

were available. These rabbits received 200 r.u. of oestradiol in oil subcutaneously, and a total of 6.6 rb.u. of progestin in 10 days commencing the third day after the oestradiol was given. Despite the fact that the endometrium in each of these rabbits was well proliferated (proving that the oestrin and progestin were effective) the distention sites failed to show appreciable enlargement as compared with the undistended portions of uterus. The degrees of distention were comparable to those which yielded growth responses at 13 distention sites in a total of 7 mature progestin-treated rabbits.<sup>1</sup>

These results show that local uterine growth, which takes place with suitable degrees of chronic distention in sexually mature rabbits, depends upon some factor or conditions associated with maturity. It is not dependent, however, upon influences of the known ovarian hormones since oestrin and progestin administered to immature rabbits were effective, causing endometrial proliferation without inducing the distention-growth response.

## 9270

### Action of Synthetic Vitamin B<sub>1</sub>.

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The antineuritic vitamin, B<sub>1</sub>, has been crystallized by different groups of investigators,<sup>1-6</sup> but it remained for Williams and his associates<sup>7</sup> to arrive at a correct structural formula, confirmed by successful synthesis.<sup>8</sup>

For some time, the crystalline vitamin B<sub>1</sub>, both the natural and the synthetic, has been available in the form of the hydrochloride in our

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<sup>1</sup> Jansen, B. C. P., and Donath, W. F., *Geneesk. tijdschr. v. Nederl. Indie*, 1927, **66**, 810.

<sup>2</sup> Otake, S., *Proc. Imp. Acad. Tokyo*, 1931, **7**, 102.

<sup>3</sup> Windaus, A., Tschesche, R., Ruhkoff, H., Laquer, F., and Schultz, F., *Z. f. physiol. Chem.*, 1932, **204**, 123.

<sup>4</sup> Seidell, A., and Smith, M. I., *J. Am. Chem. Soc.*, 1933, **55**, 3380.

<sup>5</sup> Kinnersley, H. W., O'Brien, J. R., and Peters, R. A., *Biochem. J.*, 1933, **27**, 232.

<sup>6</sup> Williams, R. R., Waterman, R. E., and Keresztesy, J. C., *J. Am. Chem. Soc.*, 1934, **56**, 1187.

<sup>7</sup> Williams, R. R., *J. Am. Chem. Soc.*, 1935, **57**, 229; 1936, **58**, 1063.

<sup>8</sup> Williams, R. R., and Cline, J. K., *J. Am. Chem. Soc.*, 1936, **58**, 1504; 1937, **59**, 216.

laboratory for pharmacological studies. Our results appear so uniform that they can probably serve as an additional proof to that established by chemical means, concerning the identity of the two products.

To determine the potency of the synthetic vitamin B<sub>1</sub>, rats were made definitely polyneuritic and treated with various doses according to the technique of Smith,<sup>9</sup> except that a small amount (0.4%) of Brewers' yeast was added to the diet, as suggested by Dann,<sup>10</sup> in order to reduce the incidence of deaths. Tests were also carried out in pigeons rendered polyneuritic by the diet proposed by Cowgill<sup>11</sup> for confirmative purposes.

TABLE I.  
Potency of Synthetic Vitamin B<sub>1</sub>.

Species Used	No. of Polyneuritic Animals Used	Dose mg.	No. of Animals Cured
Rats	$\left\{ \begin{array}{l} 3 \\ 7 \\ 13 \\ 5 \end{array} \right.$	.003	1
		.004	6
		.005	10
		.006	4
Pigeons	9	.005	9

As shown in Table I, doses of 0.004 to 0.006 mg. of synthetic vitamin given by mouth were sufficient to cure 77 to 85% of the rats. An amount of 0.003 mg. was effective in one case but failed in the other 2, so that the minimal curative dose of the vitamin by mouth in rats was 0.004 mg. in the present series of experiments. In 9 pigeons, a dose of 0.005 mg. injected intravenously abolished the symptoms of polyneuritis in 4 animals for 2 to 3 days, and in 5 for 4 days or more. The figures in this investigation with both rats and pigeons correspond closely to those reported by other workers for the natural crystalline vitamin,<sup>6, 12-14</sup> and fully confirm those claimed by Williams and Cline<sup>8</sup> for their synthetic product.

Other pharmacological effects are also similar when the natural and the synthetic compounds are compared side by side. In an etherized cat weighing 2.63 kg., doses of 5, 20, and 50 mg. of the synthetic product, injected intravenously, caused a slight decrease

<sup>9</sup> Smith, M. I., *Pub. Health Rep.*, 1930, **45**, 116.

<sup>10</sup> Dann, F. P., *J. Nutrition*, 1936, **12**, 461.

<sup>11</sup> Cowgill, G. R., *The Vitamin B Requirement of Man*, Yale Press, New Haven, 1934, 29.

<sup>12</sup> Jansen, B. C. P., Kinnersley, H. W., Peters, R. A., and Reader, V., *Biochem. J.*, 1930, **24**, 1824.

<sup>13</sup> Ammerman, M., and Waterman, R. E., *J. Nutrition*, 1935, **10**, 25.

<sup>14</sup> Waterman, R. E., and Ammerman, M., *J. Nutrition*, 1935, **10**, 161.

in respiratory volume, but no changes in respiratory rate, blood pressure, or heart rate. Similar effects were observed with the same doses of the natural vitamin. Neither substance produced congestion or necrosis in the rabbit's ear by subcutaneous injection in the dosage of 1 mg. dissolved in 0.1 cc. of saline. The minimal lethal dose (M.L.D.) in guinea pigs is the same with both the natural and the synthetic products, by intravenous injection, as shown in Table II. The vitamin solution employed for the toxicological study was 2% in each case, and the weight of the animals varied from 210 to 265 gm. Clonic convulsions occurred after doses of 150 mg. per kg., or more, had been administered. Those animals which survived the sublethal doses recovered completely within 1½ and 5 minutes, apparently without any after effects.

TABLE II.  
Toxicity of Natural and Synthetic Vitamin B<sub>1</sub> Hydrochloride.

Vitamin B <sub>1</sub> HCl	Dose mg. per kg.	No. of Pigs Died Over No. Used	M. L. D. mg. per kg.	
Synthetic	{	300	1/1	180
		200	1/1	
		180	2/3	
		160	0/2	
		150	0/1	
		100	0/1	
Natural	{	180	2/2	180
		160	1/3	

*Summary.* Results obtained in animals indicate that the natural crystalline vitamin B<sub>1</sub> and the synthetic product are identical.

## 9271 P

### Increased Estrogenic Potency of Human Urine after Hydrogenation.

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A method described by us<sup>1</sup> for the hydrolysis and extraction of urine for estrogens involves boiling with 15 vol. % of HCl for 10

<sup>1</sup> Smith, G. V., and Smith, O. W., *Am. J. Physiol.*, 1935, **112**, 340.