creased uptake of NEFA following administration of insulin.

Summary. Concentrations of NEFA in plasma of blood obtained from splenic artery, portal and hepatic veins were measured in unanesthetized dogs together with hepatic plasma flow. Rates of hepatic and non-hepatic splanchnic uptake or output of NEFA were calculated before and after insulin administration. A marked increase in rate of hepatic uptake of NEFA was observed after insulin administration. No significant change in rate of NEFA output from non-hepatic splanchnic area was found.

1. Fredrickson, D. S., Gordon, R. S., Jr., *Physiol. Rev.*, 1958, v38, 585.
2. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.
3. Gordon, R. S., Jr., Cherkes, A., *ibid.*, 1956, v35, 206.
4. ———, PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 150.
5. Bierman, E. L., Schwartz, I., Dole, V. P., *Am. J. Physiol.*, 1957, v191, 359.
6. Gordon, R. S., Jr., *J. Clin. Invest.*, 1957, v36, 810.
7. Stein, Y., Shapiro, B., *Am. J. Physiol.*, 1959, v196, 1238.
8. Shoemaker, W. C., Walker, W. F., Van Itallie, T. B., Moore, F. D., *ibid.*, 1959, v196, 311.
9. Shoemaker, W. C., *J. Appl. Physiol.*
10. Blalock, A., Mason, M. F., *ibid.*, 1936, v117, 328.
11. Havel, R. J., Fredrickson, D. S., *J. Clin. Invest.*, 1956, v35, 1025.
12. Shoemaker, W. C., Mahler, R., Ashmore, J. *Metab.*, 1959, v8, 494.
13. Bloch, K., Kramer, W., *J. Biol. Chem.*, 1949, v178, 811.
14. Brady, R. O., Gurin, S., *ibid.*, 1950, v186, 461.
15. Haft, D. E., Miller, L. L., *Am. J. Physiol.*, 1958, v192, 33.
16. Balmain, J. H., Folley, S. J., Glascock, R. F., *Biochem. J.*, 1952, v52, 301.
17. ———, *ibid.*, 1953, v53, 26, 27.

Received December 8, 1959. P.S.E.B.M., 1960, v103.

Effects of Physico-Chemical State of Vit. B_{12} Preparation in Digestive Tract on Its Absorption.* (25605)

KUNIO OKUDA, ELBA DURAN V. AND BACON F. CHOW
Dept. Biochemistry, The Johns Hopkins University, School of Hygiene & Public Health, Baltimore, Md.

D-sorbitol in large doses can enhance intestinal absorption of Vit. B_{12} under appropriate conditions in man(1) and rats(2). This phenomenon may be (a) due to formation of a chemical derivative of sorbitol in the gastrointestinal tract which can enhance absorption (3), or (b) due to physical characteristics of the concentrated sorbitol solution. Therefore, some commercially available surfactants which are derivatives of sorbitol and the importance of the physical characteristics of the administered solution in enhancement of Vit. B_{12} absorption were studied. This communication reports the results of such studies and presents a hypothesis on the mechanism of enhancement of Vit. B_{12} absorption by such compounds.

Methods. Measurement of Vit. B_{12} absorption. Adult rats were fed by stomach tube 50 mμg or 1000 mμg of aqueous radioactive $Co^{60}B_{12}$ with a total radioactivity of 50 and 100 mμc, respectively. Absorption was measured by fecal recovery of radioactivity in 4 days, or only by organ uptake of radioactivity 2 days after administration when the latter dose was used. Determination of radioactivity in feces and organs has already been described(4). In some instances "Intestinal Loop" technic(5) was used.

Results. Effect of surface active agents on Vit. B_{12} absorption.† One group of rats was given by a stomach tube 1 cc of undiluted Tween 80 and immediately afterwards 1 cc of $Co^{60}B_{12}$ by another stomach tube. Another group of control animals received

* Supported by Grant-in-Aid from U.S.P.H.S.

† Surface active agents used were obtained from Atlas Powder Co.

TABLE I. Effect of Tween 80 on Intestinal Absorption of $Co^{60}B_{12}$.

Study	B_{12} dose (mμg)	Group	No. of rats	Fecal absorption test (mμg)	Organ uptake of radioactivity (mμg) Liver	Kidneys
A	50	Control*	3	17.6 ± 1.3	2.50 ± .06	1.95 ± .39
		Tween 80†	3	30.7 ± 2.7	4.92 ± .73	2.75 ± .39
B	50	Control*	5	14.0 ± 2.3	1.70 ± .37	1.49 ± .15
		Tween 80†	3	36.2 ± .3	3.75 ± .49	3.41 ± .29
C	1,000	Control*	3		8.8 ± .7	19.4 ±2.2
		Tween 80†	3		26.1 ±2.0	48.2 ±2.4
D	50	Control‡	4	22.1 ± .5	2.42 ± .40	6.30 ±1.52
		Tween 80‡ (diluted)	4	25.9 ± .2	2.43 ± .45	5.74 ±1.43

* One cc water immediately followed by 1 cc of $Co^{60}B_{12}$.
† One cc undiluted Tween 80 immediately followed by 1 cc of $Co^{60}B_{12}$.
‡ Ten cc water (control) or 1/10 diluted Tween 80 followed by 1 cc of $Co^{60}B_{12}$.

1 cc of water instead of Tween 80. The results (Table I) demonstrate clearly that administration of $Co^{60}B_{12}$ preceded by administration of undiluted Tween 80 results in greater absorption ($p<0.05$) at both doses of 50 and 1000 mμg. However, when the same amount of Tween 80 was diluted first 10-fold with water and administered, this effect was no more demonstrable, in spite of the fact that the same amounts of Tween 80 and $Co^{60}B_{12}$ were administered.

Several other surface active compounds with the nucleus of sorbitol (Span 85, Tween 20, Tween 85, G-1096 and G-672) as well as those without it (Arlacel A and Myrj 52) were tested. They were selected because of their hydrophile-lipophile balance values ranged widely from 1.8 to 16.9. The typical results from a series of experiments are presented in Table II. The results of Study A demonstrated that only G-1096 among the compounds tested increased absorption significantly, and Study B demonstrated ability of Tween 85 to enhance absorption. Taken collectively, the results show that not all sorbitol-containing compounds are capable of enhancing absorption, and confirmed our previous results that dilution of Tween 80 abolished the ability to enhance the absorption of Vit. B_{12}.

Since surfactants and $Co^{60}B_{12}$ were not premixed before feeding, it was of interest to examine the physical state of such a mixture, even *in vitro*. When any one of these 3 sur-

TABLE II. Effects of Various Surface Active Agents on Absorption of $Co^{60}B_{12}$.*

Study	Agents† (1 cc)	Chemical form	No. of rats	Fecal absorption test (mμg)	Organ uptake of radioactivity (mμg) Liver	Kidneys
A	Control		3	21.7 ± 1.6	3.39 ±.14	1.71 ±.97
	Tween 80 (10%)	Polyoxyethylene sorbitan monooleate	3	23.8 ± 4.7	3.65 ±.65	1.93 ±.41
	Span 85	Sorbitan trioleate	3	22.2 ± .5	2.88 ±.15	1.62 ±.29
	G-1096	Polyoxyethylene sorbitan hexaoleate	3	36.2 ± 1.0	4.86 ±.29	2.62 ±.29
	G-672	Glycerol sorbitan laureate	3	23.6 ± 2.6	2.55 ±.83	1.43 ±.28
B	Control		5	17.4 ± 2.7	3.01 ±.14	2.17 ±.29
	Tween 80 (20%)		4	17.7 ± 1.5	3.11 ±.29	3.11 ±.26
	" 20	Sorbitan monolaureate	3	24.8 ± 2.2	3.02 ±.28	2.81 ±.58
	" 85	Sorbitan trioleate	3	37.8 ± .6	4.98 ±.09	5.51 ±.09
	Arlacel A	Mannide monooleate	3	31.0 ± 4.2	4.16 ±.81	3.86 ±.44
	Myrj 52	Polyoxyethylene stearate	3	25.2 ± 3.9	2.94 ±.33	4.86 ±.99

* 50 mμg $Co^{60}B_{12}$ administered orally.
† Undiluted surface active agents were used except Tween 80.

TABLE III. Effects of Salts on Absorption of $Co^{60}B_{12}$.

Study	Vit. B_{12} dose (mμg)	Group	No. of rats	Organ uptake of radioactivity (mμg) Liver	Kidneys
I	50	Control	3	1.54 ± .32	4.60 ± .72
		30% $MgSO_4 \cdot 7H_2O$—1 cc	4	2.14 ± .12	6.47 ±1.08
II	1,000	Control	4	8.0 ±2.4	12.5 ±2.8
		30% $MgSO_4 \cdot 7H_2O$—1 cc	4	17.7 ±2.0	31.5 ±4.5
III	"	Control	5	7.9 ±1.7	24.1 ±3.5
		4% $MgSO_4 \cdot 7H_2O$—1 cc	3	15.0 ±2.5	35.1 ±4.1
		25% $Na_2HPO_4 \cdot 12H_2O$—2 cc	4	17.4 ±1.3	37.8 ±2.1

factants which could enhance absorption was mixed with water in the proportion used, a very thick vaseline-like product was formed. The other surfactants which did not enhance absorption, either did not mix with water or increased fluidity without jelly formation. Thus, such a difference in physical characteristics may have some bearing on ability to enhance Vit. B_{12} absorption by reducing the rate of descent into the intestine.

Rate of passage of $Co^{60}B_{12}$ in digestive tract after administration. A study was designed to measure rate of passage of $Co^{60}B_{12}$ from the stomach in presence or absence of Tween 80. Twelve rats were divided into 2 groups: 6 received 1 cc of undiluted Tween 80 and then 1 cc of $Co^{60}B_{12}$. The other 6 controls received the same volume of water and $Co^{60}B_{12}$. Two rats in each group were sacrificed at 1, 2 and 4 hours after oral administration. The digestive tract was divided into stomach, proximal-, middle- and distal ⅓ of the small intestine, and cecum, and radioactivity in each section measured. It was found that 1 hour after administration, more than 70% of radioactivity was already in the distal ⅓ of the small intestine and only 1% remained in the stomach in control rats; in experimental group, the stomach still retained 53% of radioactivity. This trend of delayed passage of $Co^{60}B_{12}$ in the Tween 80 group was also apparent 2 and 4 hours after administration. The physical state of the mixture containing $Co^{60}B_{12}$ was such that the stomach could not evacuate it as fast as it could when present in solution.

Effect of salt solution on absorption. The effect of co-administration of Vit. B_{12} and a cathartic which may accelerate passage of Vit. B_{12} in the intestine was studied. When 1 cc of 30% $MgSO_4 \cdot 7H_2O$ was fed immediately before oral dose of $Co^{60}B_{12}$, absorption was enhanced (Table III). The effect was even more easily detectable when a larger Vit. B_{12} dose (1000 mμg) was used. When 2 cc of 25% $Na_2HPO_4 \cdot 12H_2O$ was used, absorption was also increased. However, when 1 cc of isotonic solution of $MgSO_4 \cdot 7H_2O$ (4%) was given, the effect was less marked. It was noted that the animals receiving hypertonic solutions of both salts developed mild to moderate diarrhea.

Effect of Tween 80 and magnesium sulfate on intestinal wall. We have recently described a technic using a surgically prepared intestinal loop for assessment of absorption of Vit. B_{12} in the presence of known amounts of intrinsic factor or other substances which influence absorption (5). This procedure was used to study the effect of surfactants and hypertonic salt solutions. In Study A, the effect of Tween 80 solution was compared with that of stomach extract when 50 mμg of $Co^{60}B_{12}$ was administered. The results (Table IV)

TABLE IV. Absorption of Vit. B_{12} as Measured with "Loop" Technic.

Study	B_{12} dose (mμg)	Group	No. of rats	Radioactivity recovered (mμg) Loop	Liver	Kidneys
A	50	Control	4	47.1 ± .11	.96 ± .14	.72 ± .10
		Tween 80 (20%—0.5 cc)	6	49.2 ± .49	.64 ± .11	.24 ± .07
		Stomach extract	5	29.4 ± .88	5.40 ± .40	2.47 ± .46
B	1,000	Control	3	809 ±11	23.9 ±1.7	33.5 ±4.3
		$MgSO_4 \cdot 7H_2O$ (15%—1 cc)	4	941 ±10	6.0 ±1.0	5.2 ±2.1

indicate that absorption by the loop was not enhanced as it was when the whole animals were studied. There was even a slight decrease in absorption by Tween 80, as indicated by kidney uptake of radioactivity. One cc of 15% of $MgSO_4 \cdot 7H_2O$ markedly reduced absorption of the test dose of 1000 mμg (Study B).

Discussion. Absorption of Vit. B_{12} can be enhanced by a large dose of D-sorbitol, but the mechanism is not understood. It is possible that the active agent is some chemical derivative of sorbitol formed in the gastrointestinal tract. For this reason, we have studied the effect of some readily available derivatives of sorbitol with surface activity. It was found that pre-administration of certain but not all of sorbitol containing compounds would increase Vit. B_{12} absorption. This increase is not related to surfactant activity. The 3 tested surfactants with ability to enhance absorption (Tween 80, Tween 85 and G-1096) have the common physical characteristics of becoming a jelly-like mixture when mixed with an equal volume of water. Thus, the observed phenomenon may be due to the physical rather than chemical state. When the effective dose of Tween 80 was pre-diluted with water to a fluid state and then administered, no increase in absorption was observed. The aqueous $Co^{60}B_{12}$ solution will probably become a semi-solid mixture upon contact with one of the effective compounds in the stomach and the speed of descent of $Co^{60}B_{12}$ from the stomach is greatly reduced. Since no correlation was observed between chemical structure and enhancement of absorption, it is presumed that the time during which Vit. B_{12} remains in the stomach might play a dominant role in enhancement of absorption. It is conceivable that the slow passage in the gastrointestinal tract allows maximum time for intrinsic factor in the stomach to react with Vit. B_{12} or a longer reacting time between Vit. B_{12} and the intestinal mucosal receptor. The former mechanism seems unlikely because of the results of the experiment in which $Co^{60}B_{12}$ was allowed to be retained in the stomach for 2 hours by ligation of the pylorus and absorption of such a dose of Vit. B_{12} was not increased by fecal measurement or by organ uptake.

Intestinal absorption is a function of time; therefore, it is likely that absorption of Vit. B_{12} is dependent upon its mobility through the digestive tract. A slower descent of Vit. B_{12} in the intestine will provide a better chance for the receptor to bind with Vit. B_{12}. If this were the only controlling factor, administration of cathartics might have the opposite effect. Contrary to such expectation, absorption of Vit. B_{12} at 1000 mμg dose administered with a hypertonic salt solution resulted in absorption 2 times greater than that of Vit. B_{12} without the salt solution. Since both magnesium sulfate and disodium phosphate exerted a similar effect, the phenomenon may not be limited to these 2 salts. Uptake of $Co^{60}B_{12}$ by resting *Lactobacillus leichmannii* organisms(6) is inhibited by high salt concentrations *in vitro*. Addition of salt would favor intrinsic factor in its competition for Vit. B_{12} binding in the digestive tract with microorganisms. However, absorption of Vit. B_{12} in presence of magnesium sulfate from the small intestine when tested with the "loop" technic was markedly reduced. It is conceivable that absorption of 1000 mμg dose of Vit. B_{12} in presence of hypertonic salts is partly achieved from the cecal wall, and that hypertonicity of the salt administered orally is reduced by the time it reaches the active site of absorption and thus adverse effect of hypertonicity on the intestinal wall is minimized. D-sorbitol in higher concentrations also interferes with absorption of Vit. B_{12} from the small intestine by the "loop" technic(7). Therefore, the effect of hypertonic salt solution on Vit. B_{12} absorption is similar to that of D-sorbitol in that both exert absorption enhancing effect in its high concentrations when administered orally to intact rats and yet both inhibit absorption when applied directly into an isolated loop of the small intestine.

Summary. Two means of enhancing Vit. B_{12} absorption in rats have been described. It was found that certain surface active agents, such as Tween 80, Tween 85 and G-1096, which form a semi-solid matter with an equal volume of water markedly enhanced absorption of $Co^{60}B_{12}$ when fed by stomach tube immediately before oral dose of $Co^{60}B_{12}$. The effect was abolished upon dilution of the agent with water before administration. Oral Vit.

B_{12} preceded by one of these undiluted compounds evacuated from the stomach at a much slower rate than without pre-administration of the compound. Saline cathartics such as hypertonic solutions of magnesium sulfate and disodium phosphate also enhanced absorption of Vit. B_{12} when administered in the same way. However, when tested with "intestinal loop" technic, neither surfactants nor salt solutions were able to substitute for gastric intrinsic factor. The possible mechanisms for the observed phenomenon of enhancement of absorption have been discussed.

1. Chow, B. F., Meier, P., Free, S. M., Jr., *Am. J. Clin. Nutr.*, 1958, v6, 30.

2. Greenberg, S. M., Herndon, J. P., Rice, E. G., Parmelee, E. T., Gulesich, J. J., Van Loan, E. J., *Nature*, 1957, v180, 1401.

3. Von Heinrich, H. C., Skibbe, R., Staak, M., *Z. f. Naturforsch.*, 1959, v14b, 42.

4. Okuda, K., Wider, J. A., Chow, B. F., *J. Lab. and Clin. Med.*, 1959, v54, 535.

5. Okuda, K., *Am. J. Phsyiol.*

6. Davis, R. L., Chow, B. F., *Science*, 1952, v115, 351.

7. Okuda, K., Chow, B. F., *Fed. Proc.*, 1959, v18, 588.

Received December 9, 1959. P.S.E.B.M., 1960, v103.

Plasma Protein VII. Site of Degradation of Serum Albumin.* (25606)

F. B. ARMSTRONG,† S. MARGEN AND H. TARVER
Dept. of Biochemistry, School of Medicine, University of California, San Francisco

We reported(1) inability to find significant breakdown of homologous albumin labeled with C^{14} *in vitro* in any slice or homogenate system prepared from rat tissues, with the exception of spleen homogenate. These results are in accord with those obtained by Katz and Sellers(2), but disagree with those of Roberts and Kelley(3) who reported rapid breakdown of serum albumin in slices prepared from rat livers. However, it is necessary to postulate that breakdown occurs at some site in the animal to account for the rapid turnover of serum albumin observed in numerous investigations *in vivo*(4). It is possible that albumin and perhaps other plasma proteins continually 'leak' into the gastro-intestinal tract where they are hydrolyzed. Cellular barriers are permeable in many cases to plasma proteins. Thus, both albumins and globulins pass rapidly from plasma into lymph in all species which have been investigated(5,6,7); they pass from the circulation into the peritoneal cavity and back(8,9,10) and into milk (11,12). It has also been shown that, in the guinea pig, plasma proteins are transferred across the placenta to the fetus(13), and, as is well known from immunological investigations and recent isotopic work, globulins are absorbed from the gut of newly born animals (14). To demonstrate such a loss of albumin into the gut from the blood stream, 2 types of experimental method were used, (a) the washing method, and (b) the freezing method. In both methods rabbits were first injected with either albumin-I^{131} or iodide-I^{131}. In the washing method, the lumen of an isolated section of the gut was continuously washed for several hours with a dilute albumin solution; in the freezing method an isolated section of gut was injected with an albumin solution; the section was immediately excised and frozen in an acetone-dry ice bath. While still frozen the tissue was removed leaving the core of frozen albumin-containing solution. Distribution of radioactivity in the albumin solutions so obtained was investigated. It is assumed that any radioactive albumin passing from the circulating blood into the gut would be trapped in the large volume of unlabeled albumin solution, and in the short time before enzyme action was stopped with trichloro-

* Aided by grants from Am. Cancer Soc. and U.S.P.H.S.

† Present address: Genetics Foundation, Dept. of Zoology, Univ. of Texas, Austin.