Mosetig, E., *Prog. Cardiov. Dis.*, 1960, v2, 525.
3. Nowaczynski, W., Goldner, M., Genest, J., *J. Lab. Clin. Med.*, 1955, v45, 818.
4. Kittinger, G. W., *ibid.*, 1960, v55, 796.
5. Roberts, H. R., Carleton, F. J., *Anal. Chem.*, 1956, v28, 11.
6. Wexler, B. C., Kittinger, G. W., Miller, B. F., *Endocrinology*, 1961, v68, 545.
7. Peron, F. G., *ibid.*, 1961, v69, 39.
8. Van Maanen, E. F., Blohm, T. R., Kuhn, W. L., Greslin, J. G., Smith, J. K., Lerner, L. L., Holtkamp, D. E., *Fed. Proc.*, 1959, v18, 454.
9. Holloszy, J., Eisenstein, A. B., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 347.
10. Peron, F. G., *Program of Endocrine Soc.*, 1961, p44.

Received January 19, 1962. P.S.E.B.M., 1962, v109.

Orally Administered Cation Exchange Resin-Sorbital Regimen for Treatment of Ammonia Intoxication in Dogs.* (27322)

GEORGE D. ZUIDEMA,† MARGARET M. FLETCHER AND WILLIAM D. BURTON
(Introduced by H. W. Davenport)
Department of Surgery, University of Michigan Medical School, Ann Arbor

Ammonia intoxication, a complication of advanced liver disease, is believed responsible for part of the clinical syndrome of hepatic coma. When massive hemorrhage occurs from gastroesophageal varices, intestinal microorganisms break down blood proteins to ammonia. Portal-systemic venous collateral channels permit portal blood with high ammonia content to bypass the liver and enter the systemic circulation, thereby avoiding conversion to urea. Elevated levels of blood ammonia in the systemic circulation act on the central nervous system, and are thought to contribute to confusion, irritability and stupor.

Ammonia intoxication may be produced experimentally in dogs by oral administration of blood or protein substances to Eck-fistula animals. Certain cation exchange resins are known to have an affinity for ammonium ions. Oral administration of such resins following administration of the challenging dose of blood would place the resin at the site of ammonia formation within the intestines. This should permit the resin to act with maximal efficiency, and prevent intestinal absorption of ammonia.

This report presents our laboratory experience with a sodium cycle sulphonic polystyrene resin,‡ which exchanges sodium ions for ammonium and potassium ions. It is available in particles of small size (5-10 μ diameter), thereby providing large surface area for interaction and exchange.

These experiments tested the ability of this cation exchange resin to prevent development of ammonia intoxication in Eck fistula dogs given a challenging dose of blood by gastric tube.

Methods. Eight healthy, mongrel dogs weighing 9 to 15 kg were selected and end-to-side portacaval shunts were constructed under intravenous pentobarbital sodium anesthesia (30 mg/kg). The dogs were allowed 5 to 7 days to recover from the operation before the specific experiments were performed.

The first experiment consisted of obtaining baseline ammonia response curves to a standard dose of blood. Each dog was fasted for 18 hours, and a 20 cc sample of venous blood was taken to measure ammonia, blood urea nitrogen, total serum protein, sodium, potassium and chloride. Stored human blood (40 ml/kg) was given by gastric tube in 3 equally divided doses over a period of one hour. Blood samples were taken at hourly intervals for the following 8 hours for analysis of ammonia-nitrogen concentration. Electrolytes, serum proteins and blood urea nitrogen were again measured at the end of the experiment.

* Supported by a grant from Nat. Inst. of Arthritis and Metab. Dis., N.I.H.

† John and Mary R. Markle Scholar in Medical Science. U.S.P.H.S. Career Development Awardee.

‡ Kayexalate, product of Winthrop Laboratories, New York.

TABLE I. Whole Blood Ammonia-Nitrogen Values (μg/100 ml). Mean ± stand. error

	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr
Baseline results, blood only	148 ±14	250 ±33	270 ±31	330 ±38	410 ±48	310 ±42	240 ±28	260 ±34	240 ±32
Results following admin. of blood + Kayexalate, 32 g; Sorbital, 30 ml	145 ±11	205 ±15	190 ±24	200 ±16	180 ±28	180 ±21	175 ±23	150 ±12	150 ±13

(Results of 8 experiments in 8 Eck-fistula dogs given whole blood, 40 ml/kg, by nasogastric tube.)

The results of the determinations of blood ammonia-nitrogen of these Eck-fistula dogs in response to oral administration of blood are displayed in Table I. The mean results of the serum protein, BUN and potassium determinations are shown in Table II.

The second series of experiments was performed on the same dogs and was identical except that Kayexalate, 8 g dissolved in 30 ml of water was given by gastric tube preceding each of the 3 doses of blood. The last dose of blood was followed by an additional 8 g of resin and 30 ml of Sorbital, 70%.§ The total dose of resin was 32 g and of Sorbital, 30 ml. Mean values of blood ammonia determinations resulting from this experiment are shown in Table I, and the same serum chemistries are listed in Table II.

Electrolyte, protein and blood urea nitrogen determinations were done by standard laboratory methods. Ammonia-nitrogen concentrations were measured by the modified microdiffusion titration technic of Conway(1,2).

Results. Baseline ammonia-nitrogen levels ranged from 140-240 μg%. This is considerably higher than in normal dogs and reflects the hepatic decompensation typically seen in portacaval shunted dogs(3,4). Peak levels of blood ammonia were found to occur approximately 4 hours after blood was administered (Table I). The second series of experiments showed that Kayexalate was effective in preventing a rise in ammonia level. At the 4-hour period the concentration of ammonia-nitrogen was essentially the same as the original baseline value.

No significant differences in total serum protein were observed when baseline control values were compared with specimens drawn at the conclusion of each experimental period (Table II). Blood urea nitrogen levels were increased slightly at the close of the experiments, probably reflecting an increase in urea synthesis derived from the digestion of blood within the gastrointestinal tract. Serum potassium levels remained essentially unchanged. This ion exchange resin has an affinity for potassium as well as ammonium ions, but the potassium it takes up may be derived from lysed erythrocytes(5).

TABLE II. Table of Mean Chemistry Determinations. (Each experiment based on 8 dogs.)

	Baseline		Kayexalate exp.	
	Control	Final	Control	Final
Serum potassium (meq/l)	4.5	4.3	4.8	4.8
Total serum proteins (g/100 ml)	6.8	6.5	7.2	6.7
Blood urea nitrogen (mg/100 ml)	9.9	16.8	9.5	19.4

Statistical analysis of these experiments supports the effectiveness of this cation exchange resin in preventing an elevation of blood ammonia after ingestion of whole blood. Since control experiments showed that peak levels of blood ammonia occurred 4 hours following injection of the blood load, the 4-hour level was selected for comparison with levels drawn 4 hours after ingestion of Kayexalate. When Kayexalate was given, the 4-hour level was reduced by 86% (p is $<.001$).‖

Discussion. Ammonia intoxication has been approached therapeutically in various ways. Hemodialysis has been effective in removing

§ Sorbo. Courtesy of Biological Sciences Section, Atlas Powder Co., Wilmington, Del.

‖ p values calculated from standard t-test.

excesses of ammonia from the systemic circulation. Intravenous administration of arginine or glutamic acid aids in conversion of blood ammonia to non-toxic substances and urea. Both of these technics remove ammonia rather than prevent its formation or absorption. Both are variable and unpredictable in their effectiveness. Orally administered neomycin decreases the gram negative intestinal bacterial flora which is responsible for conversion of protein material to ammonia. Theoretically this has advantages for it prevents ammonia formation. Its disadvantages, however, lie in the relatively long lag period before it becomes effective; in the risk of developing staphylococcal enteritis; and with its toxic effects which are associated with long term use.

One important side-effect of oral cation exchange resin therapy is the tendency of resins to produce severe constipation. Flinn, Merrill and Welzant(6) and Scherr and his associates(7) resolved this problem by adding Sorbital to the cation exchange resins which they used in treatment of renal failure. Sorbital was also used in our regimen, and constipation did not develop in any animal.

This regimen, combining a cation-exchange resin with Sorbital, has been shown to be effective in preventing a rise in blood ammonia in Eck-fistula dogs following administration of whole blood by nasogastric tube. These data suggest that clinical experience, employing either Kayexalate-Sorbital alone or in combination with neomycin, is warranted.

1. Reif, A. E., *Analytical Biochem.*, 1960, v1, p531.
2. Natelson, S., *Microtecniques of Clinical Chemistry for the Routine Laboratory,* Springfield, Ill., Charles C Thomas, 1959, p484.
3. Levine, R. S., Rigler, S. P., *Surg. Forum,* 1957, v8, 235.
4. McDermott, W. V., Wareham, J., Riddel, A. G., *Ann. Surg.,* 1956, v44, 318.
5. Griffin, C. L., Schloerb, P. R., *ibid.,* 1960, v152, 274.
6. Flinn, R. B., Merrill, J. P., Welzant, W. R., *New Eng. J. Med.,* 1961, v264, 111.
7. Scherr, L., Ogden, D. A., Mead, A. W., Spritz, N., Rubin, A. L., *ibid.,* 1961, v264, 115.

Received October 18, 1961. P.S.E.B.M., 1962, v109.

Effect of Amethopterin on Visceral Concentration of $Co^{60}B_{12}$ in the Mouse.* (27323)

JACK D. WELSH, EDWARD N. BRANDT, JR. AND PAUL T. CONDIT
(Introduced by Stewart Wolf)
Department of Medicine and Biostatistical Unit, Dept. of Preventive Medicine and Public Health, University of Oklahoma Medical Center, and Cancer Section, Oklahoma Medical Research Foundation, Oklahoma City

The interrelationship of folic acid and vit. B_{12} in the metabolic functions of the liver has not been well defined(1). Nutritional studies have shown that the amount of supplemental vit. B_{12} stored by the liver and kidneys of the rat is decreased by a folic acid deficient diet(2). The use of the folic acid antagonists has provided another method of producing deficiencies of this substance. These antagonists have been demonstrated to produce histochemical and biochemical alterations in the liver of experimental animals(3), and have been incriminated in production of hepatic damage in children with acute leukemia(4). Their effects on the distribution of vit. B_{12} in the liver and other viscera have not been previously reported. The purpose of the present study was to contrast the effects of a nonlethal dose of amethopterin with those of a lethal dose on the storage and 24-hour uptake of $Co^{60}B_{12}$ in the liver and kidneys of the mouse.

Methods. Male mice, (C57 x DBA), F_1, weighing 20 to 25 g were allowed Rockland

* This work was supported by Florence L. Fenton Memorial Fund for Cancer Research from Am. Cancer Soc.