

tendency to spontaneous agglutination, while the original S culture showed no such tendency.

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The Chloride Content of the Tissues.*

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In normal animals the chloride content of the blood is maintained at 280-320 mg. percent, even under the conditions of a negative chloride balance. Distilled water, injected intraperitoneally in rabbits rapidly acquires a chloride concentration up to 376 mg. percent and becomes isosmotic.¹ Consequently, by the transperitoneal perfusion of distilled water, it is possible to produce an experimental hypochloremia by dialysis.² The blood chloride falls and a large amount of chloride, as well as other electrolytes and organic crystalloids, is found in the dialysate. Rabbits thus treated develop muscular fibrillation, tremors and convulsions and die in from 2 to 5 hours. In a series of 13 rabbits so dialyzed, we found an average of 619 mgs., (from 205 to 896 mgs.), of chloride was removed from the animal as determined by analyses of the perfusate. During the corresponding period of dialysis, the average fall in blood chloride was 79 mgs. percent, (from 300 to 221 mgs.). As the blood volume in the rabbits used was empirically about 200 cc., not more than 175 mgs. of the dialyzed chloride could be accounted for as coming from the blood itself. The source of the other 440 odd mgs. of chloride was evidently other body tissues. As a result our attention has been turned to the chloride content of the tissues.

Two of us (C. B. D. and M. E. H.) have recently determined the chlorides in various tissues from the rabbit. As our primary interest at the time lay in the various lipoidal fractions, these analyses were all made upon dried tissues. The animals were killed by bleeding. The desired tissues were quickly removed, cut to shreds and dried in a current of warm air (40°C) for 10 hours.

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¹ Curtis, G. M., *Biochem. Z.*, 1927, clxxxvi, 95.

² Curtis, G. M., and Pacheco, G. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, xxvi, 874.

They were then ground to pass through an 80 mesh sieve and kept in a desiccator until used. After thorough drying they were weighed into an extraction thimble and extracted with hot ether in a Lansiedl apparatus for 20 hours. The ether was then drawn off and the residue extracted for 30 hours with absolute alcohol. Except for a small amount of chloride remaining in the residue in chloride rich tissues, such as blood, practically all of the chloride was found in the alcohol extract. Duplicate analyses were made on each extract, on the tissue residue and on the original unextracted dried tissue. Two methods of chloride analysis were used. The method of Van Slyke³ gave excellent results with the extracts. In the case of the whole tissue, however, not all the chloride present was recovered. To overcome this loss the method was modified in that the oxidation was carried out in a sealed Carius bomb.⁴ The two methods checked well in the analyses of all the extracts. Whole dried tissue analyses were made by the sealed tube method in a large number of cases. These agreed within 7% with the amounts found in the various extracts as determined by the Van Slyke method.

TABLE I.
Chloride Percentage in Dried Rabbit Tissue.

Tissue	7 normal animals	4 animals, given 35 cc. N/10 HCl intravenously	2 animals, given 50 cc. N/2 NaCl intravenously
Fundus mucosa	0.76	1.72	0.64
Liver	0.24	0.27	0.19
Intestine	0.42	0.54	0.56
Kidney	0.73	0.91	1.03
Vol. muscle	0.29	0.22	—
Sm. muscle	0.38	0.65	—
Lung	0.57	0.94	—
Blood	1.34	1.58	1.53

The results of these analyses are presented in Table I. To determine the redistribution of chloride in the tissues after injection, 4 animals were given 35 cc. of N/10 HCl, containing 125 mgs. of Cl, intravenously. Two animals were given 50 cc. of N/2 NaCl containing 875 mg. of chloride, in the same manner. The injections took from 20 to 30 minutes. The animals were killed 5 minutes after the end of the injection. Changes in the distribution of chloride within the tissues are shown in the table.

No analyses of skin or subcutaneous fatty tissue of the rabbit are included in the table. Analyses made by M. E. Hanke on these

³ Van Slyke, D. D., *J. Biol. Chem.*, 1923, lvii, 523.

⁴ Herman, W. K., personal communication.

tissues in the dog show a chloride percentage of from .10 to .15% for the subcutaneous adipose tissue and of from .80 to .90% for the skin.

While the chloride content of the liver is low, yet the size of the liver (18-30 gm. dry weight) emphasizes its importance as a reservoir for chloride. The chloride content of voluntary muscles is also low as compared to some of the other tissues. Owing to their great bulk, however, they must be regarded as an important chloride reservoir. When chloride is dialyzed away from the blood stream in rabbits, it is restored in part from some body source. The tissues which appear to be the more important as such a source are the skin, voluntary muscles and liver.

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Variations in the Blood of Rabbits With Age (Birth to Maturity).*

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The total blood volume, red cell count, volume ratio of corpuscles to plasma, reticulocyte count, hemoglobin and size of the red cells of rabbit from 6 hours after birth to 8 months of age have been studied. At birth the R.B.C. are 4 to 5 million per cu. mm.; the Hb is 13 to 16 gm., the volume ratio of corpuscles to plasma and the total blood volume are higher than at maturity. The size of the R.B.C. is 1/3 to 1/2 times larger than the adult R.B.C. The reticulocytes are 25% to 30% of the R.B.C. at birth and 1% to 3% at maturity

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