

## Proportion of Cystine Yielded by Hemoglobins of the Horse, Dog, and Sheep.\*

HUBERT BRADFORD VICKERY AND ABRAHAM WHITE.†

*From the Biochemical Laboratory, Connecticut Agricultural Experiment Station, New Haven.*

The presence of cystine among the products of hydrolysis of horse hemoglobin has escaped the notice of most investigators although Abderhalden,<sup>1</sup> about 30 years ago, reported the isolation of 0.3% from this protein. We have recently applied the cysteine cuprous mercaptide method<sup>2</sup> to the analysis of horse hemoglobin and have found 0.41% of cystine to be yielded by a highly purified preparation; it therefore seemed of interest to investigate the hemoglobins of other species, particularly in view of the fact that differences in sulfur content of various hemoglobins have long been a matter of record.

Hemoglobin was prepared from the blood of the sheep and the dog, and was recrystallized twice by the method of Hoppe-Seyler. Each preparation was dissolved in water and the solution was poured into a large volume of boiling water containing a trace of sodium chloride. The coagulated proteins were separately washed by repeated centrifugation with water until free from electrolytes, and were then dried in a current of warm air and ground to a fine powder.

The yield of cystine after hydrolysis in the presence of tin was determined by the cuprous mercaptide method,<sup>2</sup> iron was determined by the method of Kennedy<sup>3</sup> with the modifications of McFarlane,<sup>4</sup> sulfur was determined by the method of Denis<sup>5</sup> after a preliminary oxidation of the protein with hot concentrated nitric acid, and nitrogen was determined by the Kjeldahl method with mercury as catalyst. The results of these analyses are shown in the table where they are calculated on an ash- and moisture-free basis.

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† Porter Fellow of the American Physiological Society.

<sup>1</sup> Abderhalden, E., *Z. physiol. Chem.*, 1903, **37**, 484.

<sup>2</sup> Vickery, H. B., and White, A., *J. Biol. Chem.*, 1933, **99**, 701.

<sup>3</sup> Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.

<sup>4</sup> McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1034.

‡ We are indebted to Dr. R. O. Brooke for the iron analyses.

<sup>5</sup> Denis, W., *J. Biol. Chem.*, 1910-11, **8**, 401.

TABLE I  
Analysis of Hemoglobin.

	Cystine %	N %	S %	Fe %	Atomic ratio S: Fe
Horse	0.41	16.70	0.39	0.33	2.05
Sheep	0.60	16.83	0.73	0.32	4.00
	0.61				
Dog	1.17	16.48	0.57	0.33	3.02
	1.24				
	1.09				
	1.15				
Average	1.16				

The differences in the yields of cystine from these 3 hemoglobins are particularly striking and suggest that the yields of other amino acids from hemoglobins of different origin may likewise vary widely.

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### Experimental Fetal Nephritis.

MEYER BODANSKY, PHILIP FARIS SAHYŪN AND ELEANOR BLISH.

*From the Departments of Biological Chemistry and Pathology, American University of Beirut, Syria, and the John Sealy Memorial Research Laboratory, Galveston, Texas.*

The problem of uranium poisoning in the pregnant animal has been investigated by MacNider, Helms, and Helms,<sup>1</sup> who found that during gestation susceptibility to this poison is especially marked, and that the changes in the blood, urine, liver, and kidneys, as well as the clinical course, resemble certain major toxemias of pregnancy in man.

Our interest in this problem has been to determine whether the fetus likewise suffers from the intoxication. The series studied at Beirut comprised 4 dogs, 10 rabbits, one normal pregnant dog and one normal pregnant rabbit for control. More recently observations have been made on 2 dogs, 6 rabbits, and one cat. The results with the latter group are essentially the same as those in the first group, the data for which are outlined in Tables I and II. Uranium acetate was used in our experiment. MacNider and his associates employed uranium nitrate.

<sup>1</sup> MacNider, W. deB., Helms, S. T., and Helms, S. C., *Bull. Johns Hopkins Hosp.*, 1927, **40**, 145.