appearance outside the epithelial cells of bile ducts and there is a disappearance of schizogony. Further study is necessary to determine whether the increase in liver metabolic activity is due to a change in the parasite or whether the parasite change results from a metabolic alteration in the host. In a few experiments, the medium was enriched with adenosine triphosphate; this addition neither accentuated nor diminished the oxygen uptake.

Infection appeared to have little effect upon percentage of protein (nitrogen x 6.25) but percentage dry weight of infected livers was very significantly lower ($P<.01$) than that of the non-infected. This is in agreement with findings of Smith and McShan(6). These authors suggested that decrease in dry weight percentage was due to liver edema. Although total liver weights were not obtained in our experiments, an 8 to 9% decrease in dry weight (Table I) accompanied by 1 to 2% decrease in protein would imply either a considerable increase in total protein or a decrease of some other component. Since rabbit liver contains very little fat(7), glycogen depletion seems to be a logical explanation. Our oxygen uptake findings tend to confirm this possibility.

*Summary.* Oxygen uptake of liver slices from rabbits infected with *Eimeria stiedae* was measured at various times after infection with a sublethal number of organisms. Oxygen uptakes of slices from non-infected litter mates were determined concurrently. Measurements were made in a medium consisting of Krebs-Ringer phosphate buffer at pH 7.4 in a water bath at 38°C. Oxygen uptake of infected livers was significantly greater than that of non-infected controls. There was a significant decrease in percentage dry weight of infected livers while percentage protein was little changed.

1. Becker, E. R., Dilworth, R. I., *J. Infectious Diseases*, 1941, v85, 230.

2. Becker, E. R., PROC. SOC. EXP. BIOL. AND MED., 1941, v46, 494.

3. Becker, E. R., Smith, L., *Iowa State College J. Sci.*, 1942, v16, 237.

4. Becker, E. R., Manresa, F., Smith, L., *ibid.*, 1943, v17, 431.

5. vonBrand, T., *Chemical Physiology of Endoparasitic Animals*, 1952, Part III. Academic Press, Inc., N. Y.

6. Smith, B. F., McShan, W. H., *Annals of N. Y. Acad. Sci.*, 1949, v52, 496.

7. Spector, W. S., *Handbook of Biological Data*, 1956, p74, W. B. Saunders Co., Philadelphia.

Received March 25, 1958. P.S.E.B.M., 1958, v98.

## Differential Excretion of Cobalt-containing Products Following Administration of $Co^{60}$-labelled Vit. $B_{12}$ to Rats. (23982)

S. WIDER,* J. A. WIDER AND E. P. REINEKE

*Department of Physiology and Pharmacology, Michigan State University, East Lansing*

Radioactivity excreted *via* the urinary tract after administration of $Co^{60}$-labelled Vit. $B_{12}$ has been demonstrated by microbial and radiometric(1), chromatographic(2), and extraction(3) studies to be the intact vitamin. While similar studies conducted on feces, following both oral and parenteral administration of radioactive vitamin, reveal a considerable amount of ionic $Co^{60}$, the results have been attributed(1) to microbial action on the unabsorbed vitamin as it passes through the intestine.

Our data show that after parenteral administration of radioactive Vit. $B_{12}$ there is a differential excretion pattern between urine and feces that indicates alteration of the vitamin prior to its entry into the intestine. Neither sex nor testosterone propionate administration influences excretion of the radioactive substance.

*Methods.* A. Four male litters born to mothers maintained during pregnancy and lactation on a diet low in Vit. $B_{12}$, were used.

* Present address: Medical School, University of Basel, Switzerland.

From day of birth until 24 days of age, one-half of each litter (controls) received daily subcutaneous injections of 0.02 cc of cottonseed oil, whereas their littermates received a subcutaneous injection of 1 mg testosterone propionate† daily. From the 24th day of age until termination of experiment, controls received 0.06 cc of cottonseed oil and the testosterone-treated littermates received 3 mg of the androgen. All injections were administered with syringe controlled by a micrometer. When 30 days old, both groups received a subcutaneous injection of 0.25 $\mu$g radioactive Vit. $B_{12}$.‡ The specific activity of this Cobalt$^{60}$-labelled preparation was 824 $\mu$c/mg. Under the maintained injection technic and counting conditions, the total radioactivity administered was equivalent to 2,566 cpm $\pm$ 0.9%. Animals were weaned at 28 days of age and housed in individual cages. Immediately following administration of radioactive vitamin, each animal was placed in a similar metal cage to which absorbent paper had been affixed in such manner as to catch urine and feces. The wire-mesh bottom of the cage was siliconed. The paper, containing the 24-hour urine sample, was changed daily. To avoid reciprocal radioactive contamination of excretion products, the feces were removed every 4 hours. Two of 4 litters received no further treatment following injection of tagged vitamin while the other 2 litters, beginning with the third day subsequent to radioactive injection, received a subcutaneous dose of 16.6 $\mu$g stable Vit. $B_{12}$§ throughout the remainder of the 10-day collection period. Each absorbent paper was burned and quantitatively transferred to a counting planchet. To ascertain percent recovery of radioactivity by this method, 0.017 cc of $Co^{60}$-Vit. $B_{12}$ was delivered to a planchet containing 2 cc urine, while a similar preparation was transferred to absorbent paper. The former preparation was dried; the latter was burned and quantitatively transferred to a planchet. Upon determination of radioactivity in each preparation, an average of 5 runs yielded 226 $\pm$ 2 cpm and 228 $\pm$ 2 cpm, respectively. Each 24-hour feces sample was counted separately from the urine sample. Radioactivity was determined with a scintillation detector (NaI crystal) encased in a lead shield and connected to a laboratory scaler. B. Individual 24-hour excretions of radioactivity in composite feces-urine samples of 7 females were compared with individual excretions of 8 males. To note whether there was an effect of exogenous androgen on either sex, the radioactive excretions of 7 androgen-treated females and 10 androgen-treated males were collected for comparison with radioactive excretions of control animals.

*Results.* A. Fecal excretion (Table I) following a subcutaneous injection of 0.25 $\mu$g of tagged Vit. $B_{12}$ was low in the first 24-hour collection and increased to 3rd day, at which time there was a decrease in radioactive excretion. The urinary radioactivity was extremely high in the first 8-hour collection and then decreased markedly. Administration of unlabelled vitamin to animals previously injected with cobalt-tagged vitamin, was followed within 24 hours by approximately 6-fold increase in urinary radioactivity, above the level of animals not receiving the stable vitamin. An elevated level was maintained throughout additional 48-hour collection period. There was little or no effect on fecal excretion. The androgen (Table II) did not significantly affect either fecal or urinary radioactivity.

B. Sex had no significant effect on the 24-hour composite fecal-urine excretion of $B_{12}$ during various 24-hour periods (Table III). Likewise, androgen had no effect on radioactive $B_{12}$ excretion of either sex.

*Discussion.* The greatest urinary excretion of Vit. $B_{12}$, following its subcutaneous injection, occurs during the first 8 hours; the second 8-hour period contains less than 10% of the first period. This is, perhaps, attributable to the animal's lack of ability to retain the administered level of tagged vitamin. Chow *et al.*(4) noted a similar tendency in

† Testosterone propionate generously supplied by the Upjohn Co., Kalamazoo, Mich.

‡ We are grateful to Merck & Co., Inc., Rahway, N. J. for the generous supply of $Co^{60}$-labelled vit $B_{12}$.

§ The preparation employed was distilled water solution of 0.1% trituration of crystalline vit $B_{12}$ (Merck & Co.). As a preservative, phenol crystals were added to make 0.5% solution.

TABLE I. Control Rats. Excretion of radioactivity following parenteral administration of $Co^{60}$-labelled vit. $B_{12}$.

| | Pathway of excretion | | | |
|---|---|---|---|---|
| | Urine, cpm | | Feces, cpm | |
| Time sequence | Radioactive $B_{12}$ only | 16.6 $\gamma$ non-labelled $B_{12}$ daily from day 3 | Radioactive $B_{12}$ only | 16.6 $\gamma$ non-labelled $B_{12}$ from day 3 |
| 0*– 8 hr | 147 ±10.2† ( 4) | | 8 ± 1.9 (12) | |
| 8 – 16 | 12 ± 1.7 ( 4) | | 26 ± 4.0 (12) | |
| 16 – 24 | 5 ± .4 ( 4) | | 37 ± 5.5 (12) | |
| Total 1st day | 174 ± 6.0 (12) | | 71 ± 5.4 (12) | |
| 2nd | 17 ± 1.1 (12) | | 110 ± 8.9 (12) | |
| 3rd | 13 ± .3 (12) | | 155 ± 8.7 (12) | |
| 4th | 10 ± 1.3 ( 4) | 66 ± 3.0 (8) | 116 ±15.9 ( 4) | 125 ± 5.5 (8) |
| 5th | 10 ± .8 ( 4) | 36 ± 2.4 (8) | 87 ± 7.7 ( 4) | 102 ± 5.2 (8) |
| 6th | 9 ± .5 ( 4) | 21 ± 1.5 (8) | 76 ± 9.7 ( 4) | 53 ± 2.8 (8) |
| 7th | 9 ± .3 ( 4) | | 54 ± 2.8 ( 4) | |
| 8th | 8 ± .0 ( 4) | | 43 ± 1.9 ( 4) | |
| 9th | 8 ± .3 ( 4) | | 33 ± 3.3 ( 4) | |
| 10th | 7 ± .0 ( 2) | | 23 ± .5 ( 2) | |
| Total excr. % of inj. dose | 10.3 | | 29.9 | |

* Time of inj. of labelled vit. $B_{12}$ is referred to as "zero" time. † Stand. error of mean.
Figures in parentheses represent No. of animals.

humans. Although subsequently recorded urinary radioactivity was low, the fecal radioactive count increased until approximately the end of the third day, then steadily decreased. Table I shows that, at all collection points past the first 8-hour period, fecal radioactivity was many times that of the relatively insignificant radioactivity in urine. Then, it appears that a plentiful supply of $Co^{60}$-labelled substance is available for excretion both during the first 8-hour period and any subsequent first, second, or third-day period. But it is also apparent that during the first collection period, radioactivity is excreted mainly in the urine, while during any subsequent period fecal excretion predominates. Thus we can sur-

TABLE II. Testosterone-Treated Rats. Excretion of radioactivity following parenteral Administration of $Co^{60}$-labelled vit. $B_{12}$.

| | Pathway of excretion | | | |
|---|---|---|---|---|
| | Urine, cpm | | Feces, cpm | |
| Time sequence | Radioactive $B_{12}$ only | 16.6 $\gamma$ non-labelled $B_{12}$ daily from day 3 | Radioactive $B_{12}$ only | 16.6 $\gamma$ non-labelled $B_{12}$ from day 3 |
| 0*– 8 hr | 164 ±5.8† ( 4) | | 8 ± 1.9 (12) | |
| 8 – 16 | 11 ± .9 ( 4) | | 32 ± 3.5 (12) | |
| 16 – 24 | 8 ± .6 ( 4) | | 33 ± 4.8 (12) | |
| Total 1st day | 161 ±6.6 (12) | | 73 ± 4.8 (12) | |
| 2nd | 19 ±1.7 ( 8) | | 114 ± 5.2 (12) | |
| 3rd | 11 ±1.1 ( 8) | | 153 ± 5.8 (12) | |
| 4th | 11 ± .2 ( 4) | 61 ± 2.8 (8) | 136 ± 7.9 ( 4) | 158 ± 10.2 (8) |
| 5th | 10 ± .2 ( 4) | 34 ± 1.6 (8) | 84 ± 4.5 ( 4) | 99 ± 8.3 (8) |
| 6th | 9 ± .6 ( 4) | 21 ± .4 (8) | 62 ± 4.6 ( 4) | 59 ± 5.4 (8) |
| 7th | 9 ± .6 ( 4) | | 55 ± 5.0 ( 4) | |
| 8th | 8 ± .3 ( 4) | | 50 ± 5.6 ( 4) | |
| 9th | 7 ± .0 ( 4) | | 33 ± 4.8 ( 4) | |
| 10th | 7 ± .4 ( 4) | | 23 ± 1.6 ( 4) | |
| Total excr. % of inj. dose | 9.8 | | 30.5 | |

* Time of inj. of labelled vit. $B_{12}$ is referred to as "zero" time. † Stand. error of mean.
Figures in parentheses represent No. of animals.

TABLE III. Total Radioactive Excretion* of Female and Male Rat following Parenteral Administration of $Co^{60}$-labelled Vit. $B_{12}$.

| Time sequence | Control rats | | Testosterone-treated rats | |
|---|---|---|---|---|
| | Female (7) | Male (8) | Female (7) | Male (10) |
| 1st day | 266 ± 18.4† | 283 ± 23.5 | 289 ± 29.8 | 270 ± 29.2 |
| 2nd | 162 ± 24.1 | 152 ± 19.3 | 169 ± 13.9 | 164 ± 14.8 |
| 3rd | 165 ± 14.6 | 157 ± 17.1 | 180 ± 9.3 | 166 ± 17.8 |
| Further treatment | Non-labelled vit. $B_{12}$, subcut., daily | | | |
| 4th day | 215 ± 9.4 | 223 ± 16.2 | 228 ± 15.5 | 219 ± 9.3 |
| 5th | 159 ± 10.2 | 154 ± 7.7 | 161 ± 8.2 | 154 ± 6.3 |
| 6th | 124 ± 9.3 | 139 ± 16.8 | 118 ± 9.9 | 115 ± 5.1 |

* Avg values for composite urine-feces samples are reported as cpm.
† Stand. error of mean.

mise that there are at least 2 different $Co^{60}$-labelled substances, one excreted by the urinary tract, the other by the intestinal tract; whether these routes of exit are decidedly exclusive cannot be ascertained from the data on hand. The feces-destined substance appears to be an altered one, since its elimination continues to increase to the end of the third day. The tagged urine molecule may be the intact vitamin or a close associate of it since: 1) it rapidly appears in urine, and 2) upon a large injection of non-radioactive preparation of Vit. $B_{12}$, urinary excretion of radioactivity is increased approximately 5 times above its preceding level, whereas fecal radioactivity is not appreciably altered. Microbiological assays(1), extraction(3), and chromatography(2), appear to eliminate the possibility that tagged urinary substance might be inorganic cobalt.

The explanation for the interesting observation, that fecal radioactivity progressively increases (rather than decreases) past the first day until the fall noted upon examination of the 4th day collection remains at present unanswered. These results are at variance with Barbee's and Johnson's data(2) which show a decrease in fecal radioactivity subsequent to the first day's administration of radiovitamin. The disparity may, perhaps, be accounted for by difference in dosage level used.

Becker, Lang, and Chow(5) found that although radioactive Vit. $B_{12}$ content was decreased in liver and kidney, the urinary radioactivity following testosterone administration was not significantly altered. Our data show no effect of androgen on either urinary or fecal excretion patterns.

*Summary.* 1) The excretion pattern of radioactive substance following parenteral administration of 0.25 $\mu$g of Cobalt$^{60}$-labelled Vit. $B_{12}$ to the young rat has been studied. 2) Urinary excretion of radioactivity reaches a maximum during the first 8 hours after labelled Vit. $B_{12}$ administration, then rapidly declines; fecal excretion rises progressively through the 3rd day. Moreover, administration of a large dose of unlabelled Vit. $B_{12}$ subsequent to radioactive $B_{12}$ significantly increases urinary but not fecal excretion of radioactivity. From this differential excretion pattern, it is inferred that labelled vitamin is altered prior to its excretion into the intestinal tract. 3) The fecal route is the major excretory pathway for radioactivity resultant from parenteral introduction of $Co^{60}$-labelled Vit. $B_{12}$; after the 1st 8 hours, the ratio of radioactivity excreted for a 10-day period *via* fecal and urinary pathway is 7:1, respectively. 4) Sex does not appear to influence excretion of $Co^{60}$-tagged products. Similarly, administration of large quantities of testosterone propionate to either females or males does not alter the excretion pattern.

1. Rosenblum, C., Chow, B. F., Condon, G. P., Kamamoto, R., *J. Biol. Chem.*, 1952, v198, 915.

2. Barbee, K. W., Johnson, C., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 720.

3. Chow, B. F., Rosenblum, C., Silber, R. H., Woodbury, D. T., Yamamoto, R., Lang, C. A., *ibid.*, 1951, v76, 393.

4. Chow, B. F., Lang, C. A., Davis, R., Conley, C. L., Ellicott, C. E., *Bull. Johns Hopkins Hosp.*, 1950, v87, 156.

5. Becker, B., Lang, C. A., Chow, B. F., *J. Clin. Nutr.*, 1953, v1, 417.

Received February 20, 1958. P.S.E.B.M., 1958, v98.