fraction Rl. A significant increase in rate of urine flow in dogs was obtained by intravenous administration of fraction RSl in a dose range of 0.5-10 μg/kg. On a weight basis fraction RSl is much more potent than acetazolamide or chlorothiazide, under the conditions of the assay. Fraction UFO did not result in an increase in urine flow when given in a dose range of 0.5-2 μg/kg. Fraction Rl could not be assayed in the dog because of toxicity, but when administered as a suspension intraperitoneally in doses of 20-25 μg/100 g to the rat it resulted in appreciable increases in rate of urine flow and electrolyte excretion.

1. Little, J. M., *J. Pharmacol. Exp. Therap.*, 1947, v91, 124.

2. ———, *J. Lab. Clin. Med.*, 1952, v39, 518.

3. ———, *Am. J. Physiol.*, 1955, v180, 173.

4. Stephan, F., Jahn, H., Stahl, J., *C. R. Soc. Biol.*, Paris, 1957, v151, 1580.

5. Jahn, H., Stephan, F., Gruner, J., Stahl, J., *C. R. Soc. Biol.*, Paris, 1958, v152, 1798.

6. Jahn, H., Stephan, F., Stahl, J., *Arch. Sci. Physiol.*, 1960, v14, 421.

7. ———, *C. R. Soc. Biol.*, Paris, 1956, v150, 1449.

8. Milies, E., *Acta Physiol. Lat. Am.*, 1960, v10, 178.

9. Boyce, W. H., King, J. S., Jr., Little, J. M., Artom, C., *J. Clin. Invest.*, 1958, v37, 1658.

10. Little, J. M., *An Introduction to the Experimental Method,* Burgess Publishing Co., Minneapolis, Minn., 1961.

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Mucosal Adsorption and Absorption of Vitamin B_{12} in the Intestine of the Rat. (27779)

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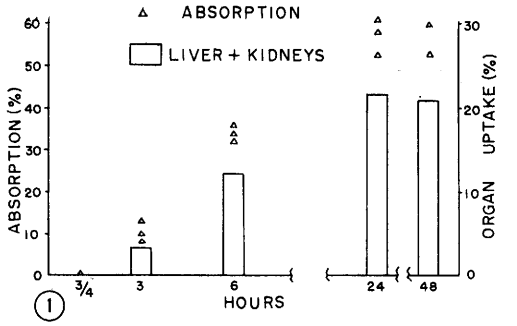
It is established that intrinsic factor (IF) mediates the process of physiological absorption of Vit. B_{12} (B_{12}) in the intestine. Recent *in vitro* studies(1,2) have indicated that IF first brings about adsorption of B_{12} to the mucosal surface with the aid of calcium ion. The subsequent steps for absorption of B_{12} have not yet been elucidated. One of the difficulties in such studies lies in the lack of a means to differentiate surface adsorption and absorption past the mucosal barrier.

The present investigation was undertaken to study quantitatively the adsorption of Co^{60}-labeled cyanocobalamin (Co^{60}-B_{12}) and its transport past the intestinal serosa in relation to time, using the intestinal loop technic(3). This *in vivo* system lends itself to such differential measurement with a functioning bowel and controlled amounts of IF. The results indicate that not all B_{12} molecules that are first adsorbed to the surface pass the mucosal barrier, and that there is a nonspecific adsorption of B_{12} that does not represent the absorptive process.

Methods. Young adult rats of the Wistar strain were used. The preparation of a washed loop with the entire jejunum and ileum by operation, and of rat intrinsic factor concentrate (IFC) from the rat stomach mucosae has been described(3). Such a loop has no more access to endogenous IF. Co^{60}-B_{12} having a specific activity of 1 μc/μg* was applied into the loop with or without IFC, and the animal was killed after certain intervals. The dose of IFC was so chosen as to yield a maximum absorption.

The transserosal transport of Co^{60}-B_{12} is herein referred to as "absorption" and was calculated directly from the difference between the dose given and the radioactivity recovered in the loop including the content, and indirectly from the radioactivity in the liver and kidneys. The adsorption was assessed by washing the loop lumen a short time after administration of the dose and determining the radioactivity that was not washed away.

* Kindly supplied by Dr. K. C. Mezey, Merck, Sharp & Dohme Laboratories, Rahway, N. J.



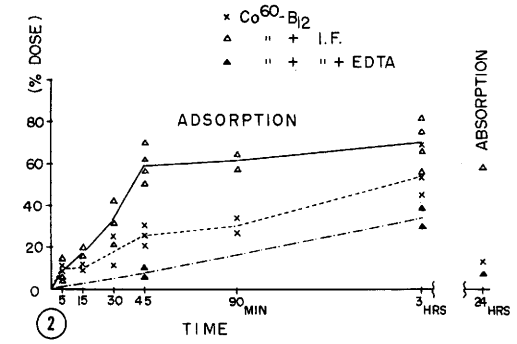


FIG. 1. Transserosal absorption of Co^{60}-B_{12} from the loop in relation to time. The dose consisted of 10 mμg Co^{60}-B_{12} plus IFC capable of binding 15 mμg B_{12}.

FIG. 2. Adsorption of Co^{60}-B_{12} by the loop mucosa in relation to time. For reference, 24 hr absorption is shown on the extreme right. Dose: Co^{60}-B_{12} = 10 mμg, IFC = 15 mμg binding, EDTA = 0.5 ml of 0.1 M solution.

For the washing, the loop was detached intact from the mesentery, untied, and the proximal end was connected to a large syringe. A total of 100 ml warm saline was run through the loop by its own gravity while the loop was hung from the syringe, and the washing was collected for radioactivity measurement. No pressure was applied with the plunger unless it was necessary to start the run. Large mucous flocculi were readily removed under these conditions. The term "adsorption" herein is not in a strict sense, and it includes surface adsorption as well as that portion of Co^{60}-B_{12} which has passed the mucosal barrier but has not left the intestine. In some animals, the loop was washed *in situ* under anesthesia using the same amount of saline to remove unadsorbed Co^{60}-B_{12}, the loop was closed, and the animal was kept alive for the determination of absorption. The radiometric measurement of organs and fluid specimens using a gamma-scintillation counter has been reported(4).

Results. A mixture of 10 mμg Co^{60}-B_{12} and an amount of IFC capable of binding 15 mμg B_{12} was applied into the loop and absorption was measured 3/4, 3, 6, 24 and 48 hours afterward, using 2 to 3 rats in each. The result (Fig. 1) showed that absorption was very small before 3 hours and was still incomplete 6 hours after the administration. Maximum absorption occurred sometime between 6 and 24 hours, and there was no absorption thereafter.

Adsorption was measured at 5, 15, 30, 45, 90 and 180 minutes after administration of the same dose of Co^{60}-B_{12} with or without added IFC. In each group 2 to 4 rats were employed. The data (Fig. 2) indicated that adsorption proceeded quickly in the presence of IFC and that about 60% of the dose was adsorbed to the mucosa in 45 minutes, during which time practically no absorption occurred. There was a further small increase in adsorption afterward. Washing the loop with the same amount of saline containing 5 ml of neutralized 0.1 M disodium ethylenediaminetetraacetate (EDTA) slightly increased the radioactivity washed out. If the washed loop was opened lengthwise and further rinsed for 10 minutes in saline containing 0.05 M EDTA at 4°C, considerably more radioactivity was released. Thus, a large portion of radioactivity measured as "adsorbed" was still outside the mucosal barrier.

Without IFC, adsorption was slow, but it increased gradually to reach a level not much lower than that obtained with IFC in 3 hours. The 24-hour absorption without IFC was about 1/5 of that with IFC. When 0.5 ml of 0.1 M EDTA was added to the dose containing IFC, most of the radioactivity was washed out at 45 minutes, yet some adsorption was measured after 3 hours; absorption after 24 hours was only 4 to 7%.

In the next study, 12 loops were divided into 4 groups of 3 each (Table I). Group A received in the loop the same dose as above, the abdomen was reopened after 3 hours for a sham operation and the animal was sacrificed 24 hours later. In B, the dose was not given at first but the loop was rewashed *in*

TABLE I. Effect of Removal of Unadsorbed Co^{60}-B_{12} from the Loop upon Transserosal Absorption.

Group	0 hr	3 hr		24 hr			
		Treatment	Co^{60} washed out	Transserosal absorption	Co^{60} washed out	Loop	Liver + kidneys
A	Dose*	Sham operation	—	58	23	19	21
B	—	Washed, then dose* given	—	54	28	18	20
C	Dose*	Washed	21	32	34	13	12
D	"	Washed with B_{12}† saline, then IFC instilled	23	30	35	12	12

* 10 mμg Co^{60}-B_{12} + IFC (binding 15 mμg). Figures are % of dose and avg of 3 rats.
† Nonradioactive.

situ with saline 3 hours after its preparation, then the dose was introduced. In C and D, the dose was applied first, the loop was washed *in situ* 3 hours later, and absorption was measured 24 hours afterward. In D, a saline containing unlabeled B_{12} (20 mμg/ml) was used for washing, and then IFC (15 mμg binding) was instilled into the loop, to prevent any conceivable loss of adsorbed Co^{60}-B_{12} due to a negative concentration gradient between the mucosa and lumen. All loops were removed 24 hours later, and radioactivities in the loop and in the washing were determined separately.

The result demonstrated that with the aid of IFC, about 58% of the dose of Co^{60}-B_{12} was absorbed past the serosa in 24 hours. Washing of the loop *in situ* 3 hours after its preparation did not appreciably reduce the capacity of the loop to absorb. Removal of the unadsorbed portion of Co^{60}-B_{12} at 3 hours resulted in about 40% reduction in over-all absorption as compared with that obtained without washing, and such reduction was not prevented by introduction of a second dose of B_{12}.

Discussion. The adsorption study demonstrated that about 60% of Co^{60}-B_{12} administered was adsorbed onto the mucosa with the aid of IFC in 45 minutes, and about 80% in 3 hours. The transserosal absorption measured 24 hours later was approximately 60% and transmucosal absorption was probably in the neighborhood of 70%. The immediate adsorption as well as 24-hour absorption were markedly inhibited by EDTA. It is tempting to postulate that when Co^{60}-B_{12} was adsorbed to the surface in the presence of added IFC, such molecules were already destined to cross the barrier. If all the adsorbed B_{12} molecules were to remain as such before passing the mucosal barrier, washing of the unadsorbed portion at 3 hours as was done here should not affect the over-all absorption. Or, if the unwashable Co^{60} at 3 hours represented completed transmucosal absorption, washing should not affect the absorption measured 24 hours later. The results were otherwise. It seems possible, therefore, that some of the washable Co^{60} at 3 hours was in an absorbable form of B_{12}.

Radioactivity which remained in the intestine after washing under the present conditions was no measure for the transmucosal absorption, insofar as about 50% of Co^{60}-B_{12} given without IFC was found adsorbed after 3 hours. Only 10 to 15% would subsequently be absorbed, therefore a large portion of such adsorption represented an unabsorbable form of Co^{60}. Even if EDTA inhibited adsorption of Co^{60}-B_{12} at the beginning, adsorption later proceeded slowly. This adsorption may be of a similar nature. The Co^{60} which remained with the loop after washing was liberated further if the loop was opened and rinsed. Therefore, some of the adsorption measured with this method was derived from the portion that was loosely attached to the mucosal surface. It may be that there are at least 2 types of surface adherence of B_{12} in the intestine; one is the kind which is mediated by IF and the other, a nonspecific type probably effected by non-IF mucoproteins secreted from the mucosa that also bind B_{12}. Inasmuch as the intestine had been cleaned at the time of dose administration, bacterial breakdown of the vitamin was negligible and most of the ad-

sorbed Co^{60} must have been still in the form of B_{12}.

The intestinal mucosa is such that the free ends of the villi desquamate while new cells regenerate in the crypt at a rapid rate(5). Complete separation of adherent mucus and mucosal debris from the absorptive mucosa seems infeasible even *in vitro* at present. The washing technic employed in this study for adsorption measurement was, admittedly, not ideal, but it was necessary to apply *in situ* and was good enough to demonstrate that in the intestine the bulk of a physiological dose of B_{12} is first adsorbed to the mucosal surface, whether closely or loosely, and that such adsorption is of complex nature. Cooper(6) recently observed, using everted guinea pig intestine and human gastric juice, that part of the radioactive B_{12} that was taken up by the intestine and not washed with saline could be removed further with EDTA. He called it S-E fraction. Whether his fraction is related to the demonstrated nonspecific adsorption remains to be seen.

Summary. Using the rat intestinal loop technic, it was demonstrated that a large part of a physiological dose of Co^{60}-B_{12} was adsorbed to the mucosal surface in the presence of rat intrinsic factor (IF) at a faster rate than it was without IF, and ethylenediaminetetraacetate markedly inhibited this *in vivo* adsorption. The early mucosal adherence of Co^{60}-B_{12} did not represent transmucosal absorption, and transserosal absorption was further delayed. It was concluded that there are at least 2 kinds of surface adsorption; one represents a phase of physiological absorption involving IF, and the other, a nonabsorptive process.

1. Herbert, V., *J. Clin. Invest.,* 1959, v38, 102.
2. Cooper, B. A., Castle, W. B., *ibid.,* 1960, v39, 199.
3. Okuda, K., *Am. J. Physiol.,* 1960, v199, 84.
4. Okuda, K., Wider, J. A., Chow, B. F., *J. Lab. Clin. Med.,* 1959, v54, 535.
5. Leblond, C. P., Messier, B., *Anat. Rec.,* 1958, v132, 247.
6. Cooper, B. A., Paranchych, W., Lowenstein, L., *J. Clin. Invest.,* 1962, v41, 370.

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Induction of Malignancy *in vitro* in Newborn Hamster Kidney Tissue Infected with Simian Vacuolating Virus (SV40). (27780)

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Studies of *in vitro* induction of malignancy by oncogenic viruses have been recently reviewed by Dawe and his associates, and they have described their own experiments with organ cultures of mouse salivary gland infected with polyoma virus(1). Morphologic "transformations" of hamster embryo cell cultures infected with polyoma virus have been reported by several groups(2,3,4) and transplantation of the transformed cells to hamsters has generally resulted in sarcomas at the inoculation sites. In the present studies, cells from monolayer cultures derived from explants of newborn hamster kidney tissue infected with SV40 have produced tumors when transplanted to newborn hamsters. Histologically, these tumors were composed predominantly of undifferentiated carcinoma with areas of adenocarcinoma, sarcoma and well-differentiated epidermoid carcinoma.

Materials and methods. Virus. SV40 virus, strain Vac777 L1 6/14/61, was obtained from Dr. Paul Gerber, Division of Biologics Standards, National Institutes of Health. It was passaged in our laboratory in tube cultures of primary African green monkey kidney cells (GMK) obtained from Microbiological Associates, Bethesda, Md. Preparation of virus pools, limiting dilution titrations, and virus isolations were carried out using methods described previously(5).

Newborn hamster tissues. Newborn Syrian