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**On the Use of Sulfanilamide in Measurement of Body Water
in the Dog.***

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Sulfanilamide has been advanced for use in the measurement of the total water content of dogs.¹ The suggestion was based upon the

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demonstration that its volume of distribution was equal to that of urea as well as to the total water content of animals, as measured by desiccation. Unfortunately, the whole blood concentration was used in calculating the volume of distribution of sulfanilamide, thus introducing an error due to the localization of sulfanilamide in the erythrocytes.² Such localization had been demonstrated in human bloods³ and it is now known that a similar situation obtains in other species. One may reason, from these considerations, that sulfanilamide is not valid for the purpose suggested. However, the concept is of sufficient importance to require a more direct examination in the dog as the basis for its rejection or acceptance.

The volume of distribution of sulfanilamide, and its distribution in a series of representative tissues have been determined in the dog, using the concentration in the plasma water as the standard of reference.

Experimental. The renal pedicles were ligated in Dogs 1-5 under light nembutal anesthesia. Dog 6 was normal. Sulfanilamide was injected immediately after the ligation in 3 animals, the experiments being terminated in 2 (Dogs 1 and 2) and 24 hours (Dog 3). It was administered to Dogs 4 and 5 24 hours postoperatively. Dogs 3, 4 and 5 had recovered completely from the anesthetic and from the acute effects of the operative procedure at the time of the distribution studies. All animals were quickly exsanguinated at the termination of the experiment and the tissues immediately sampled for analysis; blood samples were taken one hour after the intravenous administration of the sulfanilamide and just prior to sacrificing the animal.

The sulfanilamide determinations⁴ were on protein-free filtrates of plasma, erythrocytes, and tissue obtained by the precipitation of protein at high dilution (1-50) with trichloroacetic acid. Small pieces of tissue were thoroughly ground with silica and trichloroacetic acid and then extracted for one hour with frequent agitation. Complete recovery of sulfanilamide added to plasma, erythrocytes and tissue was regularly obtained with these procedures. The sulfanilamide concentrations in plasma water were calculated from the plasma concentrations on analysis and the water content of plasma. No corrections were made for the nondiffusible sulfanilamide which is small in the case of dog plasma.^{5, 6}

¹ Painter, E. E., *Am. J. Physiol.*, 1940, **129**, 744.

² Peters, J. P., *Ann. Rev. Physiol.*, 1942, **4**, 89.

³ Sise, H. S., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 451.

⁴ Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

⁵ Waterhouse, A., unpublished.

⁶ Davis, B. D., *Science*, 1942, **95**, 78.

TABLE I.
The Distribution of Sulfanilamide in the Dog.

Dog No.	Wt. kg	Sulfanilamide administered, mg	Duration of exper., hr	Plasma sulfanilamide, mg per 100 g plasma water	Vol. of distribution		Ratio	Sulfanilamide concentration per 100 g wet tissue							
					Liters	% body wt		Sulfanilamide concentration per 100 g plasma water							
								Erythrocytes	Cerebrospinal fluid	Brain (cerebrum)	Lung	Liver	Pancreas	Muscle	Nerve
1	7.27	229.7	2	3.44	6.68	91.9	1.38	.62	.66	1.07	1.29	1.08	1.13	.76	
2	7.27	232.7	2	3.10	7.49	102.9	1.38	.59	.68	1.09	1.23	1.08	1.10	.76	
3	10.0	324.0	24	2.58	12.56	125.6	1.23	.65	.72	1.02	1.47	.88	.88	.77	
4	9.95	324.0	2	3.74	8.66	87.0	1.22	.56	.65	1.05	1.06	.93	.98	.72	
5	12.7	422.0	2	3.78	11.17	88.0	1.25	.67	.67	1.17	1.50	1.00	.97	.94	
6	12.3	333.0*	2	3.01	11.06	90.1	1.33	.67	.70	1.06	1.22	1.05	1.01	.77	
Water content of tissue															
Ratio															
Wet weight of tissue															
								.65	.78	.79	.72	.73	.76	.66	

*333 mg represents the difference between the sulfanilamide injected and that excreted by the kidney in the 2-hour interval.

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Results. These are summarized in Table I. The time allowed for the establishment of plasma-tissue equilibrium of sulfanilamide was 2 hours in 5 experiments and 24 hours in one. Two hours is quite adequate since the plasma concentration was essentially constant after the first hour in all the animals with ligated renal pedicles. The concentration of sulfanilamide, other than that in the plasma, has been expressed as the ratio of its concentration per 100 g of wet tissue to the concentration in plasma water. This ratio would be equal to the fraction of wet tissue which is water if the concentration of sulfanilamide in a tissue were determined solely by its water content. Data on the water content of tissues⁷ are included in the table for convenience of comparison. The data indicate that there is a significant localization of sulfanilamide in all tissues including brain substance if, as seems probable, the latter tissue is in equilibrium with a fluid with the chemical characteristics of cerebrospinal fluid.⁸

Discussion. The data are in keeping with the qualitative view that sulfanilamide is widely distributed in tissues in a fairly even manner.⁹ They are quite contrary to the concept that its distribution is dependent solely upon the distribution of water and, that it may be used as an accurate measure of the water content of the body. The agreement previously obtained¹ between the volume of distribution of sulfanilamide and the volume of distribution of urea, or, the directly measured water content of dogs appears to be the result of compensating errors. The localization of sulfanilamide in the erythrocytes yields a high value for its concentration in the fluid of reference when whole blood is used and this approximately balances a somewhat similar localization in other tissues.

Conclusion. Sulfanilamide may not be used as a measure of total water in the dog.

⁷ Brodie, B. B., unpublished.

⁸ Wallace, G. B., and Brodie, B. B., *J. Pharm. and Exp. Therap.*, 1939, **65**, 220.

⁹ Marshall, E. K., Jr., Emerson, K., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 196.