

glycol.¹⁻⁶ The published reports, to date, agree as to the absence of toxic effects unless administered in large doses.

Discussion. A simple and economical method of administering estrogens for therapeutic purposes is described. This method consists of instilling several drops of a solution of α -estradiol in propylene glycol in the sublingual space. Definite morphologic evidence of absorption (demonstrated by characteristic estrogenic effects in the histologic sections of the vaginal mucosa and vaginal smears) was noted in all cases at the end of one week with daily doses of 0.2 and 0.3 mg of α -estradiol.

Although experimental studies appear to indicate that, in the small doses used, propylene glycol has no toxic effects, further studies should be conducted in order to determine what the effect of continued administration of these small doses would be. Should propylene glycol be found to be completely innocuous, this method of administration of estrogens offers great promise of simplifying and reducing the cost of estrogen therapy.

11828

Rumen Synthesis of the Vitamin B Complex.*†

M. I. WEGNER, A. N. BOOTH, C. A. ELVEHJEM AND E. B. HART.

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Work reported by McElroy and Goss¹ indicates the ability of sheep to synthesize several members of the vitamin B complex in

¹ Weatherby, J. H., and Haag, H. B., *J. Am. Pharm. Assn.*, 1938, **27**, 466.

² Laug, E. P., Calvery, H. O., Morris, H. J., and Woodard, G., *J. Indust. Hyg. and Toxicol.*, 1939, **21**, 173.

³ Hanzlik, P. J., Newman, H. W., Van Winkle, W., Lehman, A. J., and Kennedy, N. K., *J. Pharm. and Exp. Ther.*, 1939, **67**, 101.

⁴ Seidenfeld, M. A., and Hanzlik, P. J., *J. Pharm. and Exp. Ther.*, 1932, **44**, 109.

⁵ Hanzlik, P. J., Lehman, A. J., Van Winkle, W., and Kennedy, N. K., *J. Pharm. and Exp. Ther.*, 1939, **67**, 114.

⁶ Latven, A. R., and Molitor, H., *J. Pharm. and Exp. Ther.*, 1939, **65**, 89.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

† Thanks are extended to LaVell Henderson and Dr. Harry Waisman for assistance in the pyridoxine and nicotinic acid assays, to George Bahler for biotin determinations, and to Ann Earle and Bessie Zeman for pantothenic acid and riboflavin assays.

¹ McElroy, L. W., and Goss, H., *J. Biol. Chem.*, 1939, **130**, 439.

the rumen. Significant increases in the vitamin content of rumen ingesta were found in comparison to the amounts in the ration fed. The vitamins determined were thiamin, riboflavin, pyridoxine, and pantothenic acid. The ration used consisted of washed casein 5, washed sardine meal 6.7, cerelose 11.0, corn starch 10.6, mineral mixture 3.0, and dried plain beet pulp 63.7%. In a later report² McElroy and Goss obtained similar results when feeding this ration to a cow with a rumen fistula.

At the time the above work was being reported a similar experiment was under way at this station. The animal used was a heifer calf with a rumen fistula. The ration fed consisted of acid-washed casein 4, urea 1.0, cod liver oil 1.0, salt mixture 3.0, corn molasses 10.0, corn starch 71.0, and bleached wood-pulp 10.0%. The calf was fed daily at 8 A.M. and 8 P.M., receiving 2 pounds of ration per feeding. After the calf had received this ration for several weeks the rumen contents were sampled on alternate days at 2 P.M. until about 20 kilos of wet material had been obtained. The size of the samples was approximately 2 kilos of wet material (12-15% dry matter). Immediately upon sampling, the material was diluted with 95% ethyl alcohol to a final concentration of 45-50% and placed in a cold room (35°F) in order to stop bacterial action. Two to 3 days later this material was placed in enamel-lined pans and dried in a drying room for 24 to 40 hours at a temperature of 45 to 50°C. After grinding these samples were again stored in the cold room and removed as needed. The analyses were assays on a composite sample.

The components of the vitamin B complex assayed for were: thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and biotin. The methods of assay used were as follows: Thiamin, chick;³ riboflavin, microbiological;⁴ nicotinic acid, chemical;⁵ pyridoxine, rat;⁶ pantothenic acid, microbiological;⁷ biotin, microbiological.⁸ The results are given in Table I.

From Table I it is readily seen that an appreciable synthesis of all members of the B complex assayed for occurs.

² McElroy, L. W., and Goss, H., *J. Biol. Chem.*, 1940, **133**, lxxv.

³ Arnold, A., and Elvehjem, C. A., *J. Nutr.*, 1938, **15**, 403.

⁴ Snell, E. E., and Strong, F. M., *J. Ind. and Eng. Chem., Anal. Ed.*, 1939, **11**, 346.

⁵ Melnick, D., and Field, H., Jr., *J. Biol. Chem.*, 1940, **134**, 1.

⁶ Conger, T. W., *et al.*, unpublished method not yet in press.

⁷ Strong, F. M., *et al.*, method unpublished. Reported to the Am. Chem. Soc., Div. Biol. Chem., 1940.

⁸ Peterson, W. H., *et al.*, method unpublished. Reported to the Am. Soc. Biol. Chemists, New Orleans, March 13-16, 1940. *J. Biol. Chem.*, 1940, **133**, lxxv.

TABLE I.
Assay Results.
(γ /g dry matter.)

Factor	Basal ration	Rumen contents	Rumen contents (vitamin B ₁ added to ration)
Thiamin	0	10-12	> 20
Riboflavin	< 0.4	18.6	26.5
Nicotinic acid	60	220	172
Pantothenic acid	< 3.4	55.5	82.5
Pyridoxine	0	7	11-12
Biotin	< 0.018	0.087	0.250

In a recent report by Knight, *et al.*,⁹ the rapid destruction of ingested vitamin C in the rumen was demonstrated. The possibility existed that the same destruction might occur with members of the B complex. To investigate this possibility 200 mg of thiamin were added to the calf ration (given above) and the rumen contents again assayed for the members of the B complex as listed. The results are recorded in the last column of Table I. Since the samples were taken 6 hours after feeding and the thiamin content was still appreciably higher than found in the previous experiment destruction of this factor in the rumen, if any, is contraindicated. Further, the addition of thiamin to the ration apparently stimulated the synthesis of the other members of the B complex except nicotinic acid as can be readily seen by comparing the two experiments, with and without added thiamin respectively.

The results were obtained through the use of reliable methods with a possible exception of nicotinic acid, and even here the differences between the basal ration and rumen content are significant.

The synthesis of the vitamin B complex is very likely due to bacterial action in view of the stimulating effect obtained by adding thiamin to the ration fed.

Summary. The ability of the bovine species to synthesize significant amounts of thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and biotin when fed a ration very low in these compounds has been demonstrated. A destruction of thiamin in the rumen is contraindicated. At the same time there was an apparent stimulation of the synthesis of the other factors assayed for when thiamin was added to the "synthetic" ration.

⁹ Knight, C. A., Dutcher, R. A., Guerrant, N. B., and Bechdel, S. I., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 90.