chronic deficiency of folic acid in the rat. (4) The simultaneous parenteral administration of amounts of xanthopterin and p-aminobenzoylglutamic acid in the same molecular ratio existing in folic acid, has a complete and specific therapeutic activity almost as rapid as that of folic acid. (5) This indicates that rats can synthesize folic acid from simultaneously administered xanthopterin and p-aminobenzoylglutamic acid.

Received June 4, 1951. P.S.E.B.M., 1951, v78.

Changes in Brain Water, Brain Chloride, and Serum Chloride after Bilateral Nephrectomy in Rats. (19078)

ROBERT G. SMALL AND W. A. KREHL.

From Yale University Nutrition Laboratory, Department of Physiological Chemistry, New Haven, Conn.

In 11% of the 138 cases of cerebral edema reviewed by the authors from the autopsy files at the New Haven Hospital(1), nephritis and renal failure were found to be associated with edema of the brain. Other scattered references in the literature indicate that uremia and renal disease are often associated with cerebral swelling and edema(2-5). Hence one might expect to find edema in the brains of nephrectomized rats. The observations of Stewart-Wallace(6) suggest that chloride concentration, which he found much higher in edematous than in normal brain tissue, might serve as a biochemical index of cerebral edema. It is to be expected that in edema, in which there is an increase in the extracellar phase of a tissue, there should also be an increase in chloride, the predominant extracellular ion.

Although methods exist for the determination of tissue chlorides(7-9) preliminary experiments made it clear that a special procedure for chloride determination in small quantities of brain tissue is desirable. Enough normal and nephrectomized rats were used to justify statistical analysis of the results. Serum chloride and brain water determinations were done on both.

Experimental. Rats of the Sprague-Dawley and Yale strains were used. Bilateral nephrectomy was carried out under nembutal anesthesia and care was taken to leave the adrenal glands undisturbed. The animals were housed in an air conditioned animal room in which temperature and humidity were maintained at 75°C., and 45-55%, respectively. Purina laboratory feed and water were supplied *ad libitum*. The animals survived for 3 days after the operation, and just before death they were anesthetized with nembutal and a sample of blood was removed under oil by cardiac puncture. The skull was then removed with an electric hand saw, the pia-arachnoid was reflected, and the brain was lifted out with a spatula. The animals lived until the brain was removed. The blood covering the surface of the brain was removed by blotting with filter paper. The brain was then bisected and each half was weighed quickly. One half of the brain was used for the chloride determinations and the other for the water determinations.

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Method for brain chloride. Several modifications of the micro method of Van Slyke (8) were made to adapt it specifically for the measurement of the chloride in the brain. The following procedure, therefore, is presented as a suitable method for accurately measuring chloride in small samples of cerebral tissue. After careful weighing, each fresh half-brain (which weighs about 0.8 g) is homogenized in a Potter-Elvehjem homogenizer. During the process distilled water is added to increase the volume to 25 ml. Four 5-ml aliquots are pipetted into each of four 50-ml round-bottomed centrifuge tubes for parallel determinations on each sample. A measured amount of chloride is added to one of these aliquots as a control recovery determination. One ml of a standard nitric acid-silver nitrate reagent is then added to each aliquot, while the samples are briskly agitated. Four ml of concentrated nitric acid and a glass bead are then mixed with each sample in the centrifuge tube to provide an adequate volume for the digestion which is carried out over a bank of microburners until the solutions are a perfectly clear yellow-green color (20 min.). Decolorizing agents are not used. After digestion, 5 ml water and 0.3 g ferric ammonium sulfate indicator are added to the clear digest and 1 ml amyl alcohol is layered over the top. The amyl alcohol effectively sharpens the endpoint by concentrating the silver thiocyanate precipitate at the top of the titration mixture, making it possible to detect very slight color changes in the clear solution below. The titration is carried out after thoroughly cooling the tubes in an ice-water bath, using a microburette calibrated to 0.01 ml which delivers 150 drops per ml. The end point can be determined within 2-3 drops. The last drop brings a slight but definite color change (from yellow-green to orange) in the clear digest. All procedures were controlled, recovery determinations showing less than 1% error. When sufficient accuracy is attained, one aliquot can be used for each half-brain, eliminating the necessity for pipetting and thus decreasing the possibility of technical error. The values for brain chloride in the normal rats agree with those previously reported(9) in the literature. *Silver nitrate* (0.0521 N) is prepared in a darkroom by dissolving the weighed chemical in a minimum of distilled water and bringing the solution to volume with colorless concentrated nitric acid. Potassium thiocyanate (0.0224 N) and sodium chloride (0.0348 N) are prepared in the usual manner and standardized against the silver nitrate. Reagents of these concentrations are of the optimum strength for a clear end point and accurate readings on the microburette in the analysis of chloride in small samples of brain tissue weighing 1 g or less. *Brain water concentration* is determined by drying weighed homogenized brain halves for 48 hours at 104°C. The homogenates are placed in small weighed flat aluminum pans in the drying oven. A large surface is exposed to the air and little water can be trapped in the small particles produced by homogenization. At 104°C., brain will show small decrements in weight even after a week or more of drying, but the decrease after 48 hours is very small and probably due to chemical changes, rather than water loss. *Serum chloride* determinations on the blood collected anaerobically under oil are carried out according to the procedure completely discussed by Peters and Van Slyke(10).

Results. The experimental values are presented in Table I. The "t" test of Student and the "f" test of Fisher(11, 12) were used to evaluate the results. An increase of 1.48 meq/kg wet weight of brain chloride was found in the nephrectomized animals. The variability of values in the nephrectomized group as shown by the "f" test makes this small increase even less significant. The nephrectomized animals also show a slight decrease of brain water which is significant at

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TABLE I.

	Normal			Nephrectomized			Statistical			
	No. of animals	Mean value	Standard deviation	No. of animals	Mean value	Standard deviation	"t" value	"p" value	"t" value	"p" value
Brain chloride, meq/kg, wet wt	19	33.7	1.39	20	35.18	3.28	1.775	10%	5.58	.01%
Serum chloride, meq/l	25	98.8	4.58	13	71.8	8.30	12.58	.001%	3.40	.01%
Brain water, % wet wt	42	78.2	.835	12	77.6	.692	2.237	5%	1.37	N.S.*

* N.S. = Not significant.

the 5% level.

Discussion. It can be seen (Table I) that while no significant change took place in brain water or brain chloride after nephrectomy, a substantial reduction in serum chloride occurred. This is in keeping with other observations that brain chloride concentration is extremely stable in spite of wide fluctuations in serum chloride(13-17). Since vomiting, diarrhea, and increased fluid intake were not observed in the nephrectomized animals, one cannot explain adequately the fall in serum chloride on the basis of external loss. If some of the water supplied *ad libitum* and the metabolic water was not lost via the lungs or skin, its retention might account for some of the serum dilution.

A fall of serum chloride has also been reported in dogs after bilateral nephrectomy(18, 19) and in man in chronic nephritis(20). Wilde(21) suggests that the accumulation of potassium after nephrectomy may carry chloride into tissue cells according to the Boyle-Conway theory(22,23), while Durlacher(24) showed that a low potassium diet prolonged the life of nephrectomized rats.

Edema of the brain did not occur in these nephrectomized rats nor was there any increase in brain chloride concentration. Eichelberger, Kollros, and Walker have chemically analyzed the brains of dogs subjected to cerebral concussion(7). Although cerebral edema has long been thought to occur in brain trauma, they found no chemical evidence of edema and no increase in chloride concentration in the traumatized brains. In similar experiments Yannet and Darrow(25) did find an increase in the chloride content of the brains of dogs subjected to hyperthermia. They assumed that edema was present since the increment of chloride, an extracellular ion for the most part, indicated an expansion of the extracellular phase of the brain tissue (edema). Our own and these experiments are an approach to the cerebral edema problem which, it is to be hoped, will be clarified by further biochemical investigation. It should of course be emphasized that the results reported here are based on an acute situation; whether they would also be obtained in a chronic situation remains to be determined.

Summary. (1) An accurate procedure is presented for the determination of chloride in small samples of cerebral tissue. (2) Cerebral edema did not occur in rats subjected to

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bilateral nephrectomy, nor was there any increase in brain chloride concentration. (3) After nephrectomy, the serum chloride of rats falls markedly.

Received June 19, 1951. P.S.E.B.M., 1951, v78.

Utilization of d-Biotinol by Microorganisms, the Rat and Human.* (19079)

L. DREKTER, J. SCHEINER, E. DE RITTER, AND S. H. RUBIN.
From the Nutrition Laboratories, Hoffmann-La Roche Inc., Nutley, N. J.

Following the synthesis of d-biotinol, the alcohol analog of d-biotin, by Sternbach, Kaiser and Goldberg(1), it was of interest to compare the biological activity of this derivative to that of biotin. Alcohol analogs of other vitamins are oxidized readily to the corresponding vitamin by animal organisms as shown by urinary excretion studies. For example, d-panthenol, the analog of d-pantothenic acid, and 3-pyridinemethanol, the analog of nicotinic acid, are converted efficiently by humans(2,3); panthenol has been shown to replace pantothenic acid in the nutrition of the rat(4,5). The experiments to be described concern the activity of biotinol for growth of microorganisms and the rat and include determinations of the excretion of biotin after administration of biotinol to the rat and human.

Experimental. (1) *Microbiological Activity.* The biotin activity of several biotinol preparations was determined for *Lactobacillus arabinosus*(6), *Lactobacillus casei* (7), and *Saccharomyces cerevisiae*(8), the organisms commonly used for assay of biotin. Each lot tested showed the same activity for all three organisms, but the activity varied from lot to lot, ranging from 0.2 to 2%. This variability suggested a probable contamination with small amounts of biotin. Separation of this biotin was achieved by passage of solutions containing 1 mg of biotinol per ml through a column of Amberlite IRA-400 as described by Weiss *et al.* for panthenol(9). Microbiological assays of the column eluates showed the biotin activity of biotinol *per se* to be less than 0.05%. This inactivity is analogous to the failure of panthenol to replace pantothenate in the nutrition of *Lactobacillus arabinosus and Saccharomyces carlsbergensis* (2). Recovery tests with 2% and 20% biotin added to the biotinol solution indicated complete removal of the added biotin by the resin. Quantitative recovery of the biotinol in the column eluate was demonstrated by rat excretion assays in which equal urinary excretions of biotin were found after oral dosage of resin-treated and untreated biotinol solutions.

(2) *Rat curative assay.* Biotinol was compared to biotin as regards activity in curing egg white-induced biotin deficiency. The 30% egg white diet, vitamin supplement and test conditions were the same as those reported by Rubin, Drekter and Moyer(10).

* Roche publication No. 263.

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