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Distribution of Sulfanilamide and Acetylsulfanilamide Between Cells and Extracellular Fluid.*

HERBERT S. SISE. (Introduced by Soma Weiss.)

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

Since sulfanilamide when given to dogs has been recovered in all organs in concentrations proportional to the water contents thereof,[1] it has been suggested that this drug might offer a convenient means of estimating total body water. A method which involves the same principles as those employed in the dye technic for estimation of plasma volume[2] has been used to estimate the total body water in dogs.[3] The results indicated that sulfanilamide was dissolved in approximately the same amount of water as urea, which was administered simultaneously; and, since urea is known to be distributed uniformly in body water, the sulfanilamide method seemed applicable for dogs. In expectation that this method might apply to man as well as dogs, the following experiments were carried out. These were done in the hope that the acetylation of sulfanilamide, a process which, although absent in dogs, is normally present in man,[4, 5] might not interfere with the determination.

Four subjects in basal conditions received an intravenous injection of 40 cc of a 700 mg % solution of sulfanilamide in 0.85% saline. Two additional subjects received intravenous injections of 100 cc of a 100 mg % solution of acetylsulfanilamide.† Samples of blood for control determinations were taken before the injection, and subsequent samples were taken in bottles containing the appropriate amount of potassium oxalate (2 mg per cc of whole blood) at varying intervals following the injection. The whole blood was analyzed for both free and total sulfanilamide by the revised method of Mar-

* The author wishes to express appreciation for the help and advice of Drs. Wan-nien Bien and Florence Haynes.

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

2 Gibson, J. G., and Evans, W. A., *J. Clin. Invest.*, 1937, **16**, 301.

3 Painter, E. E., *Proc. Am. Phys. Soc., Am. J. Phys.*, 1938, **123**, 159.

4 Marshall, E. K., Emerson, K., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

5 Marshall, E. K., Cutting, W. C., and Emerson, K., *Science*, 1937, **85**, 202.

† Supplied by Winthrop Chemical Company.

shall,[6] and the final color reaction was read on an Evelyn Photoelectric colorimeter.

The resulting figures showed that in 3 of the 4 subjects who received sulfanilamide there were gross irregularities in the disappearance curves due to a 10 to 30% rise in the concentration of the total sulfanilamide in the blood occurring from 1½ to 6 hours after the injection. The concentration of the free form alone, on the other hand, showed a fairly smooth logarithmic type of disappearance curve. By subtracting the free from the total, the concentration of the acetyl form was obtained. The rise in the total concentration was thus found to coincide with the appearance and sudden rise in the concentration of the acetylated form. In the other subject, where there was no rise in the total concentration, there was no acetylsulfanilamide formed before the experiment was stopped.

When the acetylsulfanilamide was injected, there was a smooth logarithmic type of disappearance curve without any rise by the end of 6 hours; but the concentration was many times higher than would be expected assuming the drug to be equally distributed in total body water. In one subject there was no free sulfanilamide found at the end of 6 hours, and in the other a trace was detectable at 6 hours.

In order to determine the distribution of sulfanilamide and acetylsulfanilamide between the whole blood and plasma, simultaneous whole blood and plasma determinations of free sulfanilamide were carried out on 4 blood samples from 2 patients who received intravenous injections of sulfanilamide. Also 5 samples from the 2 subjects mentioned above, who received acetylsulfanilamide intravenously, were analyzed for the whole blood and plasma concentrations of the free and total fractions. The water content of the whole blood and plasma of these subjects was determined by placing accurately weighed and measured 0.5 cc samples in an oven at 37°C and continuing the drying process in a desiccator at room temperature until constant weight was reached. The results, shown in Tables I and II,

TABLE I.
Distribution of Sulfanilamide Between Plasma and Whole Blood *in Vivo*.

Subject	Whole Blood Concentration mg%	Plasma Concentration mg%	Water Content Whole Blood % by volume	Water Content Plasma % by volume
A.M.	.792 .549	.364 .218	78.6	92.6
E.M.	1.89 .687	.616 .383	83.0	92.4

[6] Marshall, E. K., and Litchfield, J. T., *Science*, 1938, **88**, 85.

TABLE II.
Distribution of Acetylsulfanilamide Between Plasma and Whole Blood *in Vivo.*

Subject	Whole Blood Concentration mg%	Plasma Concentration mg%	Water Content Whole Blood % by volume	Water Content Plasma % by volume
J.M.	.763	0	84.4	92.6
	.630	0		
	.578	0		
J.P.	.546	0.157	84.2	93.3
	.180	0.064		

indicated that a larger amount of free sulfanilamide was found in whole blood than in plasma; whereas this ratio was not anticipated from a comparison of their relative water contents. Almost all of the acetyl compound was found in the whole blood. These results indicate that these compounds are affixed to the blood cells in some form of loose combination, and that the acetyl compound is combined in relatively larger amounts than the free sulfanilamide.

In order to confirm the results *in vivo* by experiments *in vitro,* known amounts of sulfanilamide and acetylsulfanilamide were added to human blood samples, and the whole blood concentrations, plasma concentrations, whole blood water content, and the plasma water content were determined as above. These experiments showed that both sulfanilamide and acetylsulfanilamide were distributed between plasma and blood cells in the same proportion as in the experiments above, and that at high concentrations a smaller percent of the added amount was combined than at low concentrations. The results of experiments in which the concentration of sulfanilamide was kept constant while the concentration of red cells in saline suspension was varied are shown in Table III. There is some indication to believe that sulfanilamide combines with other body cells since in one of the cases in which acetylsulfanilamide was injected there was none in the plasma, which is considered to be in equilibrium with the extra-

TABLE III.
Effect of Varying the Volume of Red Cells in Saline Suspensions on the Concentration of Sulfanilamide in the Suspending Fluid When Sulfanilamide Is Added to Produce a Concentration of 33.3 mg per 100 cc of Suspension in All Experiments.

Volume Red Blood Cells %	Concentration of Sulfanilamide in Suspending Fluid mg%
0.0	33.3
11.9	31.1
23.8	29.4
35.7	28.3
47.6	26.2

cellular fluid. By determining the theoretical whole blood concentration at the moment of injection by means of extrapolating the disappearance curve back to the zero time, and from estimating the total blood volume, according to Gibson and Evans' values[7] for normal subjects, it was calculated that approximately 44% of the acetylsulfanilamide was carried in the blood cells of this individual. Since at the zero time none had been excreted and none had been changed from the acetyl form to the free form, and since none was found to be in the extracellular fluid, as represented by the plasma, the other 56% was presumably combined with other cells of the body.

It is concluded that sulfanilamide and acetylsulfanilamide in man are not distributed within the blood cells and plasma in relation to the water content. Sulfanilamide has an affinity for the blood cells while acetylsulfanilamide has an even more marked affinity for these cells. There is some indication for believing that these drugs also combine in small amounts with other cells in the body.

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Does Acetylcholine Play a Part in the Mechanism of Melanophores Expansion?

YIN-CH'ANG CHIN. (Introduced by Hsi-chun Chang.)

From the Department of Physiology, Peiping Union Medical College, and the Department of Biology, Yenching University, Peiping.

The action of acetylcholine (AC) on the melanophores was tested by Parker[1, 2] on the Fundulus and Ameiurus. When he found in 1931 that AC, unprotected by physostigmine, caused *dispersion* of the melanophore pigment in fairly large dose, he concluded that AC does not play a part in its normal control. And when he found in 1934 that AC, protected by physostigmine, caused a slight *concentration* of the melanophore pigment in fairly large amount, he made the same conclusion. Recent experiments done on the paradise fish *(Macropodus opercularis)* suggested, however, that AC may be involved in the mechanism of melanophore expansion in this species.

It was consistently demonstrated that 0.01γ AC chloride (E. Merck) injected into the body subcutaneously could produce a local

7 Gibson, J. G., and Evans, W. A., *J. Clin. Invest.*, 1937, **16**, 317.

1 Parker, G. H., *Proc. Nat. Acad. Sci.*, 1931, **17**, 596.

2 Parker, G. H., *Proc. Nat. Acad. Sci.*, 1934, **20**, 596.