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.

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

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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

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TABLE I. Effect of Amethopterin on $Co^{60}B_{12}$ Storage.

Day killed after $Co^{60}B_{12}$ admin.*		% of dose of $Co^{60}B_{12}$ Liver Group I (control)	Liver Group II (treated)†	Liver P	Kidney Group I (control)	Kidney Group II (treated)†	Kidney P
			Part 1				
2	No. mice	5			5		
	Mean	21.1			6.9		
	S.E.	.34			.16		
5	No. mice	5	5		5	5	
	Mean	23.1	22.3		5.8	6.7	
	S.E.	.36	.74	>.05	.32	.25	>.05
9	No. mice	5	5		5	5	
	Mean	24.2	25.1		5.9	6.0	
	S.E.	.44	.74	>.05	.09	.31	>.05
12	No. mice	5	5		5	5	
	Mean	25.6	25.1		7.1	7.3	
	S.E.	.43	1.14	>.05	.29	.24	>.05
19	No. mice	4	8		4	8	
	Mean	23.0	24.1		6.5	6.6	
	S.E.	.66	.44	<.05	.18	.24	>.05
			Part 2				
2	No. mice	3	3		3	3	
	Mean	23.1	22.4		8.2	7.8	
	S.E.	.53	.90	>.05	.06	.41	>.05
3	No. mice	3	3		3	3	
	Mean	20.1	19.4		6.6	7.0	
	S.E.	.21	1.39	>.05	.18	.33	>.05
4	No. mice	3	3		3	3	
	Mean	22.2	17.4		6.8	8.8	
	S.E.	.00	2.31	>.05	.22	1.24	>.05
5	No. mice	1	2		1	2	
	Mean	23.2	12.8		6.9	10.8	
	S.E.	.00	.23		.00	2.11	—
6	No. mice	3	2		3	2	
	Mean	24.2	11.8		7.2	12.4	
	S.E.	.91	.26	<.01	.65	.43	<.01

* $Co^{60}B_{12}$ administered on day 1.

† Part 1—50 mg amethopterin/kg 1 day after $Co^{60}B_{12}$.
Part 2–636 mg amethopterin/kg 1 day before $Co^{60}B_{12}$.

mouse chow and water *ad lib*. Each animal received 3×10^{-3} μg of $Co^{60}B_{12}$ subcutaneously. Previous testing had revealed that while 50 mg/kg of amethopterin was not lethal for these animals, administration of 636 mg/kg was uniformly lethal. Therefore, these two doses were selected for this study. Each was administered in a single subcutaneous injection. The animals in Groups I-IV received $Co^{60}B_{12}$ on the same day (Table I). Those in Group II received a nonlethal dose of amethopterin one day after the $Co^{60}B_{12}$ while Group IV animals were given a lethal dose of amethopterin on the day before they received $Co^{60}B_{12}$. Groups I and III served as controls for these animals and did not receive amethopterin. The animals from each of these 4 groups were killed by cervical separation at the times outlined (Table I). Animals from Groups V and VII were killed 24 hours after administration of the $Co^{60}B_{12}$ (Table II). Groups VI and VIII received amethopterin on the same day and $Co^{60}B_{12}$ was administered 24 hours before they were killed (Table II). After each animal was killed, the liver and kidneys were removed,

TABLE II. Effect of Amethopterin on 24-Hour $Co^{60}B_{12}$ Uptake.

Group	Mice	Amethopterin admin.*	Day $Co^{60}B_{12}$ given	Day killed	% dose of $Co^{60}B_{12}$ Liver	Kidney
			Part 1			
Group V (control)	5	None	1	1	21.1 † .35‡	6.9 .17
Group VI (treated 50 mg/kg amethopterin)	5	Yes	3	4	18.8 .95	6.1 .37
	5	"	7	8	20.0 .77	6.2 .23
	5	"	10	11	21.1 .82	7.2 .31
	7	"	17	18	21.8 .59	7.3 .14
			Part 2			
Group VII (control)	5	None	1	2	21.6 .88	8.2 .24
Group VIII (treated 636 mg/kg amethopterin)	3	Yes	1	2	19.9 .95	8.0 .43
	3	"	2	3	21.0 1.00	7.5 .32
	2§	"	3	4	16.6 1.9	6.8 .40
	2§	"	4	5	13.9 ‖ 1.15	11.3 ‖ 2.20
	3	"	5	6	11.0 ‖ 1.33	20.0 ‖ 3.40
	3	"	6	7	9.5 ‖ 1.41	24.2 ‖ 4.03

* Amethopterin administered day 1. † Mean. ‡ Stand. error of mean. § One animal died. ‖ Significantly different from control (p 0.05).

rinsed in tap water and blotted dry. The radioactivity of the organs was determined in a well-type scintillation counter and the results expressed as a percentage of the administered dose of $Co^{60}B_{12}$. The difference between the results obtained in the animals receiving amethopterin and their respective controls was analyzed using the standard Student "t" test(5).

Results. The influence of amethopterin on ability of the liver and kidneys to store $Co^{60}B_{12}$ is summarized in Table I. The amount of administered $Co^{60}B_{12}$ in the livers of animals (Group I) which were killed up to 19 days after receiving this radioactive material increased only slightly. The findings in the animals (Group II) which received the nonlethal dose of amethopterin were essentially the same as in Group I. Animals (Group III) killed at intervals up to 6 days after administration of $Co^{60}B_{12}$ also showed little change in their organ storage during this period. In those (Group IV) which had received a lethal dose of amethopterin, the mean percentage of administered $Co^{60}B_{12}$ in the liver had decreased from 22.4 ± 0.90% to 11.8 ± 0.26% by day 6. During this period the mean percentage of administered $Co^{60}B_{12}$ in the kidneys had increased from 7.8 ± 0.41% on day 2 to 12.4 ± 0.43% on day 6. Compared to the findings in livers and kidneys of the control animals, these were significant changes ($p = <0.01$). None of the animals in Group IV lived after day 6.

In Table II is recorded the 24-hour hepatic and renal uptake of $Co^{60}B_{12}$ in control animals (Group V and VII) and in those which received amethopterin. The 24-hour organ uptake of $Co^{60}B_{12}$ at intervals up to 18 days following administration of the nonlethal dose of amethopterin (Group VI) was similar to those in the control group (Group V). In the

animals which received the lethal dose of amethopterin (Group VIII) the mean 24-hour hepatic uptake was reduced from 19.9 ± 0.95% to a mean of 9.5 ± 1.41% by day 7 while the mean 24-hour renal uptake had increased from 8.0 ± 0.43% to 24.2 ± 4.03%. The 24-hour hepatic and renal uptake of this radioactive material was significantly different than in the controls ($p < 0.05$) on days 5, 6 and 7. Two animals from Group VIII died at the times indicated in the table so that the animals assayed at these periods are survivors.

Discussion. Interpretation of these data may be considered in the light of findings relating to an hepatotoxin, carbon tetrachloride. Experimentally-induced hepatic damage by this agent results in a release of vit. B_{12} from the liver with an initial rise in serum B_{12} and a slight increase in renal excretion (6). The decreasing storage and 24-hour uptakes of this radioactive vitamin by the livers of animals which received the lethal dose of amethopterin probably reflected progressive hepatic damage. The increased renal storage could be due to its preferential uptake of bound B_{12} released by the damaged liver or to its increased storage of this vitamin because of the liver's progressive inability to do so. The last alternative seems to be most likely since the 24-hour renal uptakes were also increased. The diminished binding capacity of a damaged liver may lead to utilization of the kidney as another important binding site for the vitamin(7). It would appear that the kidney, at least in some species, has a more fundamental role in vit. B_{12} metabolism than simply that of excretion.

Summary and conclusion. The effects of 2 doses of amethopterin on hepatic and renal storage and 24-hour uptake of $Co^{60}B_{12}$ were contrasted in the mouse. A nonlethal dose had little effect on these measurements of $Co^{60}B_{12}$ metabolism. Following administration of a lethal dose of amethopterin, a progressive decrease in storage and 24-hour uptake of $Co^{60}B_{12}$ by the liver occurred while concomitant increases were observed in the kidney. It is suggested that the lethal dose of amethopterin was hepatotoxic and decreased the ability of the liver to metabolize $Co^{60}B_{12}$. In such a situation the kidney may become a more important binding site for this vitamin in the mouse.

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Tracer Studies of Vitamin C Utilization in Men: Metabolism of D-Glucuronolactone-6-C^{14}, D-Glucuronic-6-C^{14} Acid and L-Ascorbic-1-C^{14} Acid. (27324)

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Recent studies(1) indicated that D-glucuronolactone caused increased blood ascorbic acid levels as well as increased urinary excretion of ascorbic acid in men, whereas D-glucuronic acid did not do this. To check the possible conversion of D-glucuronolactone to L-ascorbic acid, it was decided to study the metabolism of the lactone in two ways. One was to give D-glucuronolactone-6-C^{14} orally and then isolate urinary ascorbic acid to determine if any of the labeled lactone had been converted to L-ascorbic acid. The other was

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