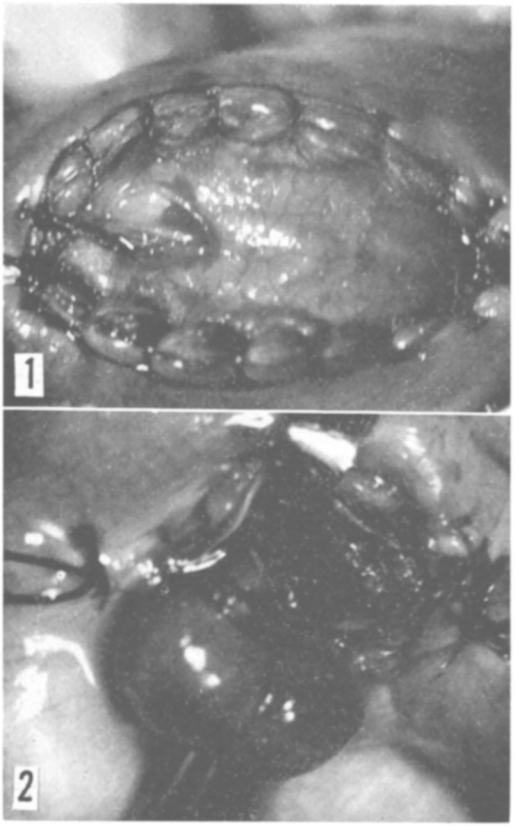

FIG. 1. Illustration of the manner in which fetal skin is sutured to fetal membranes and uterine wall to exclude the amnionic cavity from the operative site in the fetus.

FIG. 2. Mobilization and excision of the fetal kidney.

TABLE II. Creatinine Concentration in Maternal and Fetal Sera of Normal Humans and Monkeys. Creatinine Conc. mg %

	Maternal	Fetal
Man*	.99 (35)	1.02 (23)
Monkeys	1.05 (4)	.98 (4)

* McGaughey *et al.* (*Am. J. Obst. & Gynec.*, v80, 108, 1960)

( ) No. of samples.

Summary and conclusions. 1. Concentrations of creatinine in maternal and fetal sera of the monkey are within the range considered normal for pregnant humans. 2. Concentrations of creatinine in maternal and fetal sera remain in equilibrium and within this normal range 3 and 4 weeks after bilateral fetal nephrectomy. 3. It is not necessary to postulate renal excretion of creatinine into the amniotic fluid and subsequent absorption or diffusion by the chorioamniotic membrane to account for creatinine equilibrium between fetus and mother. 4. It is suggested that the placenta, rather than the fetal kidneys, is important in maintaining the composition of fetal serum.

1. Plentl, A. A., Hutchinson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 681.

2. Friedman, E. A., Gray, M. J., Hutchinson, D. L., Plentl, A. A., *J. Clin. Invest.*, 1959, v38, 961.

3. Widdas, W. F., *Brit. Med. Bull.*, 1961, v17, 107.

4. McGaughey, A. S., Corey, E. L., Scoggin, W. A., Ficklen, C. H., Thornton, W. N., *Am. J. Obst. and Gynec.*, 1960, v80, 108.

5. Clark, L. C., Thompson, H. L., *Anal. Chem.*, 1949, v21, 1218.

Received April 26, 1962. P.S.E.B.M., 1962, v110.

Intestinal Absorption of 5,6-Dimethylbenzimidazolyl Cobamide Coenzyme in the Rat.* (27530)

KUNIO OKUDA AND VICTOR TANTENGCO

Department of Medicine, Yamaguchi Medical College, Ube, Japan and Department of Physiological Hygiene and Nutrition, Institute of Hygiene, University of the Philippines, Manila, Philippines

Since the discovery of the coenzyme of vit. B_{12}(1,2), much interest has been aroused as to whether or not previous information obtained with cyanocobalamin is applicable to the metabolism of this naturally occurring

* This study was carried out in the Dept. of Biochemistry, Johns Hopkins School of Hygiene and Public Health. The author is indebted to Dr. B. F. Chow for his interest and to Miss Asuncion Gabriel for technical assistance.

form. An early report(3) suggested that intestinal absorption of 5,6-dimethylbenzimidazolyl cobamide (referred to as coenzyme) was less dependent upon intrinsic factor (IF). However, Lee and Glass(4) indicated that absorption of coenzyme with the aid of IF preparations was smaller than that of cyanocobalamin in pernicious anemia. According to Okuda and Chow(5), urinary excretion of radioactivity in Schilling test following oral administration of radioactive coenzyme was about one-half that expected with cyanocobalamin. Although tissue distribution of the coenzyme was shown to be different from that of cyanocobalamin(5,6,7), it is not understood whether the smaller urinary excretion in Schilling test with coenzyme was accounted for solely by its tighter binding by tissues or partly by poorer absorbability. To answer some of these questions, absorption of radioactive coenzyme was determined and compared with that of cyanocobalamin under various conditions in rats. This paper reports the results demonstrating that the coenzyme is very similar to cyanocobalamin quantitatively in intestinal absorption as well as in its requirement for IF.

Materials and methods. Aqueous solutions of Co^{60}-cyanocobalamin† and Co^{58}-coenzyme,‡ both having a specific activity of 0.5 to 1.0 $\mu c/\mu g$, were used in young adult rats. Extreme precaution was exercised to avoid light in handling the latter. Immediately after each experiment, the coenzyme solution used was subjected to microbiological assay for vit. B_{12} activity and for the coenzyme activity(2) to make certain that the coenzyme was still in its original form.

Fecal test for absorption. Rats were fasted overnight, then fed by a stomach tube 1 ml of radioactive cyanocobalamin or coenzyme. Three levels of 10, 50 and 2,600 mμg were used; the first 2 were considered to be physiological and the last, supraphysiological. Absorption was estimated from the fecal recovery and/or organ uptake of radioactivity. Feces were collected for 4 days in metabolism cages when the smaller doses were used, and the animals were then sacrificed. No fecal collection was made when 2,600 mμg was given because of inaccuracy of this test for large doses, and animals were sacrificed 24 hours later for measurement of organ radioactivity. The technic for determination of radio activity in feces, urine and organs has been described(8).

Urinary excretion test. The fecal test was combined with the urinary excretion test in some experiments. An intraperitoneal injection of 50 μg of unlabeled cyanocobalamin or coenzyme was given for the purpose of flushing 2 hours after the oral dose of 50 mμg of the radioactive material. The same flushing procedure was repeated 24 hours after the first flushing. Urine was collected for the first and second 24-hour periods following the oral dose and radioactivities were measured.

Intestinal loop technic. The effect of IF on absorption was studied with this method (9). Radioactive material with or without added IF was injected into the small intestine which had previously been washed free of IF and closed *in situ* to form a loop. The absorption was estimated from the decrease of radioactivity in the loop and from organ uptake of radioactivity 24 hours later. As the source of IF, a saline extract of the stomach mucosae of normal rats was prepared according to the technic previously described(9).

Results. Coenzyme vs. *cyanocobalamin in absorption.* Intestinal absorption following a single oral dose was measured from the fecal recovery and/or organ uptake of radioactivity (Table I). No significant difference was found between the 2 compounds with the 10 and 50 mμg doses. However, with 50 mμg the hepatic uptake of radioactivity was significantly higher in the coenzyme groups than in the groups receiving cyanocobalamin. It seems that the coenzyme had a greater affinity for the liver under these conditions. The radioactivity in the liver and kidneys combined following an oral dose of 2,600 mμg showed a smaller average of absorption for the coenzyme, but the difference was statistically insignificant because of large variations.

Absorption of mixture of coenzyme and cyanocobalamin. To investigate whether one

† Kindly supplied by Merck & Co.

‡ Kindly supplied by E. R. Squibb & Sons.

TABLE I. Intestinal Absorption of Coenzyme and Cyanocobalamin in Rats.

Oral dose (mμg)	Compound	No. of rats	Absorption by fecal test (mμg)	Organ uptake of radioactivity in mμg	
				Liver	Kidneys
10	Cyano.	6	5.8 ± .5	.51 ± .05	1.35 ± .16
	Coenzyme	6	4.0 ± .5	.43 ± .06	1.13 ± .20
50	Cyano.	6	15.1 ± 1.8	1.49 ± .17	2.86 ± .65
	Coenzyme	10	15.4 ± 1.6	2.19 ± .17	3.54 ± .40
50	Cyano.	6	27.8 ± 1.8	2.07 ± .24	4.73 ± .45
	Coenzyme	6	24.7 ± 2.2	4.48 ± .56	4.12 ± .21
2,600	Cyano.	5		69 ± 25.7*	
	Coenzyme	5		40 ± 10.3*	

Figures are mean ± standard error.
* The liver and kidneys were combined.

of the 2 compounds is preferentially absorbed when both are present in the gut, the following experiment was carried out. Radioactive coenzyme or cyanocobalamin was mixed with a larger dose of the same or the other compound which was not labeled, and administered. If preferential absorption occurred, it would be indicated by a difference in absorption of radioactive compound, assuming that the intestinal mucosa has a limited capacity for absorption of vit. B_{12} and its analogues. In Study A (Table II), 20 mμg of radioactive coenzyme was mixed with 80 mμg of unlabeled coenzyme or cyanocobalamin and absorption was measured by the fecal test. No significant difference in absorption was obtained. In Study B, the same amount of radioactive cyanocobalamin was mixed with 980 mμg of unlabeled cyanocobalamin or coenzyme. Again, there was no significant difference in absorption. These results suggest that preferential absorption of one compound over the other in the intestine is unlikely.

Urinary excretion after flushing. Urinary excretion test carried out in combination with the fecal test demonstrated that, although the absorption measured by the latter was about the same, urinary excretion following oral administration of 50 mμg of radioactive coenzyme was significantly lower than that obtained with radioactive cyanocobalamin. Therefore, the extent to which coenzyme is flushed out in the urine seems to be smaller than cyanocobalamin.

Effect of IF on absorption. To determine whether coenzyme requires IF for normal absorption, the "intestinal loop" was prepared in rats and one of the following solutions was applied: (a) radioactive cyanocobalamin alone, (b) same mixed with rat stomach extract containing IF activity equivalent to 100 mμg B_{12} binding power, (c) radioactive coenzyme, and (d) same mixed with stomach extract. The amount of radioactive material used was 50 mμg. The results (Table IV) clearly demonstrated that addition of rat stomach extract markedly increased the absorption about equally, of both compounds.

Discussion. Intestinal absorption of orally administered coenzyme was about the same

TABLE II. Absorption of Mixed Doses of Coenzyme and Cyanocobalamin.

Study	Materials mixed		No. of rats	Absorption of radioactivity (% of dose)	Uptake of radioactivity by liver and kidneys (%)
A	Ratioactive coenzyme 20 mμg	+ Unlabeled coenzyme 80 mμg	4	38 ± 5.5	11.0 ± 1.8
	"	+ Unlabeled cyano. 80 mμg	4	30 ± 5.8	10.2 ± 3.2
B	Radioactivity cyano. 20 mμg	+ Unlabeled cyano. 980 mμg	5	22 ± 2.7	
	"	+ Unlabeled coenzyme 980 mμg	5	19 ± 1.8	

TABLE III. Urinary Excretion Test for Coenzyme and Cyanocobalamin in Rats.

Oral dose (50 mμg)	No. of rats	Flushing material (50 μg)	Absorption by fecal test (% of dose)	Urinary excretion in 48 hr (% of dose)
Cyanocobalamin	6	Cyano.	47.4 ± 2.2	10.4 ± 2.1
Coenzyme	6	Coenzyme	43.8 ± 2.5	7.5 ± 1.8
"	6	Cyano.	41.0 ± 1.0	5.7 ± 1.1

as that of cyanocobalamin when measured by the fecal test. In more instances, absorption of the former was slightly less than the latter, but the difference was never large enough to be significant. Using an early preparation of coenzyme, we had obtained a result suggesting a smaller absorption(5). The microbiological activity of this particular preparation was found later to be much higher than we had been informed; therefore, our earlier impression of poor absorbability of coenzyme in the rat must be corrected.

The absorption of 50 mμg of coenzyme from the intestinal loop which was devoid of IF was small and addition of a rat stomach extract enhanced absorption remarkably. Thus, absorption of physiological doses of coenzyme is aided by IF like cyanocobalamin. Our human study(6) also demonstrated that coadministration of hog IF preparations enhanced absorption of radioactive coenzyme in pernicious anemia patients as determined by Schilling test. When the 2 compounds were mixed and administered in the present study, no indication was obtained for a preferential absorption of one over the other. These findings all seem to suggest that coenzyme and cyanocobalamin behave similarly, although a slight quantitative difference may exist. Since the basic structure of the coenzyme and vit. B_{12} analogues is the same(10), such a similarity is not surprising. It is likely that the active sites of the molecule for binding with IF and for absorption are shared by coenzyme and cyanocobalamin.

The reported smaller urinary excretion of radioactive coenzyme in human Schilling test (5) may be due either to an actual quantitative difference in absorption or to a different rate of urinary excretion effected by the "flushing". The urinary excretion test in rats was designed to elucidate the latter possibility. The result showed that coenzyme tended to be flushed into the urine to a lesser extent than cyanocobalamin. Our earlier study(5) demonstrated that the liver retained a greater portion of a parenteral dose of coenzyme in comparison with cyanocobalamin in rats. Such a difference in tissue retention may be responsible for the demonstrated difference in the flushing effect. No marked difference was found between the two compounds in ability to flush radioactive coenzyme. Whether or not the coenzyme is completely converted to hydroxocobalamin in the gut before being absorbed awaits further elucidation.

Summary. Intestinal absorption of Co^{58}-labeled vit. B_{12} coenzyme (5,6-dimethylbenzimidazolyl cobamide) was studied in comparison with Co^{60}-cyanocobalamin in rats. The results indicate that (a) quantitatively, absorption of orally administered coenzyme is about the same as that of cyanocobalamin. (b) Absorption of the coenzyme from the small intestine as determined by the "loop" technic is markedly enhanced by rat intrinsic factor and to the same extent as with cyanocobalamin. (c) There is no demonstrable preference in absorption between the 2 com-

TABLE IV. Absorption of Coenzyme and Cyanocobalamin from Intestinal Loop.

Material applied into loop	No. of loops	Absorption from loop	Organ uptake of radioactivity (mμg)	
			Liver	Kidneys
Cyano. 50 mμg	6	4.8 ± .45	1.31 ± .21	1.02 ± .12
" + IF*	6	23.9 ± .96	3.48 ± .42	5.64 ± 82
Coenzyme 50 mμg	4	7.7 ± 1.83	2.12 ± .30	2.06 ± .25
" + IF*	4	20.8 ± .88	4.07 ± .27	6.72 ± .60

* Saline extract of rat stomach mucosa containing vit. B_{12} binding capacity of 100 mμg.

pounds when both are mixed and coadministered. (d) Slightly less of the absorbed radioactive coenzyme is flushed out in the urine by a large parenteral dose of unlabeled compound as compared with radioactive cyanocobalamin. These findings show that coenzyme behaves much like cyanocobalamin in intestinal absorption, quantitatively as well as in mechanism.

1. Weissbach, H., Toohey, J., Barker, H. A., *Proc. Nat. Acad. Sci.*, 1959, v45, 521.

2. Barker, H. A., Smyth, R. D., Weissbach, H., Munch-Petersen, A., Toohey, J., Ladd, J. N., Volcani, B. E., Wilson, M., *J. Biol. Chem.*, 1960, v235, 181.

3. Wasserman, L. R., Ester, S., Brody, E., Herbert, V., *Lancet*, 1960, v1, 173.

4. Lee, D. H., Glass, G. B. J., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 293.

5. Okuda, K., Chow, B. F., *Fed. Proc.*, 1961, v19, 417.

6. Wong, V., Yeh, S. D., Chow, B. F., *ibid.*, 1961, v19, 415.

7. Uchino, H., Ukyo, N., Yagiri, Y., Wakisaka, K., *Vitamins*, 1962, v25, 190.

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

9. Okuda, K., *Am. J. Physiol.*, 1960, v199, 84.

10. Barker, H. A., *Fed. Proc.*, 1961, v20, 956.

Received April 30, 1962. P.S.E.B.M., 1962, v110.

Clinical Studies of Long-Term Estrogen Therapy in Men with Myocardial Infarction. (27531)

JESSIE MARMORSTON, FREDERICK J. MOORE, CARL E. HOPKINS, OLIVER T. KUZMA AND JOHN WEINER*

Departments of Medicine and Public Health, University of Southern California School of Medicine, Los Angeles County Hospital, and Cedars of Lebanon Hospital, Los Angeles

Patients with atherosclerotic heart disease often manifest abnormal blood-fat patterns which most clinicians believe are causally related to the disease(1-5). Previous investigations by the authors and others indicate that administration of certain estrogens, *e.g.*, Lynoral (ethinyl estradiol) and Anvene (mytatrienediol), cause a shift toward normal of these blood-fat patterns(6-20). The present study was designed(1) to determine whether administration of moderate doses of estrogen does in fact prevent subsequent infarctions and thus improve survival in men patients who previously have suffered at least one myocardial infarction, and (2) to evaluate the efficacy of 3 estrogen preparations, Anvene, Lynoral (ethinyl estradiol), and Premarin (mixed, conjugated equine estrogens) for this purpose.

This report summarizes our findings in the first 5 years of this study and calls attention to the unexpected finding that, in the doses used, preparations which correct abnormal blood-fat patterns (*i.e.*, Lynoral and Anvene) apparently do not increase survival in men, whereas Premarin increases survival but has no significant effect on serum lipids.

We have reported elsewhere(21) that Pre-

* Aided by grants from the Albert and Mary Lasker Foundation and from Nat. Heart Inst. We are indebted to Dr. Kenneth Ericson, Organon, Inc., for Lynoral (ethinyl estradiol), to Dr. Irwin C. Winter, G. D. Searle and Co. for Anvene (mytatrienediol, SC 6924: methoxy-16oc-methyl-1,3,5 (10)-estratriene-16B, 17B-diol) and to Dr. John J. Jewell, Ayerst Laboratories for Premarin (mixed, conjugated equine estrogens, NNR). Statistical analyses were greatly aided by the facilities of the Western Data Processing Center. Dr. W. J. Dixon provided valued statistical advice. Laboratory determinations were made under the direction of Drs. Ross Jacobs and Sara M. Myers. Clinicians participating in these studies were Drs. Ralph Alexander, Richard Anderson, Jacob Bernstein, Edmund Chesne, Richard Cohen, William Corey, Richard Coskey, Donald Feinstein, Irving Gordon, William Grant, Martin Haet, George Irwin, Harold Karpman, Albert Katz, Michael Laks, Jack Lewis, Fletcher McBroom, Irwin Miller, Fredric Mintz, Howard Rubenstein, Samuel Stone, Lailee Tecimer, Joseph Walker, Albert White, Samuel Wirtschafter, Milford Wyman and Dixon Young.