physiol. 155, 221.

Harris, E.J. & Sjodin, R.A. (1961a). To be published.

Harris, E.J. & Steinbach, H.B. (1956). The extraction of ions from muscle by water and sugar solutions, with a study of the degree of exchange with tracer of the sodium and potassium in the extracts. J. Physiol. 133, 385.

Ham, A.W. (1957). Histology: Third Edition. J.P. Lippincott, Montreal, Ch. 20.

Heppel, L.A. (1939). The electrolytes of muscle and liver in potassium depleted rats. Amer. J. Physiol. 127, 385.

Heppel, L.A. (1940). The diffusion of radioactive sodium into the muscles of potassium-deprived rats. Amer. J. Physiol. 128, 449.

Hill, A.V. (1928). The diffusion of oxygen and lactic acid through tissues. Proc. Roy. Soc. B. 104, 39.

Hines, H.M. (1952). The chemic composition of atrophic and dystrophic skeletal muscle. Proc. MDAA: First and Second Med. Conf. MDAA, Inc., New York.

Hodgkin, A.L. (1957). Ionic movements and electricl activity in giant nerve fibres. Proc. Roy. Soc. B. 148, 1.

Hodgkin, A.L., & Horowicz, P. (1959). Movements of Na and K in single muscle fibres. J. Physiol. 145, 432.

Hodgkin, A.L. & Keynes, R.D. (1955). The K permeability of a giant nerve fibre. J. Physiol. 128, 61.

Hodgkin, A.L. & Keynes, R.D. (1955a). Active transport of cations in giant axons from Sepia and Loligo. J. Physiol. 128, 28.

Horvath, B., Berg, L., Cummings, D.J., & Shy, G.M. (1955). Muscular Dystrophy. Cation concentrations in residual muscle. J. Appl. Physiol. 8, 22.

Hubbard, R.S. & Loomis, T.A. (1942). The determination of inulin. J. Biol. Chem. 145, 641.

Jenerick, H.P. (1956). The effects of calcium on several electrical properties of muscle membrane. J. Gen. Physiol. 39, 773.

Keynes, R.D. (1951). The role of electrolytes in excitable tissues. Instituto de Biofisica, Universidade do Brasil. Rio de Janeiro.

Keynes, R.D. (1954). The ionic fluxes in frog muscle. Proc. Roy. Soc. B. 142, 359.

Keynes, R.D. & Swan, R.C. (1959). The permeability of frog muscle fibres to lithium ions. J. Physiol. 147, 626.

Ling, G.N. (1952). The role of phosphate in the maintenance of the resting potential and selective ionic accumulation in frog muscle cells. Phosphorus Metabolism, Vol. II, The Johns Hopkins Press, Baltimore.

Ling, G.N. (1955). Muscle electrolytes. Amer. J. Phys. Med. 34, 89.

Ling, G.N. (1960). The interpretation of selective ionic permeability and cellular potentials in terms of the fixed-charge-induction hypothesis. J. Gen. Physiol. 43, 149.

Ling, G.N. & Gerard, R.W. (1949). The normal membrane potential of frog sartorius fibres. J. cell. comp. Physiol. 34, 383.

Locke, F.S. (1895). Towards the ideal artificial circulating fluid for the isolated frog's heart. J. Physiol. 18, 332.

Locke, F.S. (1904). The action of dextrose on the isolated mammalian heart. J. Physiol. 31, Proc. xiii.

Kruh, J., Dreyfus, J-C., Shcapira, G., & Gey, G.O. Jr. (1960). Abnormalities of muscle protein metabolism in mice with muscular dystrophy. J. Clin. Invest. 39, 1180.

McLennan, H. (1955). The transfer of potassium between mammalian muscle and the surrounding fluid. Biochim. Biophys. Acta 16, 87.

McLennan, H. (1956). The diffusion of potassium, inulin, and thiocyanate in the extracellular spaces of mammalian muscle. Biochim. Biophys. Acta. 21, 472.

McLennan, H. (1956a). Physical and chemical factors influencing K movements in mammalian muscle. Biochim. Biophys. Acta. 22, 30.

McLennan, H. (1957). The diffusion of K, Na, inulin and sucrose in the extracellular spaces of mammalian tissues. Biochim. Biophys. Acta. 24, 1.

McLennan, H. (1961). The effect of variations in the intra- and extracellular ion concentrations upon the electrical activity of normal and dystrophic mouse skeletal muscle. J. Gen. Physiol. 49, 1047.

Manery, J.F. & Hastings, A.B. (1939). Distribution of electrolytes in mammalian tissues. J. Biol. Chem. 127, 657.

Manery, J.F. (1954). Water and electrolyte metabolism. Physiol. Rev. 34, 334.

Maurer, F.W. (1938). Isolation and analysis of extracellular muscle fluid from frog. Amer. J. Physiol. 124, 546.

Meigs, E.B. & Atwood, W.G. (1916). The reactions of striated muscle to potassium chloride solutions. Amer. J. Physiol. 40, 30.

Michelson, A.M., Russell, E.S. & Harmon, P.J. (1955). Dystrophia Muscularis: a hereditary primary myopathy in the house mouse. Proc. Nat. Acad. Sci. 41, 1079.

Milman, A.E. (1954). Metabolism of glycogen in experimental muscular dystrophy. Proc. MDAA Third Medical Conf., p. 291. MDAA, New York.

Pappius, H.M. & Elliott, K.A.C. (1956). Water distribution in incubated slices of brain and other tissues. Can. J. Biochem. Physiol. 34, 1007.

Rabinowitz, J. (1960). Enzymic studies on dystrophic mice and there littermates (lipogenesis and cholesterolgenesis). Biochim. Biophys. Acta 43, 337.

Renkin, E.M. (1961). Permeability of frog skeletal muscle cells to choline. J. Gen. Physiol. 44, 1159.

Ringer, S. (1883). A third contribution regarding the influence of the inorganic constituents of the blood in the ventricular contraction. J. Physiol. 4, 222.

Rosenkrantz, H. & Laferte, R.O. (1960). A comparison of reducing systems in Vitamin E-deprived rabbits and mice with dystrophia muscularis. Arch. Biochem. Biophys. 89, 173.

Sandow, A. & Brust, M. (1958). Contracility of dystrophic mouse muscle. Amer. J. Physiol. 194, 557.

Shanes, A.M. (1955). Factors governing ion transfer in nerve. In: Electrolytes in biological systems. Amer. Physiol. Soc., Wash., D.C.

Shanes, A.M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells: Part I. Pharm. Rev. 10, 59.

Shaw, F.H., Simon, S.& Johnstone, B.M. (1957). The non-correlation of bioelectric potentials with ionic gradients. J. Gen. Physiol. 40, 1.

Shaw, F.H., Simon, S.E., Johnstone, B.M. & Holman, M.E. (1957). The effect of changes of environment on the electrical and ionic pattern of muscle. J. Gen. Physiol. 40, 263.

Simon, S., Johnstone, B.M., Shankly, K.H. & Shaw, F.H. (1959). Muscle: a three phase system. The partition of monovalent ions across the cell membrane. J. Gen. Physiol. 43, 55.

Simon, S.E., Shaw, F.H., Bennett, S. & Muller, M. (1957). The relationship between sodium, potassium and chloride in amphibian muscle. J. Gen. Physiol. 40, 753.

Steinbach, H.B. (1952). On the sodium and potassium balance of isolated frog muscles. Proc. Nat. Acad. Sci. (Wash.) 38, 451.

Stevens, L.C., Russell, E.S. & Southard, J.L. (1957). Evidence on inheritance of muscular dystrophy in an inbred strain of mice using ovarian transplantation. Proc. Soc. Exper. Bio. Med. 95, 161.

Tasker, P., Simon, S.E., Johnstone, B.M., Shankly, K.H. and Shaw, F.H. (1959). The dimensions of the extracellular space in sartorius muscle. J. Gen. Physiol. 43, 39.

Ussing, H.H. (1960). The alkali metal ions in biology. I. The alkali metal ions in isolated systems and tissues. Hdb. der Exp. Pharm. 13, 1.

Weinstock, I.M., Epstein, S. & Milhorat, A.T. (1958). Enzyme studies in muscular dystrophy. III. In hereditary muscular dystrophy in mice. Proc. Soc. Exper. Biol. Med. 99, 272.

Williams, J.D., Ansell, B.M., Reiffel, L., Stone, C.A. & Kark, R.M. (1957). Electrolyte levels in normal and dystrophic muscle determined by neutron activation. Lancet, Sept. 7, 1957, p. 464.

Young, H.L., Young, W. & Edelman, I.S. (1959). Electrolyte and lipid composition of skeletal and cardiac muscle in mice with hereditary muscular dystrophy. Amer. J. Physiol. 197, 487.

Zierler, K.L. (1957). Diffusion of aldolase from rat skeletal muscle. An index of membrane permeability. Amer. J. Physiol. 190, 201.

Zierler, K.L. (1958). Aldolase leak from muscle of mice with hereditary muscular dystrophy. Bull. Johns Hopkins Hosp. 102, 17.

Zierler, K.L. (1959). Effect of insulin on membrane potential and potassium content of rat muscle. Amer. J. Physiol. 197, 515.

Zymaris, M.C., Saifer, A., Volk, B.W. (1960). Specific activity of acid-soluble nucleotides in hindleg muscles of mice with dystrophia muscularis. Nature 188, 323.

Fig. I. Inulin Space of normal Mouse Muscle:

In Vitro Method.

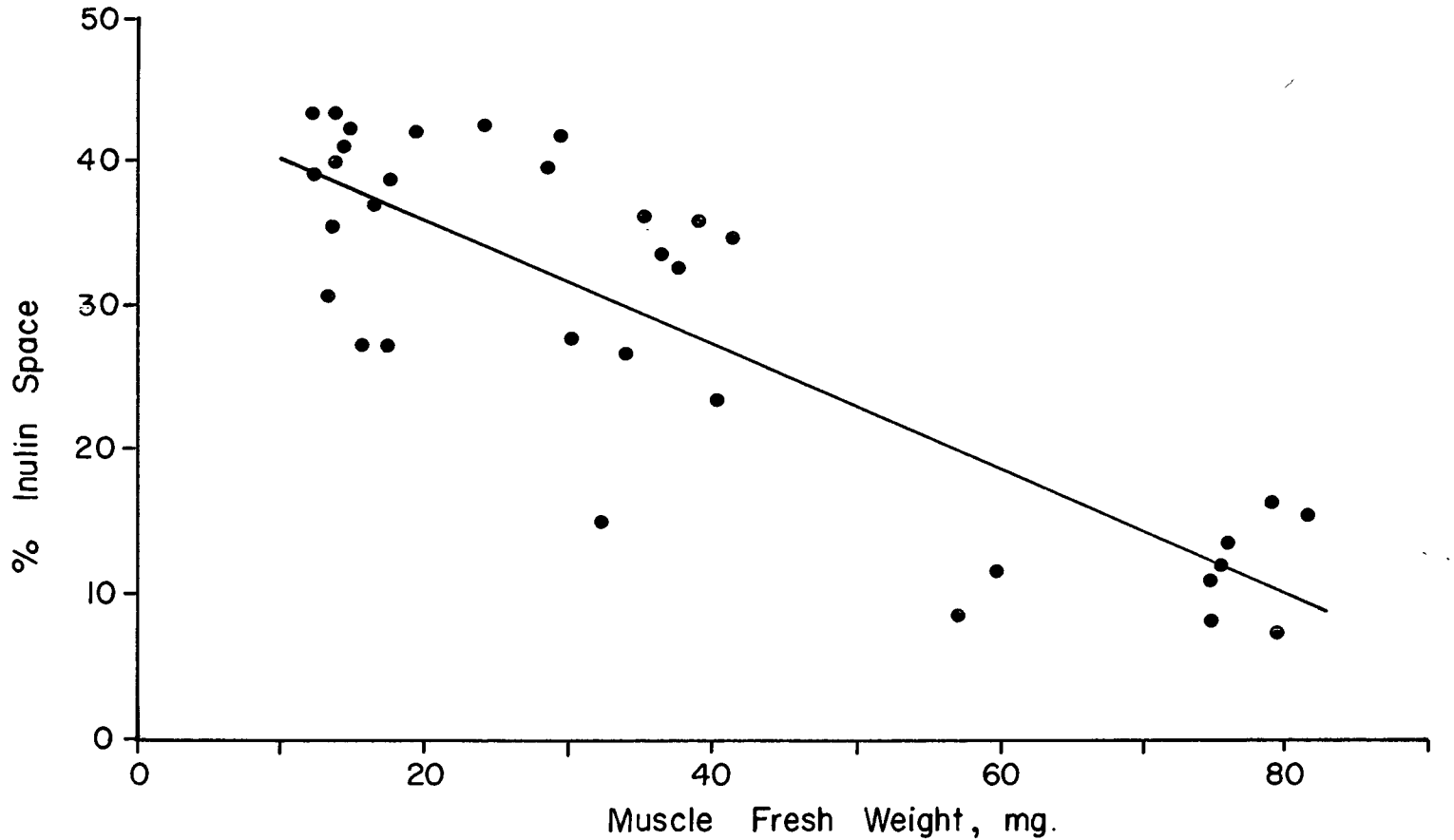


Fig. II: Inulin Space of normal Mouse Muscle: Injection Method.
• Gastrocnemii
x Peronei Longi

Figures in parentheses show the number of hours between injection and sacrifice of the animals.

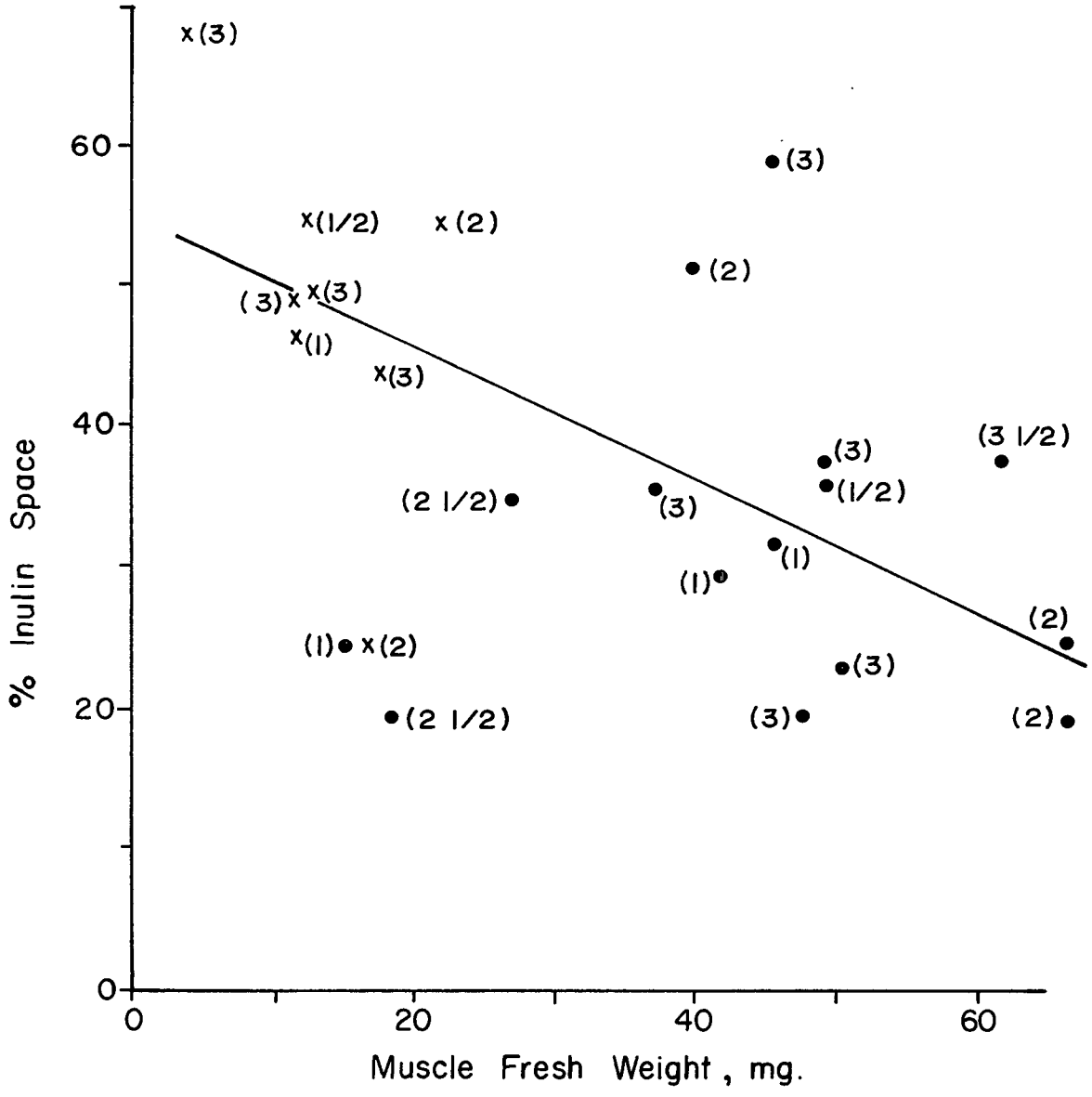


Fig. III. Inuline Space of Dystrophic Mouse Muscle : In Vitro Method.

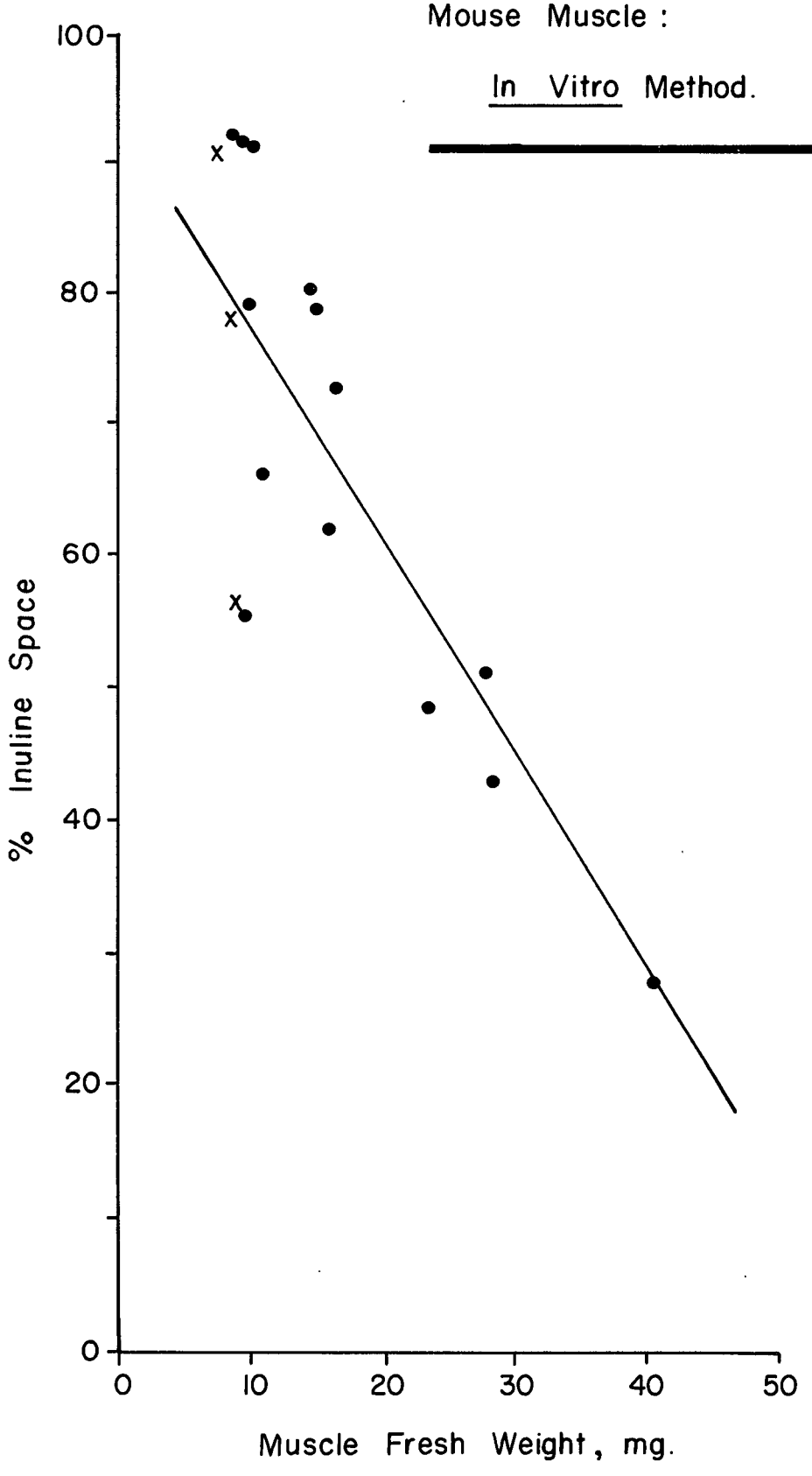


Fig. IV. Inulin Space of Swiss Albino Mouse Muscle: In Vitro Method.

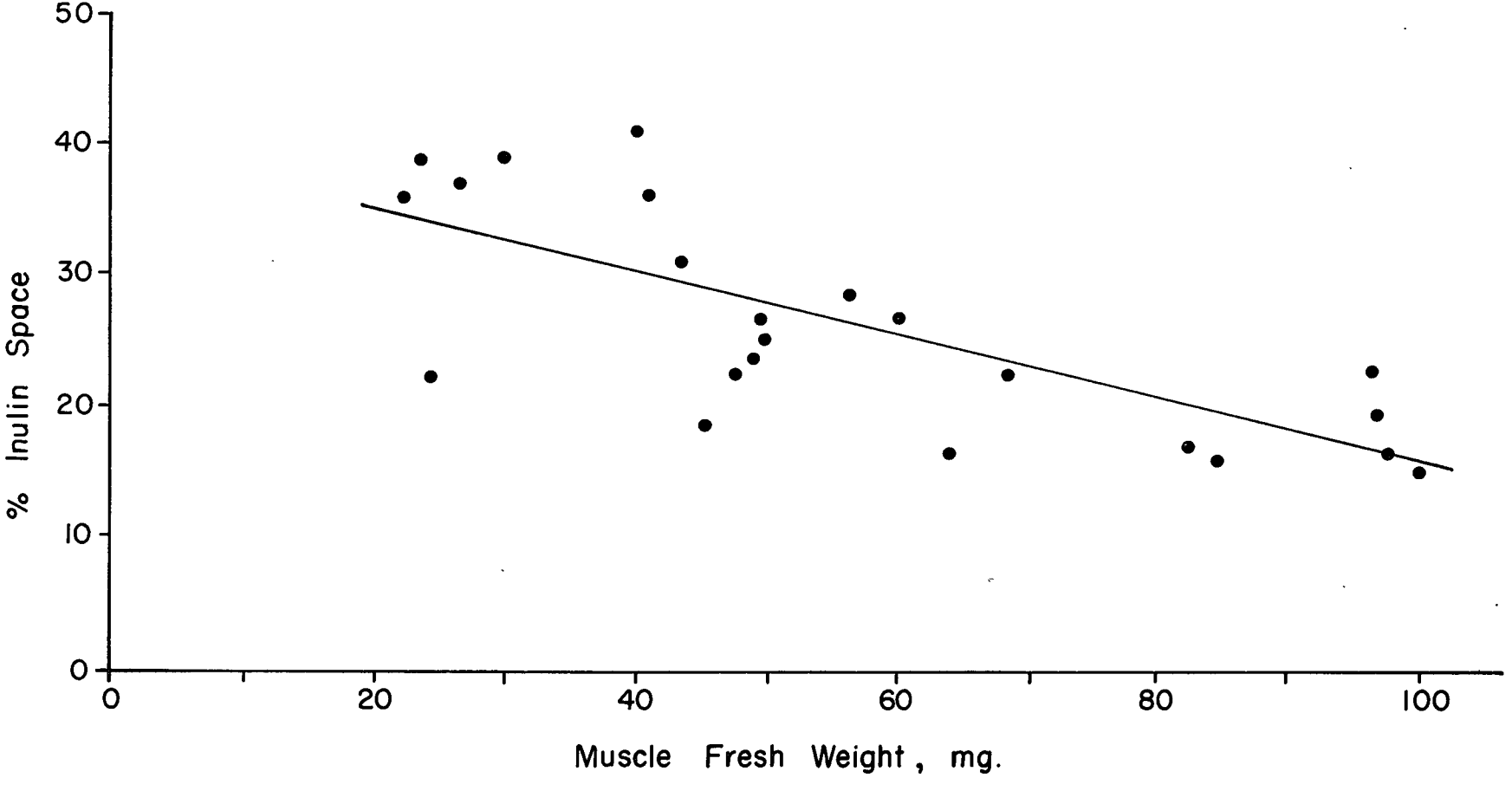


Fig. V. Thiocynate Space of normal Mouse Muscle : In Vitro Method.

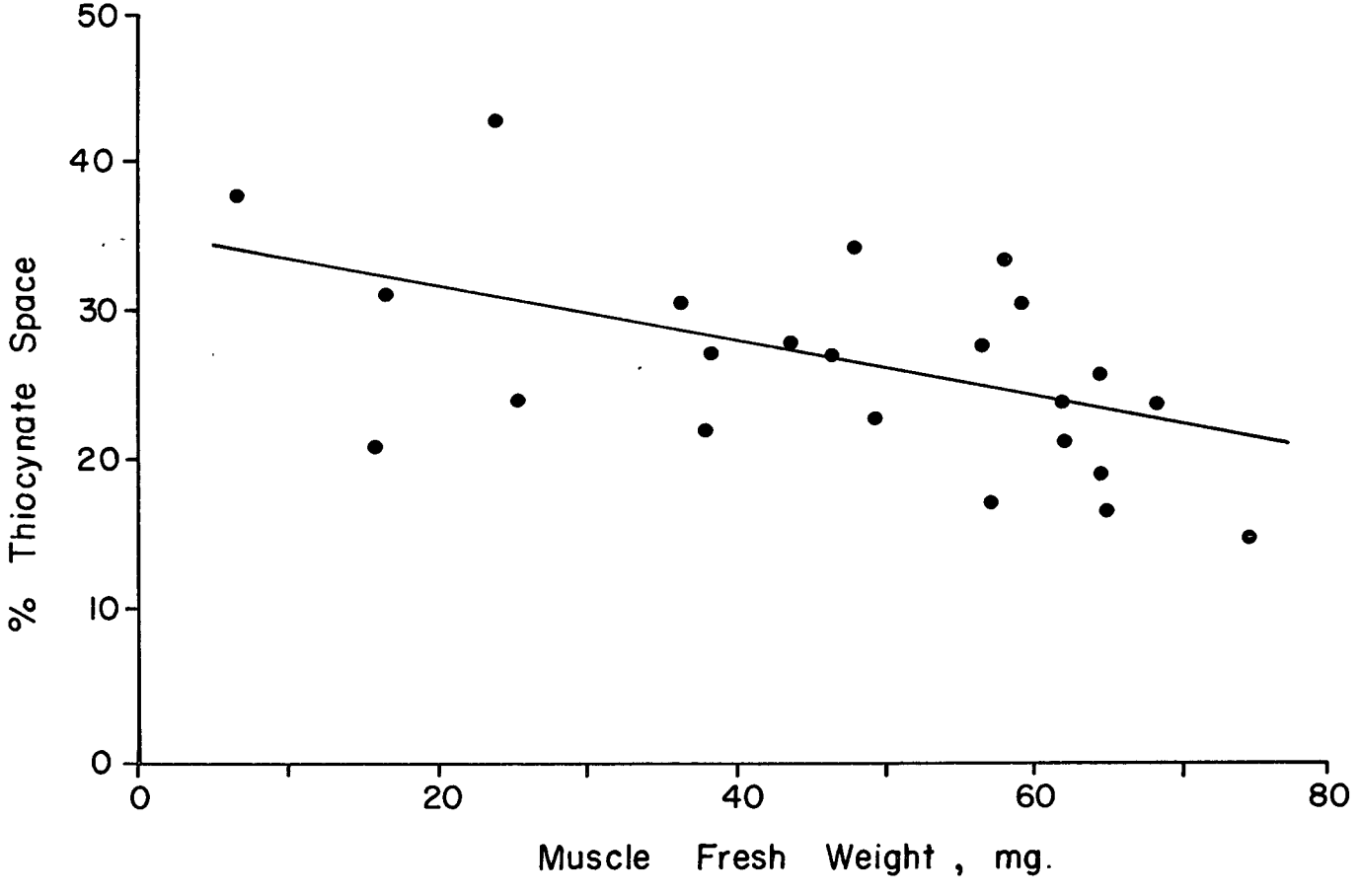


Fig. VI. Uptake of K^{42} by normal Mouse Muscle: Normal and altered K_e Incubation Media.

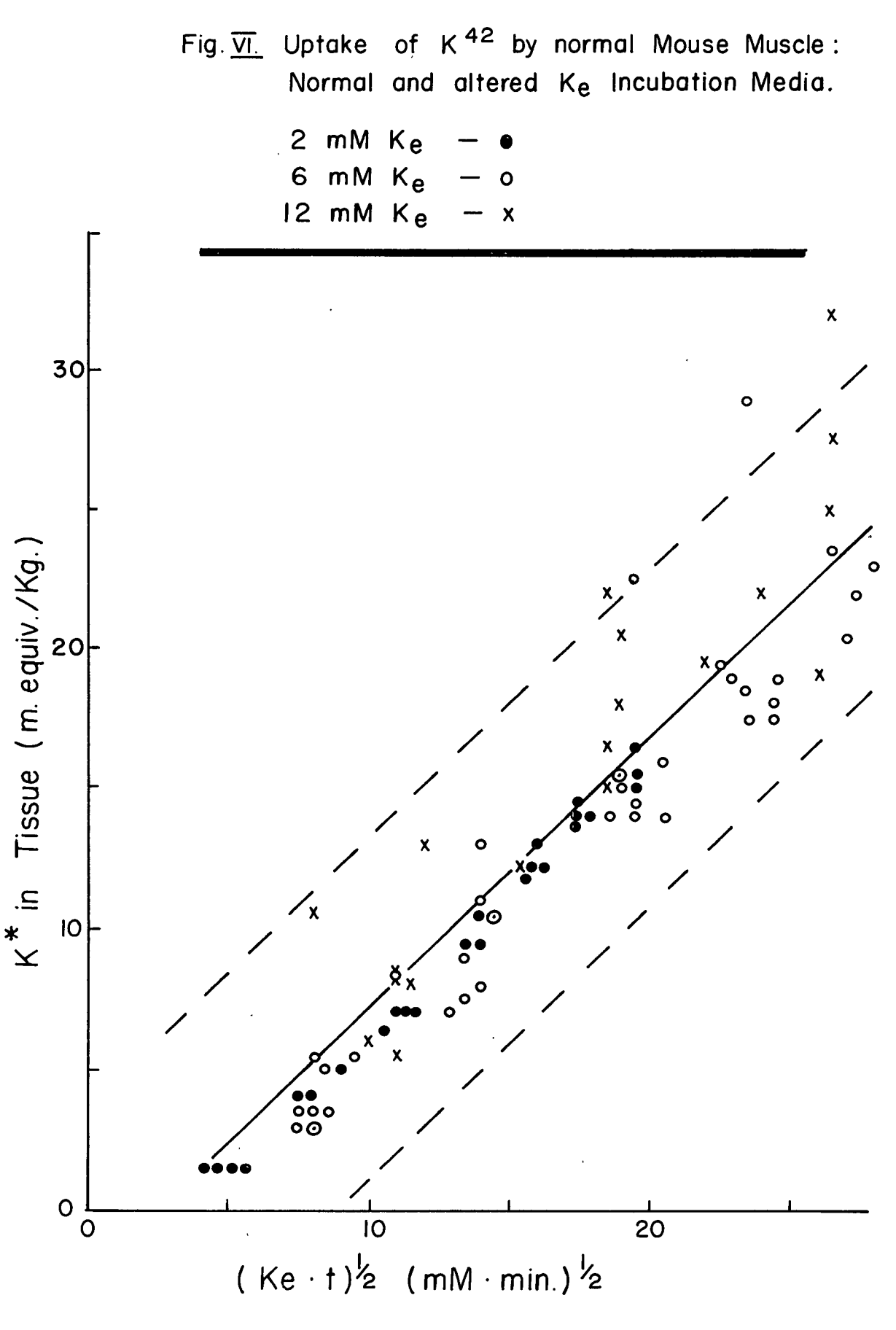


Fig. VII Uptake of K^{42} by normal Mouse Muscle: High – K Incubation Media.

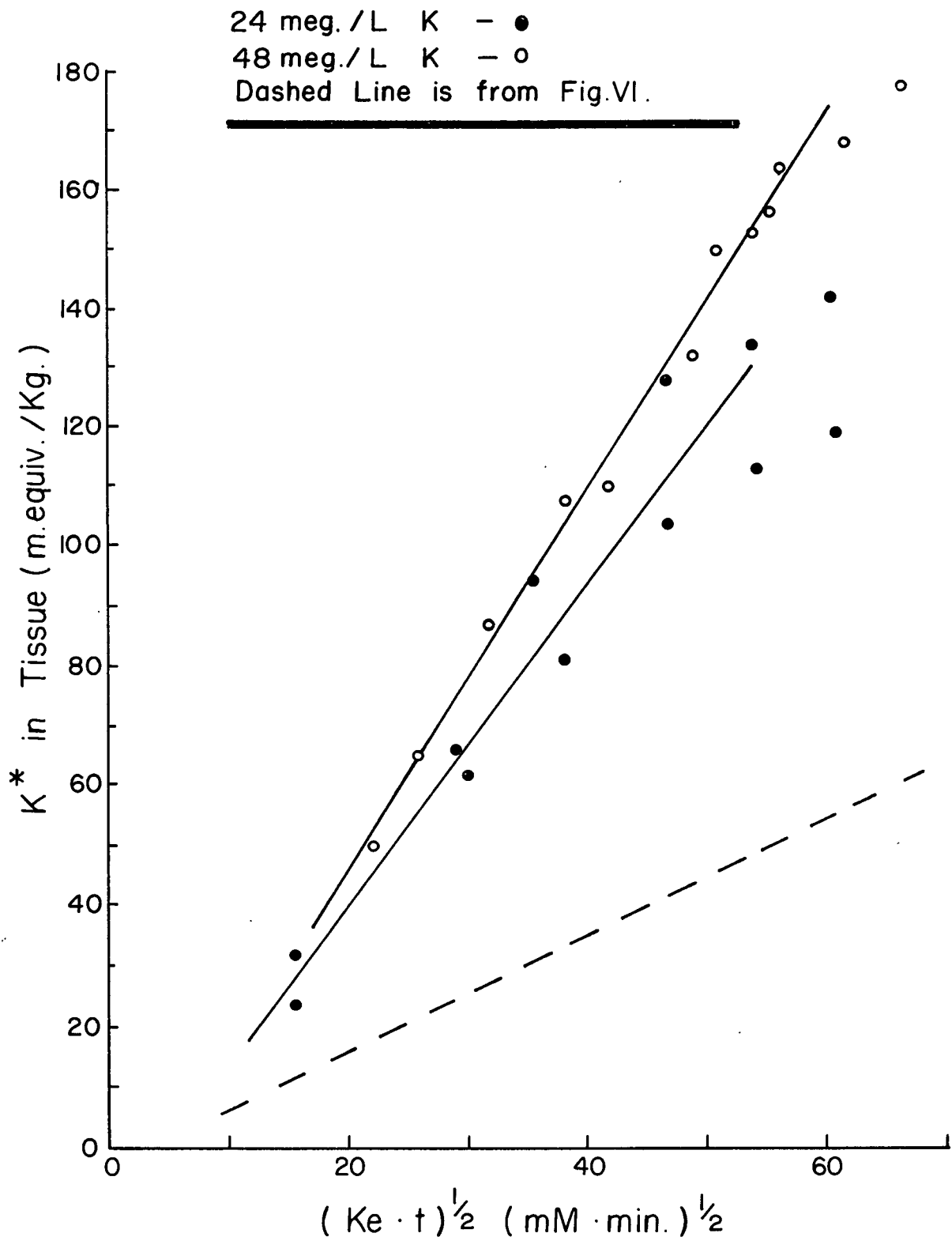


Fig. VIII Uptake of K^{42} by Mouse Muscle : Altered Na_e Incubation Media.

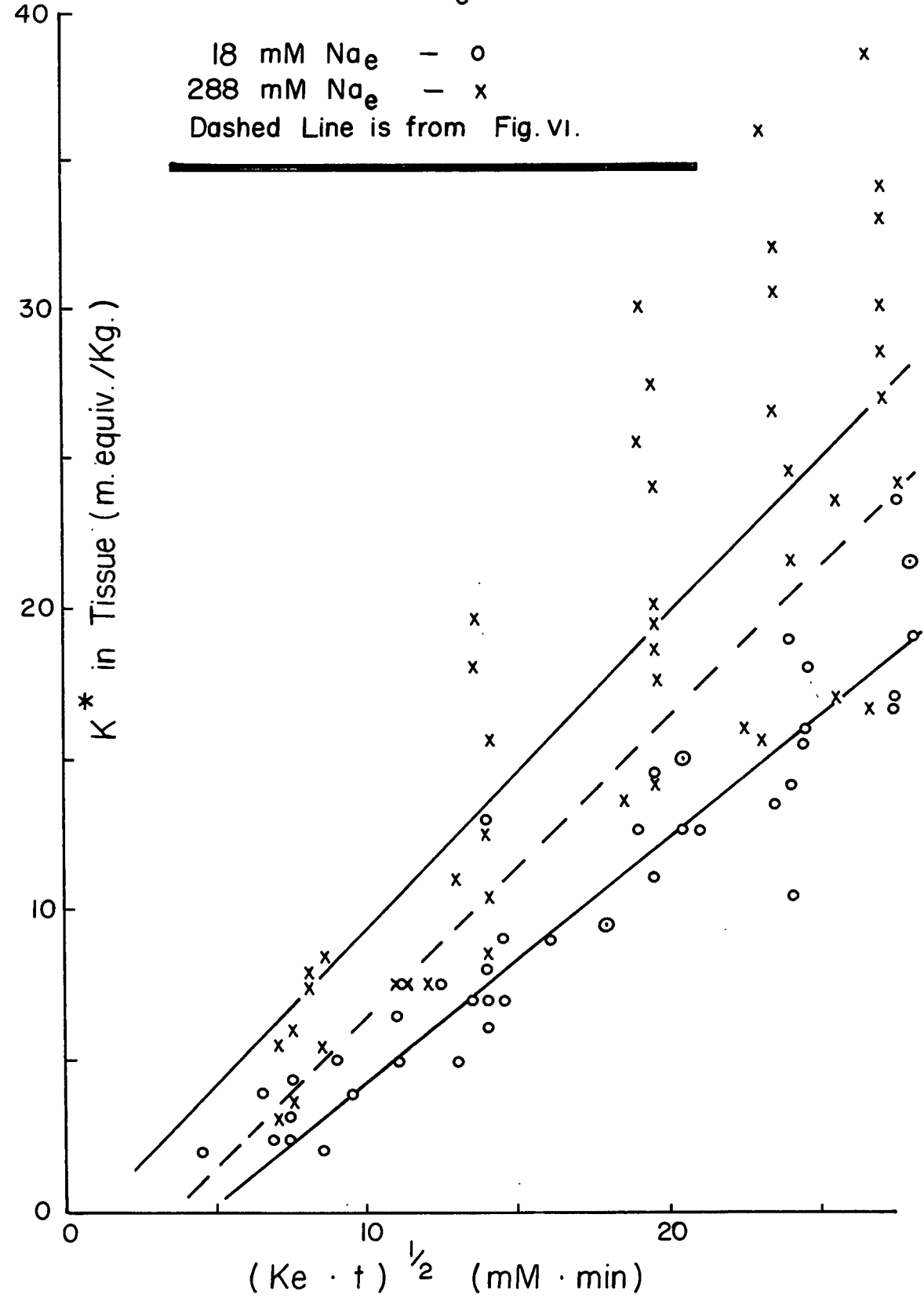


Fig. IX. Uptake of K^{42} by normal Mouse Muscle : Altered Na_e and K_e Incubation Media.

x 12 mM K_e – 288 mM Na_e

o 2 mM K_e – 18 mM Na_e

⊕ 12 mM K_e – 18 mM Na_e

• Chloride — free medium

Dashed line is normal uptake slope

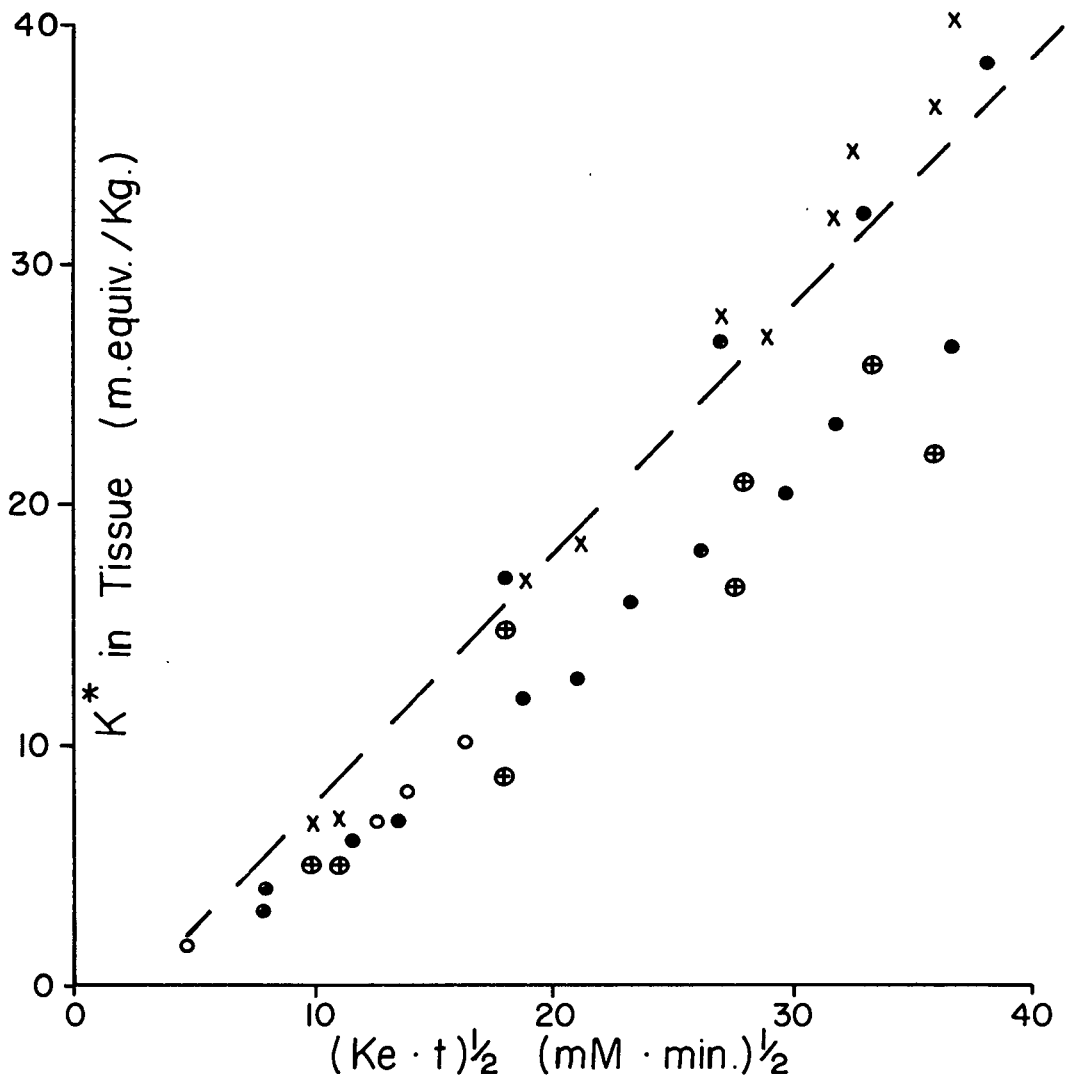


Fig. X: Uptake of K^{42} by Dystrophic Mouse Muscle: Normal and altered K_e Incubation Media.

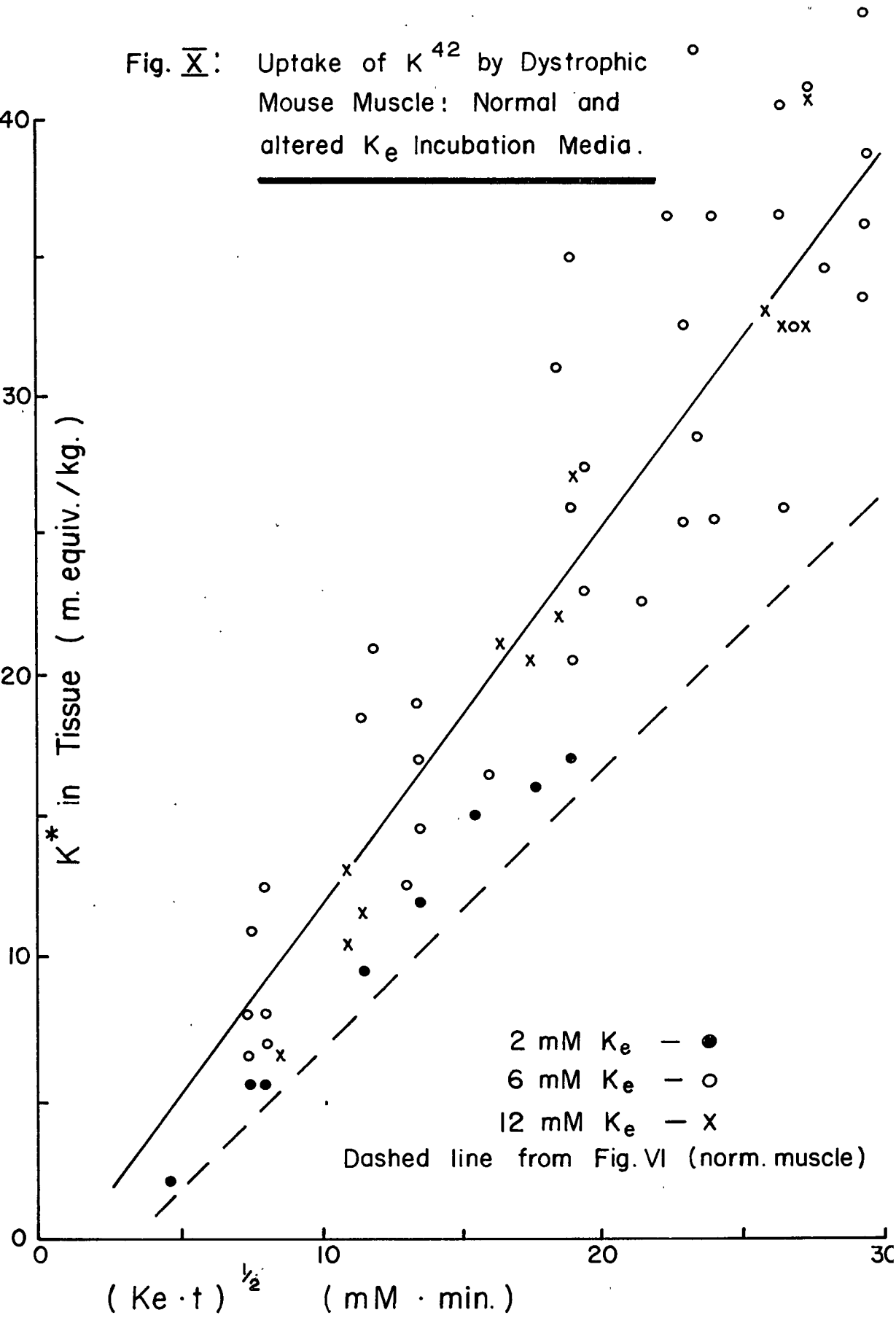


Fig. XI. Uptake of K^{42} by Dystrophic Mouse Muscle: Altered Na_e Incubation Media.

● 18 mM Na_e

o 288 mM Na_e

Dashed line is dystrophic regression line from figure X

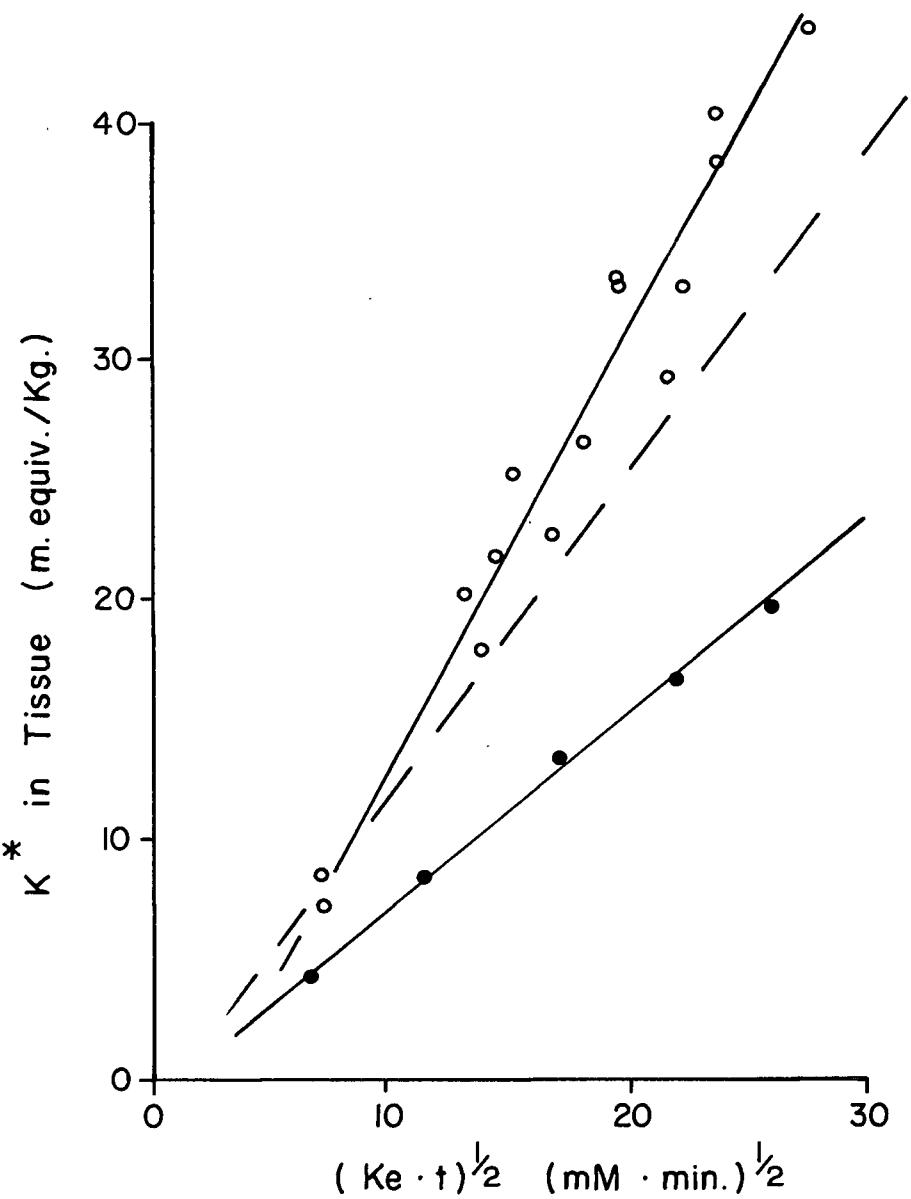


Table I K, Na, and resting potentials of excitable tissues *, Ion values expressed as m.equiv./litre of cell or plasma water. R.P. values in mV. Calculated values from Nernst Equation.

Tissue	K		Na		R.P.	
	Cell	Environ	Cell	Environ	Calc.	Actual
Frog Skel. Muscle	124.0	2.2	3.6	104.0	98	92
Rat Skel. Muscle	152.0	4.7	3.0	150.0	87	74
Crab – Muscle	146.0	12.9	54.0	513.0	61	72
Crab – Nerve	112.0	12.1	54.0	468.0	60	82
Squid – Giant Axon	369.0	13.0	44.0	498.0	83	65

* Values from: Shanes (1958), Ling & Gerrard (1949), Hodgking & Keynes (1955), Conway (1950), and Zierler (1959)