day. This estimate is substantially lower than the previous figures given in the literature for man: 300 to 400 cc/day by Dandy(2), and 600-700 cc/day given by Boyd(9). It is also proportionately lower considering the size of the species, than Flexner's(10) figure, for the cat of 12 cc/day flowing from cannulas in the aqueduct of Sylvius.

Summary. 1. Water and electrolytes enter the cerebrospinal fluid rapidly both in the ventricles and throughout the subarachnoid space. The formation of the fluid is thus not an exclusive prerogative of the choroid plexus. 2. Water and electrolytes leave directly into the blood from both the ventricles and the subarachnoid space, doing so at roughly similar rates. 3. CSF does not suddenly come into being fully formed at any point with all its constituents in their proper ratios, but each constituent is exchanging with blood at its own characteristic rate. 4. In contrast to water and electrolytes, protein is absorbed largely from the subarachnoid space, presumably from the arachnoidal villi. By virtue of this specific role played by the arachnoid villi, they serve a function in the CSF system analogous to the lymphatics of the general circulation. 5. The site and rate of protein reabsorption is an important determinant of the direction and rate of CSF flow. 6. The net amount of CSF elaborated per day in the ventricles, over and above the large and equivalent-volume exchanged, is small, and is of the order of 10 to 20 cc or less, per day in man.

1. Merritt and Fremont-Smith, *The Cerebrospinal Fluid,* Saunders, 1937.

2. Dandy, W. E., *Ann. Surg.,* 1919, v70, 129.

3. Weed, L. H., Studies on CSF, *J. Med. Res.,* 1914, v26, 51.

4. (Paper in preparation).

5. Sweet, W. H., Selverstone, B., Soloway, S, Stetten, D., *Surgical Forum Clinical Congress, Am. Coll. Surg.,* 1950, p376.

6. Key and Retzius, *Studien in der Anatomie des nervensystems unds des Bindegewebes,* Stockholm, 1875-76.

7. Weed, L. H., *Am. J. Anat.,* 1922-23, v31, 191.

8. ———, *Physiol. Rev.,* 1922, v2, 171.

9. Boyd, W., *Brit. Med. J.,* 1914, v1, 961.

10. Flexner, L. B., and Winters, H., *Am. J. Physiol.,* 1932, v101, 697.

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Natural Abundance of N^{15} in Hemin and Plasma Protein from Normal Blood.* (20660)

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Geren, Bendich, Bodansky and Brown(1) found the abundance of N^{15} in nitrogen of biological origin to be slightly higher than that of tank nitrogen used as a reference standard in the case of urinary urea, ammonia and uric acid. Urea isolated from the urine of normal individuals analysed 0.0044 $\pm$ 0.0005 atom per cent higher than tank nitrogen, and the total nitrogen, ammonia and uric acid from normal urines had N^{15} concentrations of about the same magnitude.

In connection with studies of erythropoiesis in the anemia of thermal injury(2) and of the metabolic fate of N^{15}-labelled erythrocytes transfused into normal man(3), we have had occasion to determine N^{15} abundance in a number of samples of hemin and plasma protein isolated from blood before administration of N^{15}-glycine. We have observed in hemin and plasma protein an increased abundance of N^{15} of the same magnitude found in compounds of urinary origin. For calculation of the amount of N^{15} incorporated from N^{15}-

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TABLE I. N^{15} Concentration in Hemin and Plasma Protein Isolated from Normal Blood.

	Hemin		Plasma protein
	10 dogs	9 humans	7 humans
	Atom % excess		
	.0055	.006	.004
	.004	.006	.0035
	.0055	.004	.004
	.0055	.006	.004
	.005	.0035	.004
	.003	.003	.004
	.0055	.0025	.0035
	.0045	.004	
	.004	.005	
	.0045		
Avg	.0047	.0044	.0039
S.D.	.0009	.0014	.0003

glycine into heme of the total circulating hemoglobin in different subjects for comparative purposes, correction for the slight excess of N^{15} normally present in heme may become appreciable, especially at the lower N^{15} concentrations encountered in the hemin isolated after N^{15}-glycine feeding during depressed erythropoiesis(2).

Methods. Hemin was isolated as described by Shemin and Rittenberg(4) and recrystallized according to Fischer(5). Plasma proteins were precipitated and washed 5 times with trichloroacetic acid(6), twice with acetone-ether (1:1), once with ether and dried. Duplicate samples were analyzed for N^{15} in a Nier-type isotope-ratio mass spectrometer which was built and maintained by Dr. William T. Ham, Jr., Mr. Ray Williams, and Mr. Fred Schmidt of the Biophysics Department of this institution. Each of the duplicate samples was compared with tank nitrogen as a reference standard and the N^{15} atom per cent excess over tank nitrogen determined. N^{15} atom percentages were rounded off to the third decimal place from the calculated values (7).

Results. In Table I are presented the results of analyses of 19 hemin samples analyzed along with other samples by our usual procedures at different times over a period of more than a year. The values presented are the averages of duplicate determinations which usually did not differ by more than 0.001 atom % excess. The mean values for dog hemin: 0.0047, human hemin: 0.0044, and for the 19 hemin samples combined: 0.0046 $\pm$ 0.0011 atom % excess, are very close to those found with urinary urea(1).

The different samples of normal human plasma protein were analyzed on the same day and hence the variation among samples was much smaller than in the case of the hemin samples which were determined under varying conditions. The value 0.0039 $\pm$ 0.0003 atom % excess N^{15} also shows a slightly increased abundance over tank nitrogen of a similar magnitude.

Sixteen samples of human plasma protein isolated and analysed over a 5-month period showed a wider variation among samples (mean value 0.0036 $\pm$ 0.0015 atom % excess). Although the subject in this case had received an infusion of about 500 ml of washed N^{15}-labelled red cells(3), this apparently did not affect the N^{15} concentration in the plasma proteins.

No difference from tank nitrogen was noted with nitrogen from ammonium chloride, the mean difference of 6 samples being less than 0.0001 $\pm$ 0.0007 atom % N^{15}. Fractionation of the tank nitrogen used as a reference standard was therefore not responsible for the results obtained with the biological samples. Soloway(8) also found no detectable fractionation of tank nitrogen when compared with that of ammonium sulfate or of air. Our results thus appear to lend further support to the idea that minute fractionation of the nitrogen isotopes might occur through normal biological processes(9).

Summary. Hemin and plasma protein isolated from normal blood were found to have a slightly increased abundance of N^{15} over tank nitrogen or ammonium chloride. The mean values for normal hemin, 0.0046, and for normal plasma protein, 0.0039 atom % excess N^{15}, were similar to the N^{15} concentration found in urinary urea. These results lend further support to the idea that minute fractionation of the nitrogen isotopes might occur through normal biological processes.

1. Geren, W., Bendich, A., Bodansky, O., and Brown, G. B., *J. Biol. Chem.*, 1950, v183, 21.

2. James, G. W., III, Abbott, L.D., Jr., Brooks, J. W., and Evans, E. I., *J. Clin. Invest.*, in press.

3. James, G. W., III, Abbott, L. D., Jr., Norberg,

B., Birkeland, S., and Evans, E. I., *ibid.*, 1953, v32, 578.

4. Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 1946, v166, 621.

5. Fischer, H., *Organic Syntheses*, 1941, v21, 53.

6. Fink, R. M., Enns, T., Kimball, C. P., Silberstein, H. E., Bale, W. F., Madden, S. C., and Whipple, G. H., *J. Exp. Med.*, 1944, v80, 455.

7. Abbott, L. D., Jr., and Dodson, M. J., *Analyt. Chem.*, 1952, v24, 1860.

8. Soloway, S., *ibid.*, 1951, v23, 386.

9. Schoenheimer, R., and Rittenberg, D., *J. Biol. Chem.*, 1939, v127, 285.

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Influence of pH on Toxicity of Vanadium in Mice. (20661)

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In testing various compounds as antidotes of vanadium(1,2), it was found that one of the substances (ascorbic acid) that afforded good protection against vanadium toxicity had a pH of 2.5 under the conditions of use. This suggested the possibility that H-ion concentration might alter toxic response to vanadium. Support for this possibility was found in a report that anionic vanadium is more toxic than cationic vanadium(3). Moreover, Aub *et al.*(4) showed long ago, and it has been amply confirmed since, that urinary lead excretion is increased following administration of ammonium chloride. More recently, Brown, Kolmer, and Rule(5) showed a similar effect for bismuth. It has further been postulated that an important factor in detoxication is the conversion of a weakly acidic substance, which the body excretes with difficulty, to a relatively strong acid which can be readily eliminated by the kidney(6). Accordingly, test were performed on mice to determine whether altering the pH in various ways would affect vanadium toxicity.

Materials and methods. Male mice of the Webster strain* weighing 20-29 g were used for this study. Each mouse was weighed individually and injected with the agents in doses according to body weight. The intraperitoneal route was used for all injections, since more consistent responses could be obtained by this route than by oral or subcutaneous administration. The mice were injected with substances suspected of altering vanadium toxicity into the left side of the peritoneal cavity, followed in 15 minutes by sodium metavanadate ($NaVO_3 \cdot H_2O$) injected into the right side of the peritoneal cavity. Solutions of sodium metavanadate were prepared in physiologic saline solution at a concentration of 4 mg/ml with a resultant pH of 7.0.

* Mice were obtained from Hamilton Laboratory Animals, Inc., Cincinnati, O.

TABLE I. Effect of Administering Ammonium Chloride and Bicarbonate, 15 Minutes Prior to Vanadium, on Vanadium Toxicity in Mice. Fatality rate expressed as ratio of No. of animals that died (numerator) to those used (denominator).

mg/kg $NaVO_3 \cdot H_2O$	mg/kg as V	Controls, no prior treatment	NH_4Cl, 400 mg/kg	$NaHCO_3$, 2 g/kg
25	9.1	8/20		17/20
30	10.9	12/20	5/20	

Results. Table I shows that ammonium chloride administered 15 minutes prior to vanadium resulted in a decrease of toxicity of $NaVO_3 \cdot H_2O$ at a level of 30 mg/kg. The mortality ratio of 12/20 (60%) was reduced to 5/20 (25%) by the acid chloride. Bicarbonate administered in the same manner conversely increased the toxicity of 25 mg/kg $NaVO_3 \cdot H_2O$ from 8/20 fatalities (40%) to 17/20 (85%). The doses of ammonium chloride and sodium bicarbonate used in the above tests, were the largest doses which could be used without producing signs of toxicity in the mice as determined by separate toxicity tests.

Table II shows that acidifying the $NaVO_3 \cdot H_2O$ solution to pH 1.8 before in-