Discussion. The destructive effect of 2 acute hepatitis sera on chimpanzee kidney cells, capable of serial passage, suggests the action of a viral agent or agents. Fluorescent antibody and neutralization tests indicate that the agents are antigenetically reactive with convalescent serum from clinical infectious hepatitis. Heat inactivation studies further emphasize similarity to properties attributed to hepatitis virus. Studies to characterize the agents more clearly are presently in progress.

Summary. Destruction of chimpanzee kidney cells by 2 of 12 acute sera from patients with clinical infectious hepatitis was noted. The cellular effect has been seen in serial passage. The destructive agents, tentatively named SAM-1 and SAM-2, have been shown in fluorescent antibody and neutralization tests to be serologically cross-reactive with convalescent serum derived from clinical infectious hepatitis. The agents are resistant to heat treatment at 56°C for 30 minutes.

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Dose Dependent Effects of D-Sorbitol on Intestinal Absorption of Vit. B_{12}.* (27078)

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It has been shown that D-sorbitol enhances intestinal absorption of B_{12} under certain conditions in man(1) as well as in rats(2). Morgan and Yudkin(3) reported that growth of rats can be accelerated by supplement of sorbitol to a diet deficient in the B group vitamins. They postulated that sorbitol increased intestinal synthesis of these vitamins. Our study(4) also demonstrated that dietary sorbitol promoted growth of weanling rats placed on a B_6 deficient diet, with a concomitant increase in urinary output of B_6. No such effects were obtained with B_{12}, however. Inasmuch as increased synthesis of B_{12} in the intestine will not explain the enhancement of absorption of oral doses of B_{12}, a study was carried out in rats to elucidate the mechanism by which sorbitol improves B_{12} absorption. It was found that intestinal absorption of B_{12} could be either enhanced or reduced by coadministration of sorbitol, depending upon the dose. Such diverse, dose dependent effects of sorbitol will be reported herein.

Methods. Young adult rats of Wistar strain were used. Absorption of Co^{60} labeled B_{12} (Merck) was determined by one of the following methods. a) Absorption in intact rats: Animals were fasted overnight and then fed by a stomach tube a test dose of $Co^{60}B_{12}$, and absorption was estimated from the organ uptake of radioactivity. b) Absorption in ileostomized rats: In some animals, the ileum end was surgically severed and opened outside through the abdominal wall, the cecal end being closed. Thus, the cecum was excluded from the absorptive route, and absorption measured was that from the small intestine. The technique

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has been described elsewhere(5). c) Absorption by the "intestinal loop": A loop of the small intestine isolated *in situ*(5) was prepared to determine intrinsic factor activity of sorbitol and its effect on B_{12} absorption from the loop. $Co^{60}B_{12}$ was injected with or without rat stomach extract (intrinsic factor) into the loop which had previously been washed practically free of intrinsic factor, and radioactivity remaining in the loop after 24 hours was measured.

Four different dosages of $Co^{60}B_{12}$ were used; a single dose of 10 mμg(A), 50 mμg(B), and 2600 mμg(C), and 2 doses of 1000 mμg with a 24 hour interval(D). The radioactivity in each of these doses was approximately 0.01, 0.05, 0.15 and 0.1 mμcurie, respectively. The first dosage may be considered as being within physiological limits, and the latter 2 are supraphysiological. Crystalline D-sorbitol was dissolved in 1ml of distilled water mixed with 1ml of $Co^{60}B_{12}$, and administered. The animals were sacrificed 2 days later. Because of the diarrhea in sorbitol dosed animals, no fecal collection was attempted. Determination of radioactivity in organs with a gamma-scintillation counter has been reported(6).

Results. First, the effect of sorbitol on B_{12} absorption was studied in intact rats using doses A, B and D orally (Table I). It is apparent that coadministration of 600 mg of sorbitol markedly increased absorption of the 1000 mμg dose of B_{12}. The enhancement of absorption was less marked with decreasing levels of sorbitol, no further effect being observed below 100 mg (Studies 1 and 2). When the dose of B_{12} was lowered to 50 mμg, the effect of sorbitol to enhance absorption was less pronounced (Study 3). Upon further reduction of B_{12} dose to 10 mμg, this effect was no longer demonstrable. As a matter of fact, at this dose of B_{12}, the organ uptake of radioactivity in the animals given 600 mg of sorbitol showed a definite decrease. Thus, the effect of sorbitol was dependent on its dose as well as on the dose of B_{12}, and enhancement of absorption was marked only when a large amount of sorbitol was used in combination with a large dose of B_{12}.

Since the ceca of the rats receiving a large dose of sorbitol were invariably enlarged with liquid contents, it seemed of interest to investigate the role, if any, of the cecum in sorbitol-induced enhancement of absorption. In view of the finding that the effect of sorbitol was definite only when it was used in large quantities, 600 mg of sorbitol was used. Ten days after ileostomy, sorbitol and the test dose of $Co^{60}B_{12}$ were fed by mouth. The results (Table II) clearly showed that the effect of this compound to enhance absorption of a large dose of B_{12} was completely abolished. In fact, absorption was even reduced. Absorption of the physiological dose of B_{12} (10 mμg) was also decreased by sorbitol. Since the cecum did not take part in absorption in these animals, it

TABLE I. Effects of D-sorbitol on absorption of B_{12} and dose relationship.

Study	B_{12} Dosage	Group	No. of rats	Organ uptake of Co^{60} (as mμg B_{12}) Liver	Kidneys
1	1000 mμg x 2	Control	6	17 ± 1.6	19 ± 1.4
		Sorbitol (50mg)	4	18 ± 2.1	16 ± 1.8
		" (600 ")	4	31 ± 3.2	33 ± 6.5
2	idem	Control	4	24 ± 8.0	39 ± 6.7
		Sorbitol (100mg)	4	28 ± 5.4	46 ± 9.4
		" (400 ")	4	45 ± 9.0	95 ± 8.9
		" (600 ")	4	48 ± 7.1	116 ± 7.3
3	50 mμg x 1	Control	6	1.41 ± .12	.75 ± .11
		Sorbitol (600mg)	5	1.83 ± .16	1.00 ± .06
4	10 mμg x 1	Control	6	.51 ± .05	1.35 ± .16
		Sorbitol (50mg)	4	.48 ± .06	1.37 ± .20
		" (600 ")	6	.36 ± .04	.84 ± .10
5	idem	Control	4	.65 ± .06	.98 ± .09
		Sorbitol (600mg)	4	.42 ± .03	.65 ± .08

Figures are mean ± standard error.

TABLE II. Effect of D-sorbitol on B_{12} absorption in ileostomized rats.

Study	B_{12} dosage	Group	No. of rats	Organ uptake of Co^{60} (as mμg B_{12}) Liver	Kidneys
1	1000 mμg x 2	Control	4	76 ± 13.0	157 ± 33.4
		Sorbitol (600mg)	4	52 ± 7.5	51 ± 6.5
2	10 mμg x 1	Control	3	.82 ± .10	1.21 ± .15
		Sorbitol (600mg)	3	.50 ± .09	.69 ± .11

Rats were operated on 10 days prior to test.

seems that without this part of the intestine. sorbitol does not enhance absorption. Rather. large doses of sorbitol will reduce B_{12} absorption from the small intestine.

To corroborate these findings, the effect of sorbitol was studied further using the intestinal loop technique. When 350 mg of sorbitol was added to the test dose of B_{12}, absorption from the loop was reduced regardless of the dose of B_{12} (Table III). Addition of a stomach extract (intrinsic factor) increased absorption of 50 mμg of B_{12}. Sorbitol in a slightly hypotonic solution (70 mg/2 ml) had no similar effect. Neither did this dose of sorbitol reduce absorption. It is evident that sorbitol, under these conditions, is not capable of substituting for intrinsic factor, and that at its high concentrations, it interferes with absorption of B_{12} from the small intestine.

Discussion. Greenberg et al.(2) used a large dose of B_{12} when they first reported an increase of absorption by oral D-sorbitol in rats. In human studies, the intestinal absorption of 50 μg of B_{12} was repeatedly found to be increased by coadministration of sorbitol. This dose of B_{12}, too, is far from being physiological. Employing smaller doses of B_{12} in man. Herbert et al.(7) suggested inability of this compound to enhance physiological absorption of B_{12}. Chalmers and Shinton(8) failed to repeat the observations of earlier investigators using the same 50 μg of B_{12}, but the quantities of sorbitol fed were smaller. These differences in result seem to be explained on the basis that the effect of sorbitol varies with doses of both substances, as clearly demonstrated in this study. Our data showed that sorbitol exerts an influence on absorption only when it is used in large quantities; its effect is "enhancing" on supraphysiological doses of B_{12}, but it is "suppressing" on physiological amounts of B_{12}. It is established that absorption of supraphysiological doses of B_{12} does not require intrinsic factor. It is unlikely, therefore, that the effect of sorbitol is mediated by intrinsic factor. Sorbitol was found to possess no intrinsic factor activity as studied by the "loop" technique. The results obtained with ileostomized rats and intestinal loops indicated that sorbitol, in large quantities, inhibits B_{12} absorption from the small intestine which is the natural site of absorption. This was also reflected in the decrease of absorption of a physiological dose of B_{12} in intact animals as a result of sorbitol feeding.

That the ceca of sorbitol dosed rats were

TABLE III. Effect of D-sorbitol on B_{12} absorption from intestinal loop.

B_{12} dose (mμg)	Solutions applied to loop	Amt of sorbitol (mg)	No. of rats	Absorption (mμg)	Organ uptake of Co^{60} (mμg B_{12}) Liver	Kidneys
50	B_{12}	—	1	4.6 ± .9	1.04 ± .28	.89 ± .12
	" + Sorbitol	350	1	1.5 ± 1.2	.65 ± .09	.56 ± .05
50	"	—	1	4.5 ± .7	1.66 ± .12	.55 ± .04
	" + Sorbitol	70	4	3.9 ± .5	1.42 ± .31	.42 ± .09
	" + I.F.*	—	3	21.8 ± .8	5.17 ± .72	1.74 ± .30
	" + I.F.* + Sorbitol	70	3	18.8 ± .5	4.94 ± .64	1.80 ± .21
	" + I.F.* + Sorbitol	350	3	14.2 ± .5	3.26 ± .53	1.21 ± .18
2600	"	—	3	530 ± 65	126 ± 5	123 ± 11
	" + Sorbitol	350	3	320 ± 18	44 ± 5	60 ± 4

* Intrinsic factor in the form of rat stomach extract, containing B_{12} binding power of 80 mμg.

markedly enlarged and exclusion of this organ abolished the absorption enhancing effect of sorbitol seem to provide clues for elucidation of the mechanism. Hypertonic solutions of sorbitol act like a cathartic and increase the speed of descent of coadministered B_{12} in the digestive tract. Physiological absorption of B_{12} which is executed by the small intestine is reduced by accelerated passage(9). It is possible that an enlarged cecum with liquid contents alters the physiology of the intestine in such a way as to effect unphysiological absorption of B_{12} involving no intrinsic factor. Changes of intestinal flora and hence altered availability of B_{12} molecules to the mucosa may be an important factor.

The possibility still remains that a chemical conjugation of sorbitol to B_{12} as suggested by Heinrich(10) may be involved in an unknown fashion. As to the absorption efficiency of the mucosa for B_{12}, there is no evidence that sorbitol increases permeability of the mucosa. The data of the loop experiment suggested reduced permeability by a large dose of sorbitol. Whether the mechanisms working in the rat will directly apply in man awaits further investigation.

Summary. The effect of D-sorbitol on intestinal absorption of $Co^{60}B_{12}$ was studied in rats. The data demonstrated that sorbitol, when given by mouth in large quantities together with supraphysiological doses of B_{12}, enhanced absorption of B_{12} in intact animals. However, absorption of small, physiological doses of B_{12} was reduced by the same dose of sorbitol. The dependence of the sorbitol effect on the B_{12} dose may be explained on the basis of the 2 different mechanisms for B_{12} absorption. No intrinsic factor activity was demonstrated in sorbitol as studied with isolated intestinal loops. When the cecum was excluded from the absorptive route by ileostomy, the conditions that increased absorption in intact animals resulted in reduction of absorption. Possible involvement of the cecum and the altered physiological state of the intestinal contents in enhancement of B_{12} absorption by sorbitol have been discussed.

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Depletion and Synthesis of Fatty Acids in Chickens Fed a Diet Low in Unsaturated Fatty Acids. (27079)

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Linoleic (18:2)* and arachidonic (20:4) acids are considered to be essential fatty acids (EFA). Use of this term infers that either of these compounds will meet the dietary requirement for a fatty acid(s) which cannot be synthesized by the body in sufficient quantities for normal metabolism and growth. Although arachidonate may be the only fatty acid necessary for the function of the cell(1) it is not a specific dietary essential since linoleate is readily converted to arachidonate(2, 3). In contrast linoleate either cannot be syn-

* First number denotes chain length; the second, number of double bonds.