Water, Sodium, and Potassium Content of Human, Guinea Pig, and Rabbit Lung.* (20105)

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The electrolyte content of pulmonary tissue has met with but limited interest, as is indicated by the scarcity of data found in the literature(1-5). This is remarkable in view of the variety of conditions where large amounts of fluid accumulate in the lung. Such aggregations of fluid, under the clinical term "pulmonary edema," have been extensively investigated as to their pathogenesis, and their experimental production has been recently reviewed by Gamble and Patton(6). Hemingway(7) has contributed nitrogen values for guinea pig lungs.

The experiments reported here were undertaken to establish suitable methods for the determination of water and electrolyte content of lung and vascular tissues as a background for further investigation.

Material and methods. The tissues under examination were lung, blood, and plasma of the guinea pig, rabbit, and man. The guinea pigs were of an average weight of 250 g. The rabbits averaged 4 kg body weight. The diet of the guinea pigs consisted of cabbage, salt, and oats *ad lib*. The rabbits received water in addition to the guinea pig diet. Two agents were used for *anesthesia*: ether to the point of unconsciousness to prepare for heart blood sampling, followed by ether until death occurred; sodium pentobarbital (Nembutal), 0.2 g/kg for cardiac puncture and 0.4 g/kg for sacrificing. In all instances the time that elapsed from initial unconsciousness to removal of the lungs did not exceed 15 minutes. This period was kept to a minimum to avoid lung edema formation(9). The guinea pig lung and blood specimens were pooled in sets of 2 to furnish sufficient material for analysis. Blood intended for *plasma electrolyte* studies was collected under oil with a minimum amount of heparin as anticoagulant. Centrifugation followed by separation of the plasma from the cells was performed immediately. Studies on *human tissue* were done on lungs removed surgically for carcinoma (Specimens A and C) or bronchiectasis (B). The specimen employed for analysis was taken distant from the lesion. Histologic control revealed for lung C an occasional small group of macrophages with pigment in their cytoplasm that stained positively for iron. Specimens A and B were free of lesions. *Gravimetric* sampling was used throughout. For the measurement of water content of lung, blood, and plasma, 3 different methods were used: (a) Oven drying to constant weight. This consisted, after experimentation to determine the proper drying period, of heating the sample overnight (16 hours) in an electric oven maintained at 97 $\pm$ 1°C. (b) Freeze drying for the same length of time. The vacuum attained was sufficient to maintain the samples in the frozen state until dry. (c) The Karl Fischer titrimetric method(8). Sodium acetate $3H_2O$, reagent grade, as suggested by Warren(10), was employed as the primary standard for water and yielded almost identical duplicates. The liquid specimens were weighed according to Cook *et al.*(8). The lung samples were weighed on small glass spoons suspended from the hook of the balance. The spoon plus sample was then placed in a dry 50 ml volumetric flask. Use of the glass spoon eliminated titration of a paper blank. Karl Fischer reagent was added to within about 10 to 20% of the previously estimated end point. The flasks were stoppered and allowed to stand with occasional shaking for one-half hour before completing the titration. Evidence that the one-half hour extraction period was sufficient consisted of allowing the completely titrated specimen to stand sealed overnight. Such treatment did not result in fading of the end point. *Sodium and potassium* were determined by means of a Beckman DU

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TABLE I. Comparison of Drying Methods.

Species	Oven drying % water			Chemical % water			Freeze drying % water		
	Lung	Blood	Plasma	Lung	Blood	Plasma	Lung	Blood	Plasma
Rabbit 101	80.9	86.5	92.7	81.0	84.6	91.1	80.7	85.9	92.6
102	78.6	84.8	91.5	78.9	84.3	91.5	78.9	84.8	91.3
110				80.9	83.5	90.5			
Human A	80.2	78.5	91.1			89.8	79.9	78.2	91.0
T		78.3	90.2			91.4		78.4	90.2
H		81.0	90.7					81.1	90.5
Guinea pig set 1-10*							79.7±.41	82.6±.43	
11-15†							80.2±.35	82.5±.15	93.5±.2
Rabbit 1‡	81.2	83.0	92.8						
2‡	79.2	81.9	90.6						

* Ether anesthesia. † Nembutal anesthesia. ‡ Data of Manery and Hastings.

Spectrophotometer with flame attachment utilizing acetylene and oxygen. The tissues were ashed in an electric oven at 400°C for 72 to 120 hours, cooled, taken up in 1 ml conc. HCl, and diluted to an appropriate volume for analysis. *Chloride* was measured by the method of Van Slyke and Sendroy(11). To eliminate any possible adsorption by protein, a modification of Hill's method(12) was developed for the estimation of iron. An accurately weighed sample of approximately 100 mg of dry, or a corresponding amount of wet, material was placed in a 50 ml Erlenmeyer flask. Concentrated nitric acid in 0.2 ml portions was added, followed by gentle heating over a free flame to dryness between each addition of acid. A char was formed which was later consumed upon further nitric acid digestion. After oxidation of the char, 1 ml of concentrated HCl and 2 ml water were added to the flask, which was then covered with a watch glass and heated gently on an electric hot plate for about 15 minutes. Upon cooling, the solution was rinsed with water quantitatively into a 10 ml volumetric flask and diluted to the mark with water. One ml of this solution was used for analysis. One ml of a solution containing 20 γ iron per ml (as ferrous ammonium sulfate) was employed as the standard, and one ml of water as the blank. To the test tubes containing these one ml amounts were added in turn: 2 ml water; 5 ml ethyl alcohol; 1 ml 0.2% a-a' dipyridyl; and 1 ml 2% freshly prepared aqueous iron-free sodium hydrosulfite(12). The tubes were mixed by inversion and allowed to stand for 2 hours for complete color development before reading in a Coleman Junior Photometer at 520 mμ against the blank. An occasional nitric acid blank was run. Recovery of iron was checked by adding 100 μg quantities of iron to duplicate samples of tissue. Recoveries ranged from 99.4% to 101.4%. The optical density versus concentration curve, which passed through the origin, was linear to an optical density of 0.4. At D = 0.8 the drop in the curve was such as to yield values about 7% low. In addition, a simple extraction procedure for measurement of *hemoglobin* in lung tissue was developed and used for human lungs B and C (to be published). The results are comparable to those obtained using the iron determination method.

Results and discussion. Table I lists the water content in percent of total weight of the respective specimens. The differences between the results obtained by the 3 methods are minimal and of the same order as those between individual samples. They also compare well to those reported by other authors by oven drying and by the Karl Fischer method (8). By virtue of its simplicity and speed, the titrimetric procedure of Karl Fischer seems to be the method of choice for determination of water content.

The fact that values for water obtained on freeze dried guinea pig specimens after ether or nembutal anesthesia are nearly identical indicates that, under the experimental conditions, the influence of these 2 types of anesthesia on guinea pig lung water, if any, is the same.

TABLE II. Chloride Data Obtained from Fresh, Oven Dried, and Freeze Dried Specimens.

Material			Chloride, mEq./kg Fresh	Oven dry	Freeze dry
Rabbit 101		Lung	49.4	51.3	50.7
		Blood	100.5	86.7	97.9
		Plasma	99.7	97.1	94.3
Rabbit 102		Lung	63.8	59.6	61.1
		Blood	90.5	88.7	89.2
		Plasma	101.2	97.4	98.8
Human	A	Lung	72.6	72.9	
	A	Blood		73.7	73.1
	A	Plasma	102.8	95.5	94.1
	T	Blood	79.2	82.0	73.0
	T	Plasma	102.2	102.6	95.3
	H	Blood	83.1	71.1	
	H	Plasma	92.0	85.7	88.1
	R	Plasma	88.7		85.8

TABLE III. Iron Values Obtained from Fresh, Oven Dried, and Freeze Dried Specimens.

Material			Iron, mg/kg Fresh	Oven dry	Freeze dry
Rabbit* 101		Lung	67.1	73.1	72.6
		Blood	211.0	193.0	207.0
Rabbit† 102		Lung	80.1	83.3	80.3
		Blood	241.0	236.0	249.0
Human	A	Lung	236.0	234.0	242.0
	A	Blood		505.0	515.0

* Hematocrit 15. † Hematocrit 31.

TABLE IV. Sodium and Potassium Content of Oven Dried and Freeze Dried Samples.

Material			mEq. sodium per kg wet material: Oven dried	Freeze dried	mEq. potassium/kg wet material: Oven dried	Freeze dried
Rabbit 101		Lung	76.0	74.3	57.0	61.0
		Blood	112.0	112.0	24.2	24.5
		Plasma	145.0	136.0	3.7	3.7
Rabbit 102		Lung	45.6	43.3	36.6	33.0
		Blood	74.9	81.1	19.9	20.5
		Plasma	122.0	134.0	2.3	2.7
Human	A	Lung	86.2	83.7	51.7	51.8
		Blood	63.7	64.5	53.4	54.1
		Plasma	131.0	130.0		

The chloride values obtained on rabbit and human samples are reported in Table II. Those of the dried specimens were calculated to the wet basis, using the percentage of water found by the respective drying method. The results show almost uniformly lower chloride values for the oven and freeze dried samples. This may be attributed to loss of chloride by volatilization, or an absorption of water by the dried materials during storage after the original weighings to determine water content. The latter explanation will be considered in reference to the data presented in Table III.

The values for iron (Table III) were calculated in the same way as those for Table II, and were close for oven, freeze dried, and fresh samples. This indicates that there was no absorption of moisture during storage or weighing. Thus, volatilization of chloride during drying apparently accounts for the loss of this ion.

Table IV reports sodium and potassium values obtained on oven dried and freeze dried samples which were ashed at 400°C for 72 hours. If either drying method were superior for sodium or potassium from the standpoint of volatility, the ashing eliminates the advantage. The values show variation up to (mean) $\pm 5\%$.

The data applied to lung tissue are summarized in Table V. The electrolyte values for blood-free wet tissue were calculated on the assumption that non-vascular lung iron was negligible and that lung blood has the same iron, water, and electrolyte composition as heart blood. These assumptions permitted estimation of the amount of blood, and its corresponding water and electrolytes, present per unit weight of lung. After subtraction from the total, the remaining water and electrolytes were those of the lung *per se*. Rat and rabbit lung data of Manery and Hastings (5) are included for comparison.

A relatively small range of values for the guinea pig lung electrolytes will be noted. Darrow *et al.*(13) have published values for electrolytes of muscle, heart, and liver of the cat, and Harrison's group(14), data on muscle and liver of the dog, monkey, and rabbit. Their values likewise show small variation of electrolyte content of any of the 3 tissues of the species examined, although there was considerable variation from one species to another. The electrolytes of the rabbit lung (Table V) are, on the other hand, quite variable. In this connection, it should be noted that the rabbits used in these experiments were severely anemic. Also, in com-

TABLE V. Na^+, Cl^-, and K^+ in Milliequivalents per kg of Lung Tissue.

Tissue	Na^+	Cl^-	K^+	Na^+	Cl^-	K^+	Na^+	Cl^-	K^+
	Per kg wet lung			Per kg blood free wet lung			Per kg blood free lung water		
VAHO*									
Avg G. pigs 1-10	94.1 ± 3.5		85.3 ± 4.6	88.9 ± 6.5		93.7 ± 6.1	113.2 ± 8.0		113.8 ± 15.1
11-15	92.5 ± 2.7	46.7 ± 3.6	84.1 ± 1.7	87.6 ± 4.3	36.5 ± 5.5	90.4 ± 2.6	110.8 ± 7.5	46.5 ± 7.2	105.6 ± 2.2
Human A	85.0	72.6	51.8	82.2	64.8	37.2	95.0	74.9	57.0
B	76.8	74.3	48.1	72.4	73.8	52.9	91.3	93.1	66.7
C	73.4	62.0	39.2	67.8	62.0	37.5	81.3	67.5	46.0
Human lung, mean and dev.	78.4 ± 4.4	69.6 ± 5.1	46.4 ± 4.8	74.1 ± 5.4	66.9 ± 4.5	42.5 ± 6.9	89.2 ± 4.6	78.5 ± 8.5	58.6 ± 8.2
Rabbit 101	70.7	49.4	62.8	51.9	30.8	83.4	66.7	39.6	107.3
102	44.5	63.8	34.8	21.8	36.7	33.7	27.1	45.6	41.9
110	45.2	46.0	42.8	36.6	36.0	48.2	45.6	44.8	60.0
	Per kg wet tissue			Per kg blood and fat free fresh lung			Per kg blood free lung water		
Manery & Hastings					61.6				
Rat No. 6					51.3				
No. 7								71.0	107.2
Avg, 5 rats	72.3	67.3		60.8	57.7				
Rabbit No. 1	71.5	63.6		62.6	55.4				

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paring results, a source of variation is to be found in the manner used to correct for blood values. While the foregoing authors mentioned(13,14) utilized exsanguination, probably 40 to 50% complete, the present data are based on iron determinations.

If all chloride is considered to be extracellular, the extracellular tissue water of guinea pig lungs (Fig. 1) is about 30% of the total blood-free lung water. The corresponding value, calculated from Manery and Hastings' data on rats, is 40%. The 3 rabbits vary from 27 to 52%. The calculated extracellular water of human lungs was 56.8, 58.3, and 59.5% and is, therefore, considerably greater than in guinea pig lungs. This may be accounted for by species variation as well as by the ligation of the pulmonary vein prior to that of the pulmonary artery. The latter is done to prevent escape of tumor cells into the blood leaving the lung. Although the interval between the 2 ligations is short, extravasation of plasma may take place.

Conclusions. 1. A comparison of 3 methods for water determination of lung, blood, and plasma has been given. The method utilizing the Karl Fischer reagent is considered superior. Ratios of iron, sodium, potassium, and chloride content of guinea pig, rabbit, and human lung are presented. 2. The probable volatilization of chloride during oven or freeze drying is discussed. The electrolyte concentrations and the extracellular water vary

FIG. 1.

widely in the lungs of rabbits, less so in guinea pigs, but are not as consistent as has been reported for rats. The extracellular water in the 3 human lung specimens was remarkably constant.

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Use of Trypsin in Preparing Subcultures of Monkey Testicular Tissue.* (20106)

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The use of trypsin as a method of obtaining tissue culture cells for transplanting purposes was introduced by Rous and Jones(1) and elaborated by Vogelaar and Erlichman(2). A detailed study of the stimulating effect of various proteolytic enzymes on tissue growth was reported by Simms and Stillman(3). Several interesting observations were made by these various authors: a) Contact with 3% crude trypsin for 1 hour at 37°C did not injure rodent or avian cells, but 5% did(1); b) Following solution of the plasma clot, repeated subculture was possible(1,2); c) Cell growth was rapid and at times explosive (1,3); d) The lag period for adult cells in tissue culture was shortened(3); e) Similar stimulation was obtained with both crude and crystalline trypsin, chymo-trypsin, and papain, suggesting that proteolysis of growth inhibitory substances was an essential factor (3). The purpose of this report is to present a simple tryptic digest method of transplanting roller tube cultures of monkey testicle with the objective of increasing the yield of usable explants for the propagation of poliomyelitis and other viruses.

Methods. Propagation of original tissue. Freshly obtained rhesus monkey testicles were minced in Simms-Hanks solution (1 part ox serum ultrafiltrate and 3 parts Hanks' balanced salt). Six to 8 fragments per tube were planted in chicken plasma which was then clotted with 50% chick embryo extract. The nutrient fluid consisted of 1.5 ml of a medium containing 10% chick embryo extract (1:1), 78.75% Simms-Hanks solution, 10% unheated horse serum, and 1.25% human albumin. Penicillin and streptomycin were added in a final concentration of 100 units and 100 μg per ml, respectively. The tubes were placed in the roller drum at 37°C and incubated until well developed fibroblast outgrowth was obtained (2 weeks). The cells were maintained thereafter by adding fresh medium at weekly intervals.

Subculture. The nutrient fluid and the original tissue fragments were removed leaving the outgrowth intact. To each tube was

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