Summary. Testosterone propionate and dehydroandrosterone acetate when administered daily at a 2 mg level to immature hypophysectomized rats induced sperm head or spermatozoon formation in the seminiferous tubules. Smaller doses were ineffective in this respect, even though they caused marked stimulation of the accessory organs. Testosterone propionate seemed in a few instances partially to prevent the adrenal cortex shrinkage which follows hypophysectomy; it is not known whether this effect was the result of a direct or indirect action by male hormone. An explanation which helps to reconcile the apparently discordant reports on the effects of androgens on spermatogenesis is suggested.

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Vitamin B_1 in Bacterial Metabolism.*

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Since the work of Peters[1] showing that the oxidation of pyruvic acid in pigeon-brain requires the presence of vitamin B_1, attention has been called to the function of this vitamin in the pyruvate-metabolism of yeast and bacteria. Lohmann and Schuster[2] have demonstrated that the co-enzyme of carboxylase, co-carboxylase, is diphosphorylated vitamin B_1. Lipmann[3] has reported that the phosphorylated vitamin is essential for the dismutation of pyruvate by an acetone preparation of *Bacillus acidificans longissimus.* In the same paper he states that the non-phosphorylated vitamin is without stimulating effect. Krebs[4] reports that no stimulation in the metabolism of pyruvates by *Staphylococcus aureus* could be obtained on the addition of vitamin B_1. However, Hills[5] has reported a marked stimulation in the pyruvate-metabolism of *Staph. aureus* grown in vitamin B_1-deficient media by the simple addition of crystalline vitamin B_1.

The purpose of the present investigation was to determine the

* Supported in part by Industrial Science Research funds of Iowa State College.

1 Peters, R. A., *Biochem. J.*, 1936, **30**, 2206.

2 Lohmann, K., and Schuster, Ph., *Angew. Chem.*, 1937, **50**, 221.

3 Lipmann, F., *Enzymologia*, 1937, **4**, 65.

4 Krebs, H. A., *Biochem. J.*, 1937, **31**, 661.

5 Hills, G. M., *Biochem. J.*, 1938, **32**, 383.

effect of the addition of free vitamin B_1 on the metabolism of various bacteria grown in B_1-deficient media,

Bacteria. The organisms employed were *Escherichia coli* (26), *Aërobacter indologenes* (23B), *Propionibacterium pentosaceum* (49W), and *Propionibacterium peterssonii* (11W). The propionic-acid bacteria were chosen for study since it has been shown by Tatum, Wood, and Peterson[6] that vitamin B_1 has an important rôle in the growth of these organisms. *A. indologenes* was cultured in an ammonium-sulphate, glucose, tap-water medium. The other organisms were grown in the basal medium of Tatum, *et al.*,[6] containing ethereal extract of yeast and hydrolyzed gelatin with and without the addition of crystalline vitamin B_1 in a final concentration of 10^{-8} g per cc.

The organisms were harvested by centrifuging after 60 hours of growth at 30°C. After one washing with distilled water, suspensions were made in distilled water containing 10% of cell-paste by volume. One-half cc of the suspension of propionic-acid bacteria and 0.25 cc of the *E. coli* and *A. indologenes* were employed in each of the experimental Warburg vessels according to Dixon.[7] The total volume in each vessel was 2 cc.

The pyruvic acid was redistilled twice at 59-63°C at 15 mm of mercury. Three mg of acid neutralized with sodium hydroxide were used in each cup. The buffer employed (1 cc in each cup) was M/5 monopotassium phosphate adjusted to pH 5.6 with sodium hydroxide.

All experiments were conducted anaërobically under an atmosphere of nitrogen.

Fig. 1 shows the stimulating effect of vitamin B_1 on the dismutation of pyruvate by both *P. peterssonii* (11W) and *P. pentosaceum* (49W). *P. peterssonii* (11W) grown on vitamin B_1-deficient media exhibits almost no activity. On the addition of 2 gammas of vitamin B_1, its activity is immediately enhanced. The effect of the vitamin on *P. pentosaceum* (49W) is similar, its activity being increased about 4 times. The addition of 2 gammas of the vitamin to *P. pentosaceum* (49W) cells grown in a concentration of 10^{-8} g of vitamin per cc increases its metabolic rate to almost that of the vitamin B_1-deficient cells to which 2 gammas of the vitamin has been added.

A. indologenes and *E. coli* although grown in vitamin B_1-deficient media were very active and showed no increase in the rate of dis-

[6] Tatum, E. L., Wood, H. G., and Peterson, W. H., *Biochem. J.*, 1936, **30**, 1898.

[7] Dixon, Malcolm, *Manometric Methods*, Cambridge Univ. Press, 1934.

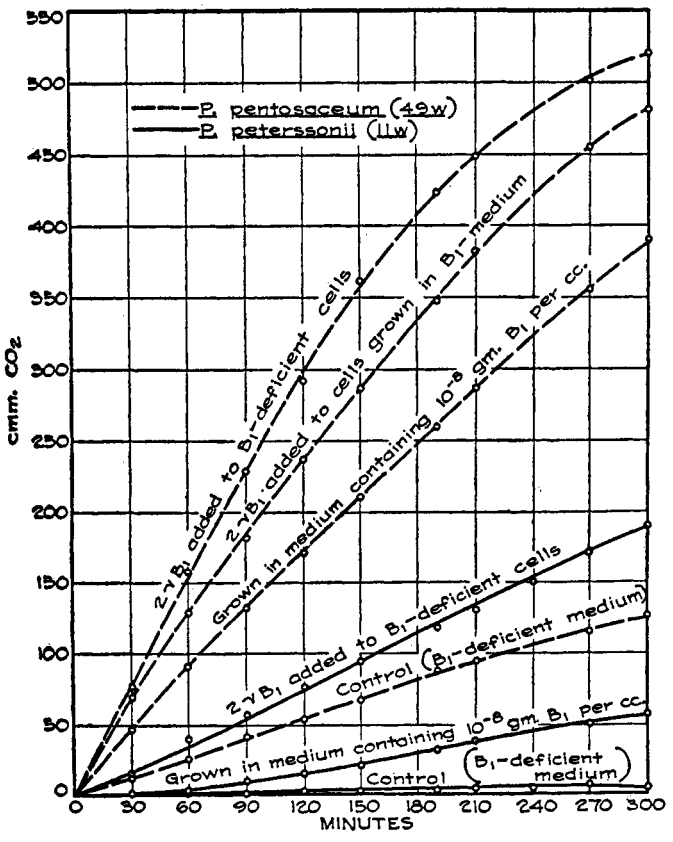

Fig. 1 EFFECT OF B_1 ON PROPIONIC ACID BACTERIA

similation of pyruvates on the addition of crystalline vitamin B_1. Since members of the Escherichia-Aërobacter group grow well in synthetic media, they are in all probability capable of synthesizing the vitamin as rapidly as it is required so the need for it cannot be demonstrated. Sunderlin and Werkman[8] reported the synthesis of vitamin B by *E. coli*.

On the other hand, propionic-acid bacteria are difficult to culture in synthetic media; one possible reason being their inability to synthesize the vitamin, therefore its need can be readily demonstrated.

The optimal concentration of the vitamin for the anaërobic breakdown of pyruvic acid is shown in Fig. 2. Under the conditions of our experiment, maximal activity is attained at a concentration in the vicinity of 0.5 gamma per 2 cc of liquid, equivalent to 2.5×10^{-7} g of vitamin per cc. Reference to Fig. 1 shows that the activity due

[8] Sunderlin, G., and Werkman, C. H., *J. Bact.*, 1928, **16**, 1.

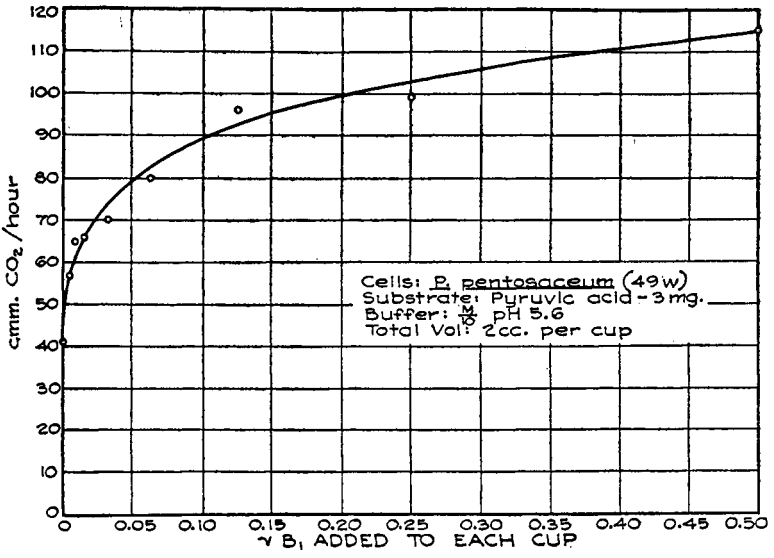

FIG. 2. EFFECT OF VARYING CONCENTRATIONS OF B_1 ON B_1-DEFICIENT CELLS

to 2 gammas of vitamin per cup does not greatly exceed that at ½ gamma per cup. In this respect it is interesting to compare this value with that of Tatum, *et al.*, for the optimal growth concentration of propionic-acid bacteria. They report a growth-optimum at a concentration of 0.5 gamma per 100 cc or 0.5×10^{-8} gammas per cc. This is 50 times less than the optimum concentration for anaërobic pyruvate-metabolism.

TABLE I.

Gammas vitamin B_1 added	0	.0039	.0078	.0156	.0313	.0625	.125	.25	.5
CO_2 evolved in mm^3/hr	41	57	65	66	70	80	96	99	115

Table I shows that the addition of 0.0039 gammas or 3.9×10^{-9} g of the vitamin increases the rate of CO_2 evolution from 41 mm^3 to 57 mm^3 per hour—or an increase of more than 35% over the original rate. In other words, employing the Warburg technic and propionic-acid bacteria grown in vitamin B_1-deficient media, it is possible to demonstrate the presence of as little as 3.9×10^{-9} g of vitamin B_1. It seems possible to use a bacteriological method for the assay of this vitamin.

The function of the vitamin here is probably not that of co-carboxylase since acetaldehyde has never been detected in a propionic-acid fermentation. More probably it is that of a hydrogen carrier

as has already been suggested by Lipman. Whether or not phosphorylation of the vitamin occurs is now being investigated.

The authors are indebted to Dr. H. G. Wood of this laboratory for suggestions and assistance in culturing the propionic-acid bacteria.

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Storage of Vitamin D in the Tissues of Growing Calves.*

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Numerous researches have been conducted with the view of determining the influence of varying intakes of vitamin D on the antirachitic potency of milk and eggs. Very few data are available, however, relative to the influence of vitamin D intake on the storage of this vitamin in the body tissues of various types of animals.

Metz and Coppens[1] have reported that some of the parenchymatous tissues from dogs contain considerable quantities of vitamin D. DeVaney and Munsell[2] have reported on the vitamin D content of ox, lamb, swine and calf livers, as purchased on the open market. Heymann[3] investigated the storage of vitamin D in various tissues of the rabbit, as resulting from feeding definite unitage of this vitamin in the form of viosterol in oil. According to this investigator, the amount of vitamin D storage was relatively small in all cases, the greatest storage being in the liver and in the blood, respectively.

It is highly probable that the lack of data concerning the ability of the larger animals to store vitamin D in their tissues is due to the amount of time and expense involved in carrying out any such investigations. Investigations of this type necessarily require an accurate measure of the total vitamin D intake of the particular animal while being maintained under carefully controlled conditions

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1 Metz, G. A., and Coppens, Ph. A., *Nederland. Tijdschr. v. Geneesk.*, 1934, **78**, 769.

2 DeVaney, G. M., and Munsell, H. E., *J. Home Ec.*, 1935, **27**, 240.

3 Heymann, Walter, *J. Biol. Chem.*, 1937, **118**, 371.