A SIMPLE. RAPID METHOD FOR THE QUANTITATIVE SEPARATIOI OF THE EXTRACELLULAR FLUID IN FROG MUSCLES

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SUMMARY

A simple, rapid and nondestructive method for the quantitative separation of th extracellular fluid in frog muscles is described.

The present report describes a method of separating the extracellular fluid from isolated frog sartorius muscles which is simple, rapid and nondestructive. This method serves at least three purposes, providing a means of (1) assaying the volume of the extracellular space; (2) assaying the contents of the extracellular space; and (3) "purifying" muscle tissues from the contamination of the extracellular space fluid in quantitative studies of the properties of muscle cells.

MATERIALS AND METHODS

Sartorius muscles of Northern American leopard frogs (*Rana pipiens pipiens*, Schreber) were studied. A humid chamber similar to that described by Elliott' was used. The chamber was large enough to accommodate a torsion balance for the accurate weighing of the muscle tissues. Na^{22} was obtained from International Chemical and Nuclear Corp., Irvine, California. Centrifugation was carried out in an International Refrigerated Centrifuge with horizontal heads.

Filter papers used to catch the spun off extracellular fluid were wetted to different degrees: the "semiwetted" filter paper was merely subjected to five minutes autoclaving at 15 lbs. per square inch; "wetted" filter paper was first soaked in Ringer solution and then spun for four minutes at 1000 g in a Gelman centrifugal filter holder (Product No. 4305, Gelman Instrument Co., Ann Arbor, Michigan).

Isolated frog sartorius muscles were blotted on wet Whatman No. 1 filter paper according to the standard procedure, described in an earlier publication by Ling et al.[2] and weighed on a torsion balance kept in the humid chamber. The muscle was then laid flat

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on four squares of "wetted" filter paper which, in turn, rested on two squares of "semi-wetted" filter paper. Muscle and paper were then carefully wrapped and sealed in Parafilm and the Parafilm packet placed, muscle side up, in the bottom of either 50 ml centrifuge shields or, in later studies, aluminum alloy carriers (for 250 ml bottles). After centrifugation, the muscles were removed and weighed again in the moist chamber.

In experiments where muscles were previously equilibrated in Na^{ZZ}-labeled Ringer solution, the Na^{22} content of the fluid spun off the muscle was assayed by packing all the filter paper on which the centrifuged muscles rested, together with the Parafilm wrap, in the bottom of counting tubes; the radioactivity was assayed in a Nuclear Chicago automatic y-scintillation counter.

RESULTS AND DISCUSSION

Constant Weight Loss at Varying Durations of:Centrifugation

Figure 1 shows that at a fixed relative centrifugal force of 1000 g, the weight loss of the sartorius muscles was constant when the duration of centrifugation varied between 2 and 16 minutes. The average weight loss of all individual determinations between these limits was 9.4%.

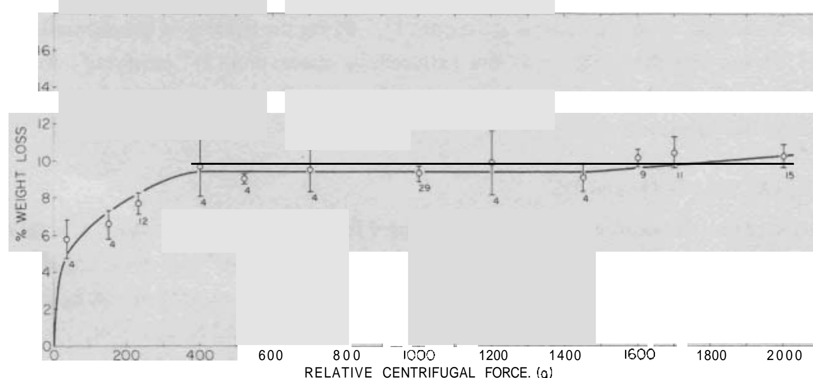

Figure 1. Weight loss of sartorius muscles after centrifugation at varying relative centrifugal force for four minutes. The numerals below the experimental points represent the number of experiments done. The distances between horizontal bars are twice the standard error. Weight loss is expressed as percentage of initial weights of the isolated muscle.

Constant Weight Loss at Varying Relative Centrifugal Forces

Figure 2 shows that the weight loss of sartorius muscles remained constant when the centrifugal force varied between 400 g and 1500 g. The average of all individual volumes between these limits was 9.3%.

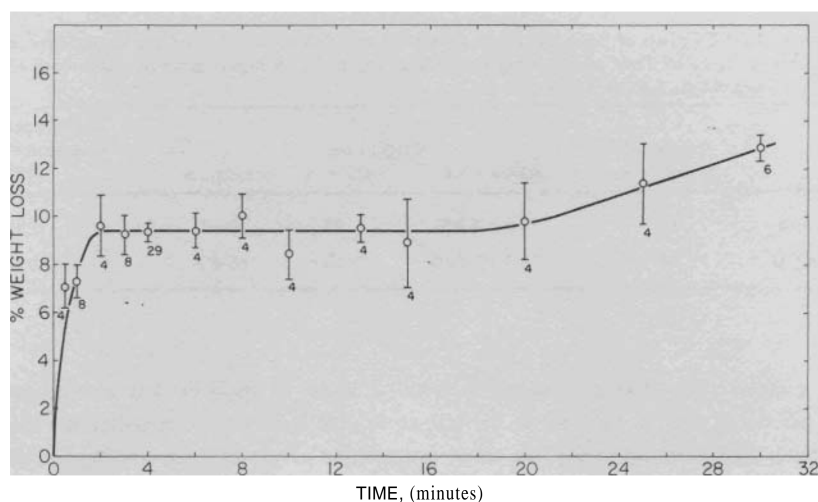

Figure 2. Weight loss of sartorius muscles after centrifugation at 1000 g for varying lengths of time. For description of symbols, see legend of Figure 1.

Na^{22} Content of the Separated Fluid as a Criterion for Recognizing Extracellular or Combined Extracellular and Intracellular Origins of the Spun Off Fluid

While the plateauings of weight loss shown in Figures 1 and 2 suggest a limiting volume being spun off, they do not prove that this limiting volume corresponds to the total amount of fluid originally present in the extracellular spaces. However, two types of evidence support this view.

Firstly, the constant weight loss averages **9.4%.**This figure agrees well with those determined by Ling and Kromash with the extracellular space probe inulin at low concentration (10.3%);[3] with poly-L-glutamate (8.9%);[3] with the single muscle fiber sucrose space method **(9%)**;[1] and from analysis by Ling of Br^{86} efflux (8.2%).[4]

Secondly, the simultaneous measurements of the weight loss and of Na^{22} loss from muscles previously equilibrated in Na^{22}-labeled Ringer solution also corroborates the idea that the limiting volume spun off represents the total amount of extracellular space fluid.

A total of 78 of these dual assays were made and the data sorted into two groups according to the magnitude of the spun off volume. The first group of **32** experiments includes all muscles with weight losses of **9.4%**or lower (the average, 7.2 ± 0.22%; the lowest value, 5%). In this group the average concentration of Na^{22}-labeled Na ion concentration was **99 ± 3.9%**of that in the original Ringer solution in which the muscles were equilibrated. This agreement between the concentration of the labeled Na^+ spun off and

Table 1. Na^{22} Contents of Spun Off Fluids from Muscles at Different Relative Centrifugal Forces and Different Lengths of Time of Centrifugation. Data include all 78 experiments in which both Na^{22} contents and weight loss were determined.

	Number of Experiments	Weight Loss means ± S.E.	minimum	maximum	Relative Na^{22} Concentration (%)
Group I	**32**	**7.2 ± 0.22%**	**5%**	**9.4%**	**99 ± 3.9%**
Group II	**46**	**12.3 ± 0.29%**	**9.4%**	**16.4%**	**73 ± 3.1%**

the concentration of labeled Na in the bathing solution in which the muscle was equilibrated shows that up to **9.4%** of the muscle volume is indeed an unadulterated extracellular fluid with essentially the same composition as the external bathing solution.

The second group of **46** experiments includes all the remaining muscles; their weight losses were more than 9.4%, the average being **12.3 ± 0.29%**, with the highest value 16.4%. The average concentration of labeled Na^+ in the spun off fluids was only **73 ± 3.1%** of that in the original bathing solution. The intracellular labeled Na^+ concentration is much less than that in the external solution. Furthermore, loss of intracellular water may not be accompanied by a corresponding amount of labeled Na^+. Therefore, any intracellular water lost must contain less Na^{22} per unit volume than that in the extracellular fluid. The average of **73%** of labeled Na^+ as compared with that in the original bathing solution shows that a small additional weight loss of 2% or **3%** beyond the **9.4%** figure can be attributed only to loss of intracellular water. The mean values from Figures 1 and 2 **(9.3-9.4%)** must, therefore, represent with reasonable accuracy the total volume of the extracellular space.

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