was produced by mouse pituitary explants even after addition of growing hypothalamic tissue. Since the assay procedures employed would have detected 3 mU of thyrotropin, less than 4% of the original thyrotropin content of the explants was produced per week.

1. Guillemin, R., and Rosenberg, B., *Endocrinol.*, 1955, v57, 599.

2. Harris, G. W., *Neural Control of the Pituitary Gland,* Williams & Wilkins, Baltimore, 1955.

3. a. Greer, M. A., *J. Clin. Endocrinol.*, 1952 v12, 1259.

b. Scow, R. O., and Greer, M. A., *ibid.*, 1953, v13, 855.

4. Bogdanove, E. M., and Halmi, N. S., *Endocrinol.*, 1953, v53, 274.

5. D'Angelo, S. A., and Traum, R. E., *ibid.*, 1956, v59, 593.

6. Morgan, J. F., Morton, H. J., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.

7. Hodge, J. R., *J. Endocrinol.*, 1955, v12, 152.

8. Florsheim, W. H., Moskowitz, N., Schwartz, J. R., and Morton, M. E., *Endocrinol.*, 1957, v60, 683.

9. Gilliland, I. C., and Strudwick, J. I., *Clin. Sci.*, 1953, v12, 265.

10. Bates, R. W., and Cornfield, J., *Endocrinol.*, 1957, v60, 225.

11. Greenspan, F. S., Kriss, J. P., Moses, L. E., and Lew, W., *ibid.*, 1956, v58, 767.

12. Emmens, C. W., *Principles of Biological Assay,* Chapman and Hall, London, 1948.

Received May 6, 1957. P.S.E.B.M., 1957, v95.

Alteration of Hepatic Storage of Radiolabeled Vit. B_{12}.* (23322)

ALFRED DOSCHERHOLMEN AND PAUL S. HAGEN
(With technical assistance of Margaret Liu)
Medical Service, Veterans Administration Hospital and Department of Medicine, University of Minnesota Medical School, Minneapolis

The liver is the most important storage organ for vit. B_{12}. After oral or parenteral administration of radiocobaltlabeled cyanocobalamin, surface counting over various sites has shown that the liver accumulates most of the radioactivity(1-4). The liver also retains the radioactivity with great avidity. Indeed, from 86 to 94% of the maximal activity was found after 3 months(1), and 50% after one year(5). Moreover, the plateaus of liver radioactivity in 4 control subjects were unaffected by single, intramuscular doses of 1 mg of "cold" vit. B_{12} given on the seventh day (6).

The objective of the present investigation was to observe the effect of a large series of 1 mg parenteral doses of "cold" vit. B_{12} on hepatic storage of radioactivity after orally administered radiolabeled cyanocobalamin.

Materials and methods. The Co^{60} labeled vit. B_{12} used in these experiments had a specific activity of 1082 μc per mg. A test dose of 0.46 μg vitamin containing 0.5 μc Co^{60} was administered to 2 healthy female volunteers after an overnight fast in approximately 100 ml of water and breakfast was withheld for 2 hours. Plasma radioactivity(7) as well as a 24 hour urinary excretion study(8) started at 24 hours showed that they both absorbed the vitamin normally. External monitoring over the organ areas was carried out according to the method of Glass(1) employing a NaI (thallium activated) probe scintillation counter with a crystal 1½ inches in diameter and 1 inch thick and with an aperture of 2½ inches in diameter. Uptake of radioactivity over the liver, spleen and precordium was determined 9-13 days after administration of the test dose and at intervals up to 7½ months after the tests. The radioactivities recorded between one and 2 weeks after the tests were taken as the 100% values. One of the subjects was given a series of one hundred 1 mg parenteral injections of nonradioactive cyanocobalamin over the ensuing 7 months, while the other subject served as a control. Twenty-four hour urine collections were made

* This investigation was supported by grant from Eli Lilly and Co. to the University of Minnesota.

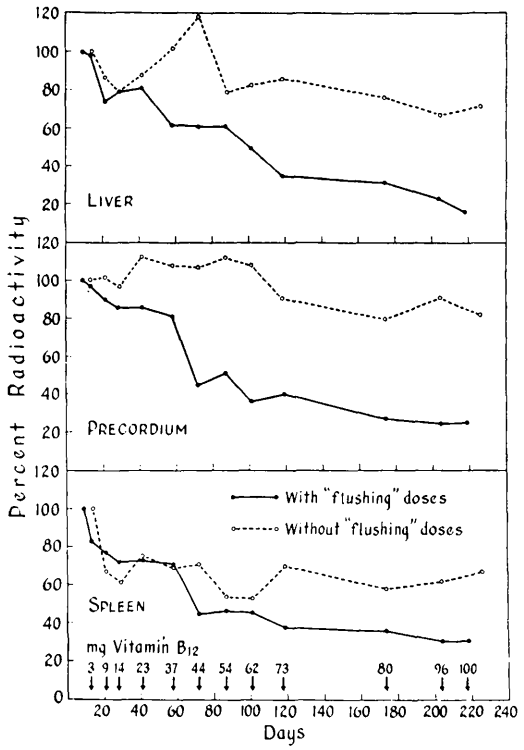

FIG. 1. Effect of parenteral injections of vit. B_{12} on body stores of radioactive cyanocobalamin. Net counts/min. for 100% values were 905, 599, and 421 for the liver, precordium and spleen, respectively, in the exp. subject. Comparable figures for control subject were 507, 382, and 295. Cumulative amounts of parenteral "cold" vit. B_{12} are shown at bottom of graph.

at intervals and the amounts of radioactivity excreted were determined according to a previously described technic(7).

Results. Fig. 1 shows that repeated 1 mg parenteral doses of "cold" vit. B_{12} caused a marked increase in the rate of disappearance of radioactivity over the liver as well as over the precordium and spleen. The radioactivity over the liver declined to 49.5% 101 days after administration of the test when 62 mg cyanocobalamin had been administered, and to 15.8% 218 days after the test when 100 mg had been given. In the control subject liver radioactivity amounted to 82.6% and 71.4% on the 101st day and the 226th day after the test.

Table I summarizes the amounts of urinary radioactivity in each subject. The control subject did not excrete measurable radioactivity 10 days after the single injection of

TABLE I. Effect of Repeated Intramuscular Injections of "Cold" Vit. B_{12} on Urinary Excretion of Radioactivity.

Urinary collection started at:	24 hr urinary radioactivity, % of oral test dose: Exp. subject	Control subject
24 hr	17.7*	4.9*
8 days	0 †	
9 "	.7*	
10 "		0 †
21 "	.5*	
30 "	.4*	
186 "	.3*	

* Vit. B_{12} parenterally immediately before start of urinary collection.
† No vit. B_{12} parenterally.

cyanocobalamin which she received. After the parenteral vit. B_{12} injections the experimental subject had definite radioactivity in the urine each time it was examined. The amount excreted was 0.7% of the administered dose at the start of the injections and decreased to 0.3% toward the end.

Discussion. The results of this study show that the radioactivity stored in the liver can be reduced by a series of parenteral injections of vit. B_{12}. Because of a simultaneous decline in the radioactivity over the spleen and the precordium, it was thought unlikely that the loss of hepatic radioactivity was due to an accumulation of radioactivity in other organs. The finding of radioactivity in the urine showed that the kidneys were at least one route of loss from the body. A rough estimation indicated that about 40% of the test dose was excreted in the urine during the series of injections which were started the ninth day after administration of the oral test. No attempts were made to prove that the radioactivity was lost in the form of cyanocobalamin, although this has been contended for the urinary radioactivity in the usual Schilling test(9). The findings in this study are in agreement with Schloesser and Schilling's report of a break in the disappearance curve for hepatic radioactivity effected by a few parenteral injections of vit. B_{12}(5).

Although the hepatic radioactivity in the control subject declined slower than in the experimental subject, the rate was still faster than expected from the physical decay of Co^{60} alone. This is in agreement with previous ob-

servations(1,5), and indicates a release of stored radioactivity. Assuming that the stored radioactivity represents radioactive cyanocobalamin(4) and with a normal intake and absorption of vit. B_{12} this finding suggests an exchange between stored cyanocobalamin in the liver and the rest of the body. Such turnover can be enhanced by parenteral injections of large amounts of vit. B_{12}. The loss of the vitamin in the urine can be explained by the assumption that all available binding capacity was exceeded whereby the free cyanocobalamin was excreted by the kidneys.

Summary. Radioactivity measurements were made over the liver, spleen and precordium for 7½ months after oral administration of test doses of radioactive vit. B_{12}. One subject was given a large series of parenteral injections of nonradioactive vit. B_{12}, while another subject served as a control. The disappearance rate of radioactivity was much faster with the repeated parenteral injections. Radioactivity was lost in the urine. These observations showed that massive parenteral doses of vit. B_{12} exchanged with cyanocobalamin stored in the liver.

1. Glass, G. B. J., Boyd, L. J., Gellin, G. A., and Stephanson, L., *Arch. Biochem. Biophys.*, 1954, v51, 251.

2. Glass, G. B. J., Boyd, L. J., and Gellin, G. A., *Blood,* 1955, v10, 95.

3. Booth, C. C., and Mollin, D. L., *Brit. J. Haemat.*, 1956, v2, 223.

4. Meyer, L. M., Berlin, N. I., Jiminez-Casado, M., and Arkun, S. N., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 129.

5. Schloesser, L. L., and Schilling, R. F., VIth Cong. of Internat. Soc. of Hematol., Boston, Mass., 1956.

6. Meyer, L. M., Arkun, S. N., and Jiminez-Casado, M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 515.

7. Doscherholmen, A., and Hagen, P. S., *Blood,* 1957, v12, 336.

8. Schilling, R. F., *J. Lab. and Clin. Med.*, 1953, v42, 860.

9. MacLean, L. D., and Bloch, H. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 171.

Received May 6, 1957. P.S.E.B.M., 1957, v95.

Preparation and Some Properties of *Neisseria gonorrhoeae* Endotoxin.* (23323)

HENRY TAUBER AND WARFIELD GARSON
(With technical assistance of C. S. Adams and D. L. Johnson)
Venereal Disease Experimental Laboratory, U.S.P.H.S., School of Public Health, University of N. Carolina, Chapel Hill.

In the present paper a rapid and simple method for the preparation of a stable fraction containing dry, soluble endotoxin from dry gonococcus cells is described. This material offers a fairly toxic product suitable for *in vivo* and *in vitro* studies. It has been observed that washed gonococcus cells dried over sodium hydroxide *in vacuo* dissolve readily at pH 13. Under these conditions presumably all of the cell-bound endotoxin is freed. Other investigators(1,2) have previously used a much less alkaline pH for the slow extraction of cell-bound endotoxin. Under those conditions only a small portion of the endotoxin is obtained. The gonococcus in these studies was grown in an atmosphere of air using a modified liquid medium which was found to be suitable for the large scale cultivation of the organism.

Materials and methods. Culturing methods. Six different strains of gonococcus were used in this study. Five were isolated from patients by Dr. James D. Thayer of this laboratory, one strain was obtained from the American Type Culture Collection (No. 9827). The strains were maintained on dextrose starch agar (Difco) supplemented with 0.1% yeast extract and 1% nutrient agar

* Presented before the Am. Assn. of Immunologists, Chicago, Ill., Apr., 1957.