THE ELECTROLYTE CONTENT OF SKELETAL MUSCLE IN CONGESTIVE HEART FAILURE; A COMPARISON OF RESULTS WITH INULIN AND CHLORIDE AS REFERENCE STANDARDS FOR EXTRACELLULAR WATER¹

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In estimating shifts of electrolytes between intracellular and extracellular fluid compartments from metabolic balance data, it is necessary to assume 1) either an initial or final volume of extracellular water, and 2) that the balance of chloride reflects changes in extracellular chloride only. Similarly, in calculating intracellular electrolyte concentrations from tissue analyses, it is necessary to assume that the chloride present in tissue is freely diffusible and is derived only from the extracellular fluid space. Recent studies (1-4) have shown, however, that the latter premise may not be entirely correct, and that a significant moiety of tissue chloride may be intracellular. Yannet and Darrow (5) have subtracted 1 mM. of chloride from the total tissue chloride per 100 grams of fat-free solids to correct for this nonextracellular chloride in muscle. This correction averages about 15 per cent. Since the volume of distribution of inulin is smaller than that of chloride or other electrolytes, it has been suggested that inulin is confined to the extracellular compartment. Therefore, in an effort to obtain a more precise measurement of the extracellular fluid of muscle, inulin was infused into experimental subjects until uniform distribution had been achieved. Then muscle was obtained by biopsy and analyzed for inulin and electrolyte content. As will be seen from the results obtained, intrinsic analytical variations may occasionally conceal any real difference between the chloride and inulin "spaces."

Metabolic balance data (6-8) on patients recovering from congestive heart failure show in most instances a retention of potassium in excess of nitrogen. This has been interpreted as a replacement of potassium lost during the development of failure. Cardiacs in whom edema is pro-

duced by the addition of salt (9) and/or the withdrawal of digitalis (9, 10) may either be in balance or have negative or positive potassium balances; thus a negative potassium balance is not a necessary concomitant of congestive heart failure. These indirect findings are substantiated in part by our direct chemical analyses of muscle of patients in heart failure.

METHODS

Six patients in chronic congestive heart failure, who had varying amounts of pitting peripheral edema but, with one exception (G. M.) no demonstrable pleural effusion or ascites, and four non-cardiac control subjects were studied. The cardiacs received no digitalis or mercurials for at least two weeks prior to study with one exception, I. F., who developed acute pulmonary congestion and who received digoxin about 36 hours prior to muscle biopsy. The controls and several of the cardiacs were given a regular hospital diet. Some of the cardiacs were on salt poor diets containing 5 to 15 mEq. of sodium and about 70 mEq. of potassium, to which 5 to 10 grams of sodium chloride were added daily for variable periods until the signs and symptoms of congestive heart failure were manifest.

While in the post-absorptive state with water permitted *ad libitum*, the control subjects were given a constant infusion of 10 per cent inulin in 0.45 per cent saline and 2.5 per cent glucose for at least six hours, at the end of which time it has been shown (11) that inulin has equilibrated in the extracellular compartment. In the cardiacs, however, the equilibration time was prolonged to 30 hours.² The priming and sustaining infusions were given at a rate calculated to result in an extracellular in-

² This time was determined by expediency and by the fact that interstitial and plasma inulin concentrations fell within the analytic error. Constancy of plasma concentration does not insure extracellular compartment saturation.

During this period about 1,800 ml. of 10 per cent inulin in 0.3 per cent saline and 3.3 per cent glucose were infused. There was no significant change in extracellular fluid volume since most of the solution was excreted. A more concentrated inulin solution suitable for infusion was not available.

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ulin concentration of about 1 mg./ml. in the cardials and about 2 mg./ml. in the controls. There were no untoward effects with these high inulin concentrations in any of the subjects. While the inulin infusion was continuing and presumably equilibration had been achieved, a biopsy of the gastrocnemius muscle was taken under spinal anesthesia. Simultaneously, blood was sampled from the femoral artery. In the cardials, edema fluid was also obtained from the leg from which muscle was removed. The inulin in plasma and edema fluid was analyzed by the resorcinol method of Roe, Epstein, and Goldstein (12) and the inulin in muscle was analyzed by a modified resorcinol method developed by the authors (13). The inulin volume of distribution was determined according to the technic of Gaudino, Schwartz, and Levitt (14) and Schwartz, Schachter, and Freinkel (11).

The muscle was freed from visible blood, connective tissue, and fat and treated in the following manner: 1) Samples of 1 to 2 grams were analyzed for inulin. 2) The blood content of the muscle was estimated on triplicate 0.3 to 0.5 gram samples taken from different sites and extracted with 3 to 5 ml. of normal saline for about one hour at a reduced temperature with occasional stirring. The red cells in the saline extract were counted in a chamber and its equivalent as whole blood was calculated from the red cell content of peripheral blood. Neither spectrometric determination of the blood in the muscle, as suggested by Lowry and Hastings (15), nor the method of Fanelli (16) for determining blood hemoglobin in the presence of muscle myoglobin was employed because the method of counting red cells was more convenient. 3) The remaining muscle was minced and the wet-dry weight determined. 4) The dried contents were then ground to a fine powder in an agate mortar and aliquots taken for the following analyses: total lipids by extraction with alcohol-ether in the Soxhlet apparatus, evaporating to dryness and weighing the residue after re-extraction with petroleum ether; chloride and phosphorus by mixing the dried solids with an excess of chloride-free sodium carbonate and ashing in a muffle at 450° C. The alkaline ashed material was dissolved in sulfuric acid and an aliquot taken for phosphorus determination according to Fiske and SubbaRow (17) and for chloride analysis according to Van Slyke and Hiller (18); sodium, potassium and magnesium on a separate aliquot ashed overnight at 450° C. in a separate furnace. Sodium was determined gravimetrically as the uranyl zinc acetate salt (19), potassium was determined titrimetrically after precipitation as the iodoplatinate (20, 21), and magnesium was determined colorimetrically as phosphate in magnesium ammonium phosphate after the removal of calcium as the oxalate (22); nitrogen by micro-Kjeldahl digestion, steam distillation, and titration.

All the muscle analyses were done on separate duplicate and triplicate aliquots and showed the following deviation from the mean: potassium, ± 0.8 per cent with a range of 0 to 2.2 per cent; chloride, ± 1.1 per cent with a range of 0 to 4.1 per cent; sodium, ± 1.9 per cent with a range of 0.2 to 4.4 per cent; fat, ± 2.5 per cent with a

range of 0 to 5.2 per cent; inulin, ± 5.3 per cent with a range of 0.5 to 9.0 per cent.

The bicarbonate content of serum was determined manometrically (23). The analytic methods described above for muscle electrolytes were employed also for serum electrolytes.

Sodium and potassium in whole blood were determined with a Perkin-Elmer flame photometer, model 52A, after the red cells were hemolyzed with distilled water. Whole blood chloride was determined on a picric acid filtrate, as described by Van Slyke and Hiller (18).

Calculations

The concentration of electrolytes in extracellular water was calculated from their concentration in serum corrected for serum water and an average Donnan equilibrium factor of 0.96. The muscle analyses were corrected for the variable amount of fat and blood present in the specimen sampled and are expressed as *fat-free, blood-free solids*. Darrow (5, 24) has suggested the use of fat-free solids as a reference base because these are practically all intracellular and are independent of the extracellular fluid volume. However, variables in the intracellular solid composition such as glycogen may at times make this reference standard inexact for describing muscle composition.

For comparative purposes, the *extracellular water* (H_2O)_e per 100 grams fat-free, blood-free solids was calculated using three different standards for measuring extracellular water as follows:

1.
$$\frac{\text{mg. inulin per 100 grams solids}}{\text{mg. inulin per gram serum water}} = \text{grams } (H_2O)_e$$
2.
$$\frac{\text{mM.Cl per 100 grams solids}}{\text{mM.Cl per gram extracellular water}} = \text{grams } (H_2O)_e$$
3.
$$\frac{\text{mM.Cl per 100 grams solids} - 1}{\text{mM.Cl per gram extracellular water}} = \text{grams } (H_2O)_e$$

as suggested by Yannet and Darrow who reported that 1 mM. of chloride per 100 grams fat-free muscle solids is not freely diffusible (5).

The *extracellular electrolyte content* of the muscle is the product of the extracellular water and the concentration in that water.

Intracellular water was calculated as the difference between total water and extracellular water. Similarly, the *intracellular electrolyte content* was calculated as the difference between the total electrolyte content of the muscle and the extracellular value; they are expressed as concentrations per kgm. intracellular water (Table II).

No attempt was made either to determine the collagen and elastin content of the muscle or to differentiate non-protein nitrogen from protein nitrogen in muscle.

The total body volume of distribution of inulin was calculated according to the technic of Gaudino, Schwartz and Levitt (14) and Schwartz, Schachter, and Freinkel (11), except that no urine correction blank was applied because the inulin equilibrium concentration in the extracellular water was about ten times the concentration usually achieved.

RESULTS

The analytical results and the derived calculations are presented in Tables I, II, and III. An orientation on the interpretation of muscle analyses is given by Darrow (24).

Extracellular water. In the four non-cardiac subjects the extracellular water in the muscle was on the average 18 per cent greater when measured with chloride than with inulin. In patients A. N. and W. Z., the chloride "space" in the muscle was as much as 31 per cent and 41 per cent greater than with inulin. If one corrects for non-diffusible chloride as suggested by Darrow, the extracellular water value on the average was only 4 per cent greater than when calculated with inulin. The range, however, was 15 per cent less to 23 per cent greater than the inulin value (Table III). In absolute terms, the amount of extracellular water associated with 100 grams fat-free, blood-free solids varied from 39 to 59 grams, 55 to 59 grams, and 47 to 50 grams estimated from inulin, chloride, and chloride corrected for the non-diffusible moiety, respectively (Table II). On a percentage basis, the extracellular water averaged 9.8 per cent, 11.6

per cent and 10.0 per cent of the wet tissue, respectively, employing the above standards for extracellular water. In the three cardiacs, I. F., Y. J., and F. P., in whom, we believe, distribution equilibrium in the extracellular water of the muscle had been achieved with inulin as shown by the equality of the inulin concentration in the plasma and in the edema fluid in the leg from which the muscle was taken, the extracellular water associated with 100 grams fat-free solids was 96, 98, and 112 grams. The corresponding values obtained with chloride in these three cardiacs were 98, 92, and 100 grams of interfiber water per 100 grams fat-free solids. Similarly, applying the correction factor for non-diffusible chloride, the values were 89, 85, and 91 grams of extracellular water per 100 grams fat-free solids. On a wet basis, the extracellular water in the muscle of the cardiacs was about twice that of the controls. The *volume of distribution* of inulin averaged 14.1 per cent of the body weight in the controls and was 27.8 per cent, 36.6 per cent and 24.5 per cent of the body weight in the three cardiacs, I. F., Y. J., and F. P., respectively.

Intracellular water. The value calculated for

TABLE I
Summary of analyzed data
A. Patients without congestive failure

Patient*	Age	Diagnosis†	Serum concentration per liter						Muscle composition									Per 100 grams tissue
									Per 100 grams fat-free, blood-free solids									
			H ₂ O	Cl	CO ₂	Na	K	Mg	H ₂ O	Cl	Na	K	Mg	N	P	K/N	Fat-free solids	
A. W.	38	Cerebellar degeneration	grams 921	mM. 105.4	mM. 21.5	mM. 137.3	mM. 4.4	mM. 0.84	grams 362	mM. 7.0	mM. 11.4	mM. 48.1	mM. 3.88	grams 15.6	mM. 31.4	mM./gram 3.08	grams 21.0	
A. N.	27	Normal	917	106.5	25.4	134.0	4.1	0.96	362	7.1	11.9	48.1		15.7	30.8	3.06	20.4	
W. J.	50	Strabismus	917	110.0	26.4	144.0	4.0	0.88	340	6.9	11.5	43.5	3.65	14.3	29.8	3.04	20.1	
W. Z.	42	Cataract	912	105.7	28.1	139.5	3.7	0.94	359	6.6	11.6	46.8	4.20	15.4	31.2	3.04	20.0	

B. Patients with congestive failure																	
M. M.	60	ASHD	917	112.6	16.2	138.2	5.8		435	17.0	25.5	41.4	4.20	15.6	30.1	2.66	17.5
I. F.	47	RHD	922	105.8	20.6	127.0	3.3		405	11.7	18.2	45.0	4.35	15.1	29.2	2.98	18.2
Y. J.	33	SHD	929	109.8	19.7	137.0	4.4	0.72	383	11.4	16.7	42.4	5.00	14.2	28.6	2.99	20.1
F. P.	25	RHD	920	107.0	22.9	140.0	3.2	0.74	433	12.1	20.3	47.3	4.95	14.6	31.0	3.24	17.7
G. M.	41	RHD and constrict. pericard.	941	80.5	42.0	130.1	4.5	0.63	553	17.6	31.0	51.0	4.38	14.9	31.0	3.32	13.7
J. A.	44	HHD	917	107.7	19.4	129.9	4.0	0.93	446	12.6	18.6	42.0	5.10	15.6	31.1	2.69	17.2

* All patients except I. F. and F. P. were males.

† ASHD = Arteriosclerotic heart disease.

RHD = Rheumatic heart disease.

SHD = Syphilitic heart disease.

HHD = Hypertensive heart disease.

TABLE II
Calculated values of intracellular electrolytes based on the various methods for measuring extracellular water
A. Controls

Patient	H ₂ O per 100 grams fat-free solids						Intracellular concentration per kgm. H ₂ O								
	Extracellular			Intracellular			Na			K			Mgt	N†	Pt
	Inulin	Chloride	Chloride-1	Inulin	Chloride	Chloride-1	Inulin	Chloride	Chloride-1	Inulin	Chloride	Chloride-1			
	grams	grams	grams	grams	grams	grams	mM.	mM.	mM.	mM.	mM.	mM.	mM.	grams	mM.
A. W.	59	59	50	303	303	312	10.0	10.0	13.5	158	158	153	12.5	51	102
A. N.	45	59	50	317	303	312	17.6	12.3	15.7	151	158	153		50	99
W. J.	48	55	47	292	285	293	14.4	12.2	15.0	148	152	148	12.4	49	103
W. Z.	39	55	48	320	294	311	18.4	11.2	14.5	145	158	150	13.5	50	101

B. Cardiacs

M. M.		133	125		302	310		20.8	24.2		135	131		51	98
I. F.	96	98	89	309	307	316	17.8	17.3	20.2	145	145	141		49	94
Y. J.	98	92	85	285	291	298	10.2	12.4	15.8	147	145	141	17.0	49	98
F. P.	112	100	91	321	333	342	12.2	17.7	20.5	146	141	137	14.6	44	94
G. M.*		195	185		358	368		15.1	17.4		140	136	11.8	41	86
J. A.*		102	94		344	352		14.2	16.5		121	118	14.4	45	89

* Inulin was not infused in patients G. M. and J. A. because the presence of massive edema, pleural effusion, or ascites in these patients precluded the attainment of equilibrium distribution within a reasonable period of time.

† These concentrations are calculated with an average value for intracellular water.

this datum as well as the value for intracellular electrolyte concentration is dependent primarily upon the accuracy with which the extracellular water can be measured. Since there is no single absolute unit of reference for extracellular water, we have calculated it with the three frequently used

reference standards. It will be seen (Table II) that in the normal subjects when the values for the extracellular water obtained with inulin are subtracted from the total water, the intracellular water varied from 292 to 320 grams per 100 grams fat-free solids. The maximum variation in intracellular water calculated with the several extracellular water values occurred in W. Z. and amounted to 8 per cent. There was no increase in intracellular water in the three cardiacs who were equilibrated with inulin. If one considers the six cardiacs as a group, the average increase of 7 per cent in intracellular water is not significant ($P > .05$) as compared to the four normals. It appears that the increased water content of the skeletal muscle in cardiacs is due to extracellular fluid accumulation, and not to any plethora of intracellular water.

The *sodium* content of the muscle averaged 11.6 ± 0.21 mM. per 100 grams fat-free solids in the four non-cardiacs and 21.7 ± 5.55 mM. per 100 grams fat-free solids in the cardiacs with variable edema. The *chloride* content averaged 6.9 ± 0.21 mM. per 100 grams fat-free solids in the normals, and 13.8 ± 2.77 mM. per 100 grams fat-free solids in the cardiacs. The Na/Cl ratio in the two groups, 1.68 in the normals and 1.58 in the cardiacs, was essentially the same. The slightly lower ratio in the cardiacs is not surprising since in this

TABLE III
Extracellular water ratio and volume of distribution
A. Controls

Patient	Extracellular H ₂ O ratio		Volume of distribution			
	Chloride Inulin	Chloride-1 Inulin	Wet muscle			Body weight
			Inulin	Chloride	Chloride-1	
			per cent	per cent	per cent	per cent
A. W.	1.00	0.85	12.4	12.4	10.5	15.7
A. N.	1.31	1.11	9.2	12.0	10.2	12.4
W. J.	1.15	0.98	9.7	11.1	9.5	14.3
W. Z.	1.41	1.23	7.8	11.0	9.7	14.0

B. Cardiacs

M. M.				23.3	21.9	
I. F.	1.02	0.93	17.5	17.8	16.3	27.8
Y. J.	0.94	0.87	19.7	18.5	17.2	36.6
F. P.	0.89	0.81	19.8	17.6	16.1	24.5
G. M.				26.7	25.4	
J. A.				17.6	16.1	

group there were increased amounts of extracellular water derived from serum with a Na/Cl ratio of 1.2. Had there been a *selective* intracellular gain of sodium in the cardiatics there should have been either an increase³ in this ratio in the muscle or a decreased interfiber Na/Cl ratio. Moreover, the intracellular sodium concentration calculated with inulin as the reference base for extracellular water varied to the same extent in both groups. In the cardiatics, the sodium concentration varied from 10.2 mM. to 17.8 mM. per kgm. intracellular water and in the normals, the range was from 10.0 mM. to 18.4 mM. of sodium per kgm. intracellular water. If the assumption is made that chloride is exclusively confined to the extracellular space, then on the average the cardiatics had a 42 per cent increase in intracellular sodium concentration as compared to normals. Similarly, with a fixed correction for non-extracellular chloride according to Darrow, the cardiatics had on the average a 30 per cent increase in intracellular sodium as compared to the four control subjects.

The *potassium* content of the muscle averaged 46.6 ± 2.10 mM. per 100 grams fat-free solids in the normals as compared to 44.9 ± 3.75 mM. per 100 grams fat-free solids in the cardiatics (Table I). This difference is not significant. Only a small moiety, 0.5 to 1.7 per cent of the total muscle potassium of all the subjects, is contained in the extracellular water; therefore, the intracellular *concentration* of potassium is determined to a greater extent by the amount of intracellular water than by the amount of potassium in the extracellular compartment. It will be seen (Table II) that in the normals the intracellular potassium concentration averaged 151 mM., 157 mM., and 151 mM. per kgm. water as calculated from inulin, chloride, or chloride-1, respectively, as the standards for extracellular water. In the cardiatics, the corresponding average values were 146 mM., 138 mM., and 134 mM. potassium per kgm. intracellular water. The difference in the average intracellular potassium concentration in the two groups is about 10 per cent.⁴

³ The increase in intracellular sodium is a small fraction of the total sodium and may not be readily apparent in the muscle Na/Cl ratio. In the cardiac a 10 per cent increase in the amount of intracellular sodium will result only in a 2 per cent increase in the muscle Na/Cl ratio.

⁴ It should be noted that although the increase in intra-

The *potassium/nitrogen* ratio in the muscle of the four non-cardiatics varied slightly from 3.04 to 3.08 mM. potassium per gram nitrogen, whereas in the cardiatics there was a greater variation of from 2.66 to 3.32 mM. potassium per gram nitrogen. The difference of the means in the two groups is not significant. Of interest are the results obtained in M. M., who had a second biopsy on the opposite leg after a 13.3 kgm. weight loss and disappearance of his peripheral edema. In failure, the K/N ratio (Table I) was 41.4/15.6 or 2.66 mM. potassium per gram nitrogen, and when compensation was restored, the K/N ratio was 48.1/15.4 or 3.12 mM. potassium per gram nitrogen.

The *magnesium, nitrogen, and phosphorus* content (Table I) of the muscle in the cardiatics and controls did not differ significantly. The figures in Table II are based on an *average* intracellular water content. It will be seen that on the average there was 7 per cent less nitrogen and 8 per cent less phosphorus in the cardiac group than in the control patients.

When the intracellular concentrations of K, Mg, and Na are added, the total cation concentration per kgm. intracellular water is equivalent to 168 mM. in the cardiatics and 179 mM. in the non-cardiatics; the difference is chiefly attributable to the lowered potassium concentration in the cardiac.

Except for the lower total CO₂ in the cardiatics, the serum electrolytes in the two groups were essentially similar. Patient G. M. always had an elevated CO₂ and decreased chloride. The blood pH was 7.40. We attributed these serum findings to a primary CO₂ retention secondary to pulmonary pathology, although at autopsy there was only a moderate interstitial pulmonary fibrosis.

DISCUSSION

In the present study an attempt has been made to limit the number of variables that may influence the results. In many instances the blood content of excised human muscle is not negligible. On the average there was 1 per cent blood in the muscle samples which if uncorrected would increase the extracellular water by about 5 per cent

cellular water found in the cardiatics is not significant, on the average it affects a 10 per cent reduction in the intracellular potassium concentration. Derived values may be magnified at times by minor differences in analyzed data.

when chloride is used to estimate this compartment. This correction was of the same order of magnitude in several patients who had high inulin blood levels. The blood contamination error becomes even more significant when the chloride content of muscle is corrected for the non-diffusible moiety. This consideration may not apply to studies in which animals have been exsanguinated and the muscle chilled prior to removal (15).

Certain factors must be considered in relation to the patients used in this study. The electrolyte intake of the subjects, while not rigidly controlled, were sufficiently similar for about 14 days prior to the study probably to preclude any differential influence on the electrolyte content of the muscle. The role of restricted protein intake for protracted periods in several of the cardiacs is unknown. A reduced potassium intake can diminish the potassium content of muscle as shown by Heppel (25) and later by Conway and Hingerty (26) who were able to replace about 25 per cent of the muscle potassium with sodium when rats were placed on a low potassium intake, equivalent to about 0.025 mM. of potassium per 70 gram rat daily for about 26 days. This consideration may not apply to the cardiacs just prior to study, but the influence of possible low potassium intake, mercurials, and digitalis for prolonged periods on the potassium content of muscle cannot be evaluated at present.

Although the etiology of the heart disease varied, all the cardiacs had fixed congestive heart failure since the edema rapidly accumulated if sodium was not rigidly restricted.

The validity of any conclusions to be drawn from the present study is dependent upon the accuracy with which the extracellular water in muscle can be determined. From the present data one cannot select the true reference standard for measuring extracellular water. Levitt and Gaudino (27) have presented evidence, albeit inferential, that the equilibrium volume of distribution of inulin approximates more closely the extracellular fluid compartment than does chloride, thiocyanate, or mannitol. It is for this reason that we have chosen inulin as a reference base for extracellular water. The chief disadvantage in the use of inulin in the edematous cardiac is the difficulty in achieving equilibrium distribution throughout all the recesses of the extracellular compartment within a reasonable period of time (11). In the absence of pleural

effusion or ascites, however, equilibrium has been achieved in 30 hours as demonstrated by equality of concentration, within experimental error, in the plasma water and edema fluid. Another criticism of the use of inulin is that the accuracy of its determination in muscle is less than that of chloride. The latter can be determined with an accuracy of about 1 per cent, whereas that of inulin averages about 5 per cent. Some of the variation in the chloride/inulin ratio may be due to these factors.

If inulin is *limited* to extracellular distribution, the average chloride/inulin ratio of 1.18 in the non-cardiacs suggests that a significant portion of muscle chloride is normally non-diffusible and presumably non-extracellular in distribution. This confirms the findings of Harrison, Darrow, and Yannet of a significant moiety of non-diffusible chloride in muscle (4). Whether this is a constant fraction of the total chloride present under normal and pathological conditions and equivalent to 1 mM. per 100 grams fat-free solids as suggested by Darrow's data in cats, is not evident from the present study. The chloride/inulin ratio of 0.95 in the three cardiacs, on the other hand, may be explained by: 1) A simple expansion of the extracellular volume, since under such conditions a given amount of non-diffusible chloride is a *smaller* fraction of the total chloride; 2) an actual shift of chloride out of the cells. Under the influence of increased adrenocortical activity it has been reported (28) that chloride shifts into the inulin space. These shifts, however, are of a transient nature and may not apply to a chronic state such as congestive heart failure, although there are data (9, 29) suggestive of hyperadrenocorticism in heart failure. It is not known, though, whether this pertains to the cardiacs in the present study since no attempt was made to obtain evidence for excessive adrenal cortical stimulation.

Conclusions drawn from metabolic balance data based on the premise that the chloride changes are all extracellular should be re-examined in the light of recent data (1-4) including the present study which suggests that chloride may also be intracellular. Elkinton, Squires, and Crosley (30) have called attention recently to the fact that at times absurd values are obtained when chloride is used for calculating extracellular volumes and that an additional reference base such as the volume of distribution of inulin should be employed. The

present data on the volume of distribution of inulin agree with those previously published by Gaudino, Schwartz and Levitt (14) and by Schwartz, Schachter, and Freinkel (11). It is also of interest to note that the inulin "space" determined by immersing frog sartorius muscle in an inulin-Barkan solution (31) was 9.6 ml. per 100 grams muscle and similarly in the present *in vivo* study it was 9.8 ml. per 100 grams muscle; the variation was the same in both studies.

The earlier work of Harrison and co-workers (32-34) and more recently of Alexander and associates (35), reported a decreased potassium content of skeletal muscle in heart failure. The analytical results were not expressed on a fat-free basis and are not entirely comparable with the present data. In the present study, if inulin is accepted as the true reference standard for measuring extracellular water and if equilibrium distribution has been achieved, then the intracellular potassium content of the three cardiacs, I. F., Y. J., and F. P., does not differ from the normals.⁵ More data similar to that obtained in M. M. are required before it is concluded that there is a decreased potassium content in the skeletal muscle of congestive failure patients. In patient M. M. there was a distinct increase in the potassium content of the muscle as heart failure waned.

In one month the intracellular potassium content of muscle in a cardiac with a daily negative external potassium balance of 10 mEq. will decrease about 10 per cent. This is equivalent to the decrease observed in the present series of cardiacs if all the values for intracellular potassium are averaged. The assumption is made that the loss is entirely from the skeletal muscle mass which in a 70 kgm. man contains about 2,800 mEq. of potassium. The liver contains only about 4 per cent and the blood about 8 per cent as much potassium as muscle. It therefore appears very unlikely that liver and blood are the major sources of the potassium that is lost when the organism is in chronic negative potassium balance.

The accuracy of the determination of the intracellular sodium will depend largely on the determination of the extracellular value for this cation because it is predominantly outside the cell. It is

for this reason that large variations in intracellular sodium concentration are obtained in the same sample depending upon what standard measures extracellular water. In biopsies from the rectus abdominis muscle, Mudge and Vislocky (36), using chloride as the reference standard, obtained values of 2, 8, and 14 mM. of sodium per liter intracellular water in three control patients.

What has emerged from the present studies is the *uncertainty of the interpretation of data relating to intracellular electrolyte concentrations*, determined either directly by tissue analyses or indirectly by metabolic balance studies or isotopic dilution techniques, *unless the extracellular compartment is accurately measured*. Inherent difficulties in methodology may at times obscure any real difference between the inulin and chloride "spaces," and the variation in the chloride/inulin ratio may be such that calculated small differences in intracellular electrolyte concentration may be more apparent than real. If future work proves that the equilibrium volume of distribution of inulin is the correct reference standard, there may be no difference in the intracellular electrolyte composition of patients in congestive heart failure as compared to normal. However, if some modification of the "chloride space" turns out to be the reference base, the cardiac in failure may have a decreased intracellular potassium and an increased sodium concentration, but the exchange between potassium and sodium is not equal.

Yannet and Darrow (5) previously have called attention to the fact that shifts of water are not determined solely by changes in extracellular electrolyte concentration. More recently Opie and Rothbard (37) have concluded that movement of water in striated muscle removed from the body occurs not only under conditions of osmotic interchange but also under conditions which have no relation to electrolyte concentrations. In the dog, Gaudino and Levitt (38) have shown that DCA causes a decrease in intracellular fluid volume and an expansion of the extracellular space. Similar findings are reported in man by Levitt and Bader (28) with cortisone and ACTH. In man and dog the effects are transient. Robinson (39) has concluded from experiments on rat kidney that mammalian cells are not in osmotic equilibrium with their environment and that "the osmotic pressure of the cell fluids is normally 50 to 100 per

⁵ It should be noted that an apparent increase in the amount of extracellular water will result in an apparent increase in the *concentration* of intracellular potassium.

cent greater than that of the extracellular fluids." Whether this applies to other tissues as well remains to be determined. While one cannot assess total osmotic pressure from cation concentration alone, it is of interest to note that the intracellular cation concentration in the muscle of the cardiacs and controls was significantly higher than the interstitial fluid. The hypothesis of inactive cell base (40) remains to be proved.

We are not in a position to corroborate with the present limited data the earlier report by Laszlo (41) that the phosphate content of skeletal muscle is significantly reduced in heart failure.

SUMMARY AND CONCLUSIONS

Biopsies of the gastrocnemius muscle were taken in seven patients, four non-cardiacs and three cardiacs with congestive heart failure, after a constant infusion of inulin had been administered for 30 hours in the cardiacs and six hours in the controls. In three additional cardiacs biopsies were taken without prior inulin infusion. On the premise that equilibrium distribution of inulin had been achieved, it was found that in normal patients 48 grams of extracellular water were associated with 100 grams of fat-free muscle solids; using "chloride space" as the reference standard, this value for extracellular water was 18 per cent higher. When a constant correction for non-diffusible chloride was applied, equivalent to 1 mM. of chloride per 100 grams of fat-free solids, the extracellular water content was 4 per cent greater than with inulin. The range, however, was wide. In the three patients with heart failure the chloride/inulin ratio was 0.95 and the chloride-1/inulin ratio was 0.87.

The true value for extracellular water is not evident from the present study. If one accepts the "chloride space" or a "corrected chloride space" as the reference standard for extracellular water, it can be shown that the cardiac with fixed heart failure has a loss of intracellular potassium and a gain of intracellular sodium. With inulin as a standard, there is no change in the intracellular electrolyte composition in heart failure from the normal.

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