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Electrolytes in Denervated and Reinnervated Muscle. (25693)

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Since the work of Hines and Knowlton(1) on ion changes in the denervated muscle it has not been clear whether the decrease of K ions in the denervated muscle is the result of a decrease in intracellular K concentration(2) or is only proportional to diminution of the muscle phase itself and to increase in relative amount of intercellular collagen phase(3). The content of extracellular ions Na and Cl increases after denervation when expressed in mequiv. Na and Cl/100 g fat-free dry solid (FFDS)(1,2,3). However, ion changes in denervated muscles have not been evaluated in absolute units, *i.e.*, total amounts of Na and Cl ions present in whole normal and denervated muscles have not been compared. Such an evaluation makes it possible to study the relationship between extra- and intracellular muscle phases in denervation atrophy. Moreover, if ion composition of the denervated muscle is changed, it is very important to know whether and how it returns to normal in the course of reinnervation. These aspects are the subject of this report.

Methods. Experiments were performed on male rats of 150-200 g body weight. Ion composition was studied in extensor digitorum longus following section of peroneal nerve. For reinnervation studies, denervation was induced by crushing the sciatic nerve; this procedure leads to reinnervation of the muscle and restitution of function about 14 days after nerve crushing(4). For these experiments the anterior muscle was used. Na and K ions were determined by flame photometry, Cl ions by modification of Sanderson's method(5). Non-collagenous protein nitrogen was extracted from the muscle with 0.1 N NaOH, precipitated with TCA, and after Kjeldahlisation was determined by Conway's micromethod(6).

Results. Decrease in non-collagenous protein nitrogen occurs in denervated muscle at the same rate as the decrease in K content

TABLE I. Potassium and Non-collagenous Protein N Content in Denervated Muscle.

Denervation period, days	Control muscle	Denervated muscle	Denervated / Control
	μequiv. K/muscle		
3	8.63 ±.76	8.89 ±.80	1.03
20	11.55 ±.06	7.32 ±.06	.63
	mg N/muscle		
3	2.39 ±.18	2.34 ±.13	.98
20	3.52 ±.88	2.20 ±.07	.63
	K/N		
3	3.61 ±.13	3.80 ±.19	1.05
20	3.28 ±.19	3.32 ±.13	1.01

All data are mean values of 6 muscles.

(Table I). The amount of K corresponding to 1 mg protein nitrogen remains unchanged after denervation. Since non-collagenous protein nitrogen corresponds to the intracellular muscle mass, our results thus confirm earlier assumptions of some authors who, finding decreased K content in the denervated muscle, concluded that this decrease is caused by a relative increase of collagenous tissue and a decrease in the muscle phase/unit weight of denervated muscle(1,3).

On evaluating Na and Cl ions in absolute units, *i.e.*, per whole muscle, one finds that total amount of Na and Cl ions remains unchanged in the denervated muscle (Fig. 1). Changes in K correspond well with results in Table I. Rate of K decrease and rate with which fat-free dry muscle solids decrease run almost parallel.

Ion composition of the denervated muscle gradually returns to normal with reinnervation (Fig. 2). When evaluated in absolute units (total amount/muscle), sodium ions remain unchanged throughout reinnervation period. The K content (μequiv./muscle) and FFDS (mg/muscle) diminish concomitantly during denervation; after restitution of muscle motor function they recover together.

Discussion. Intracellular water (ml/100 g FFDS) in the denervated muscle remains unchanged, as does intracellular sodium content (Na/100 gr. FFDS), hence the increase in Na ions, when expressed in relation to total dry mass, corresponds to the change in relative amount of extracellular fluid(7,8). Taken together with our data on the main intracellular ion, K, as given in Table I, available results show that osmotic conditions remain unchanged in muscle cells deprived of their motor innervation. Twenty-five days after denervation muscle atrophy reaches 50% (Fig. 1). At this time marked metabolic disturbances take place inside muscle cells(9). In spite of this, however, the transport sys-

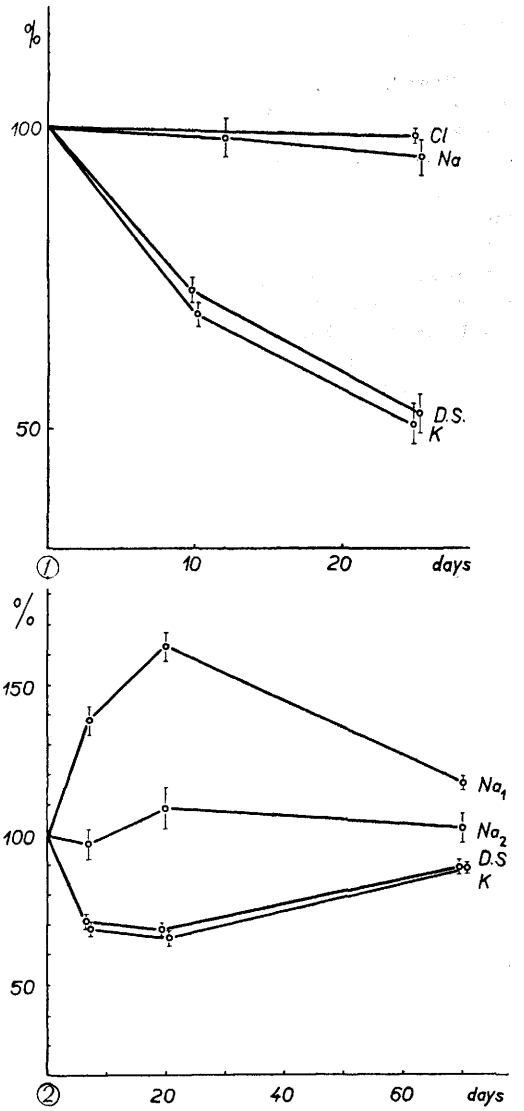

FIG. 1. Changes in electrolytes (Cl, Na, K) and fat-free dry solids (D.S.) after denervation. Na, Cl, K, D.S. represent % changes of these relative to normal innervated control muscles.

FIG. 2. Na, K, and fat-free dry solids (D.S.) changes during denervation followed by reinnervation. Na_1 represents % change of Na expressed in meq/100 g FFDS. Na_2, K, D.S. represent % changes of total amounts/muscle.

tems of cells maintain concentration gradients of Na and K ions under these conditions at normal level even though rate of K exchange in the denervated muscle is somewhat decreased(10).

Evaluation of total absolute ion changes makes it possible to estimate changes in extracellular fluid and the intracellular muscle phase. The results indicate that following denervation, only the intracellular muscle phase decreases, while extracellular space remains unchanged even when muscle atrophy reaches 50%. Conclusions in earlier reports concerning increase of Na and Cl ions in the denervated muscle(1,2,3,7,8) must be modified in view of our evidence that the increase of Na and Cl ions/unit weight of denervated muscle is only relative, due to volume of extracellular fluid remaining unchanged while volume of intracellular phase decreases.

Summary. The content of potassium expressed in relation to non-collagenous protein nitrogen is normal even 20 days after denervation in muscles of the rat. Following denervation only the intracellular muscle phase decreases. The drop in K content (K/muscle) and FFDS (weight/muscle) reaches 50%. At this time the extracellular space (Na/muscle, Cl/muscle) remains unchanged. Ion composition and dry muscle weight gradually return to normal after reinnervation and restitution of motor function.

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Histological Changes in Cells Infected with Columbia SK Virus. (25694)

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Histological evidences of Columbia SK virus infection have been of interest because the nature of pathological changes and site of multiplication of the virus have not been completely identified, and also because we hoped to identify the step at which virus propagation was interrupted by the antiviral substance "Propionin"(1). Some clarification of the first of these problems is given in this paper, in which histological appearance of infected cancer cells is described.

Procedure. After serial passage in Ehrlich ascites tumor cells, grown intraperitoneally in young white mice, an adapted strain of Columbia SK virus was obtained which produced severe oncolysis(2). Such infected ascites fluid, aspirated with 2 ml of saline from abdominal cavity before mice died at 3 to 5 days after virus inoculation, contained about 10^6 LD_{50}/ml of virus. The supernatant of this fluid, which contained the same titer of virus, was diluted 10^{-3}, and 0.1 ml mixed with 0.3 ml of fresh, uninfected Ehrlich ascites, drawn 5 to 10 days after implantation, and inoculated intraperitoneally into new mice. Three or 4 days later cells were withdrawn for morphological study, and stained as follows: *Giemsa*—Smears were fixed with methanol and stained by usual Giemsa solution. *Lipoid*—Smears were fixed with formalin and stained with Sudan III, or Sudan Black B, saturated solutions in 70% alcohol, then counterstained with Giemsa solution. *Tetrazolium*—Immediately after aspiration a drop of ascitic fluid was mixed on a slide with a drop of 2% 2,3,5-triphenyl-2H-tetrazolium