

and maintained for 8 transfers. Elementary bodies were demonstrable in the membranes from 17 of the 25 eggs employed but were usually less numerous than the elementary bodies of vaccinia. Fourteen of the virus-positive eggs contained an active embryo, in 2 of them after an incubation period of 4 days.

Most of the membranes inoculated with variola virus showed macroscopic evidence of a slight tissue reaction indistinguishable from that noted in some of the eggs inoculated with vaccinia and negligible in comparison with the reaction in membranes incubated at 37°C. At the latter temperature the development of variola virus is characterized by a focal epithelial hyperplasia which becomes confluent, resulting in a membrane many times thicker in cross-section than those incubated at 28°C. Histologically the membranes showed at most a moderate proliferation of the ectodermal cells with a few phagocytes in the mesoderm.

*Summary.* Vaccinia and variola viruses were established and maintained on transfer in the chorioallantoic membrane of embryonated eggs incubated at a temperature sufficiently low to prevent embryonic development (28°C). The number of elementary bodies present in the membrane on the 3rd day approximated that in eggs incubated at a temperature which favored growth of the embryo (37°C). At 28°C, however, the effect of the respective viruses on the membrane and the embryo as well as the cellular response of the former to them was significantly retarded.

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#### Identity of Natural and Synthetic Crystalline Vitamin B<sub>6</sub>.

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Vitamin B<sub>6</sub> was first established as a component of the vitamin B complex by György.<sup>1</sup> It was subsequently isolated from natural sources by Keresztesy and Stevens,<sup>2</sup> Lepkovsky,<sup>3</sup> Kuhn and Wendt<sup>4</sup>

<sup>1</sup> György, P., *Biochem. J.*, 1935, **29**, 760.

<sup>2</sup> Keresztesy, J. C., and Stevens, J. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 64.

<sup>3</sup> Lepkovsky, S., *Science*, 1938, **87**, 169.

<sup>4</sup> Kuhn, R., and Wendt, G., *Ber.*, 1938, **71**, 780.

and György.<sup>5</sup> It was ultimately synthesized by Harris and Folkers<sup>6</sup> and almost simultaneously by Kuhn and others.<sup>7</sup> With the exception of a short note by Möller, Zima, Jung and Moll<sup>8</sup> in which the biological identity of the natural and synthetic products was established by their influence on the growth of bacteria there is no other information in the literature on the matter of this identity. In the course of the work on the isolation and synthesis of crystalline vitamin B<sub>6</sub> in the Research Laboratories of Merck & Co., Inc., we had an opportunity to investigate the activity of both the natural and synthetic materials by the single dose curative method proposed by Moll<sup>9</sup> as well as by the rat prophylactic procedure. A summary of these results is presented in this communication.

In the curative method 21-day-old rats were placed on a vitamin B complex free diet consisting of vitamin-free casein 18%, cerelose (dextrose) 68%, Crisco 8%, cod liver oil 2%, and O & M salt mixture 4%, supplemented with 40 micrograms each of thiamin chloride and riboflavin per rat per day.\* In 5 to 8 weeks the majority of the rats developed well defined symptoms of B<sub>6</sub> deficiency characterized by swollen and edematous paws, thickened and sometimes encrusted ears, a denuded nose and occasional ulcerations about the mouth and tongue. When these symptoms became well pronounced a single dose of the test substance was administered orally to the rat. Its effect on the symptoms was observed over a period of 14 days and, in addition, weight records were kept on all animals since it is our experience that some significant although transient increase in weight accompanies the administration of an effective dose of B<sub>6</sub> to animals maintained on the above diet. Table I shows the results of the administration of various doses of natural and synthetic vitamin B<sub>6</sub>.

Table I shows that both the synthetic and the natural vitamin B<sub>6</sub> are equally effective in curing the symptoms of vitamin B<sub>6</sub> deficiency. Furthermore, though single doses of 100  $\gamma$  are sufficient to effect 100% cures in the test animals, 50  $\gamma$  will completely cure 70 to 80% of the animals and cause some improvement in the remaining animals

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<sup>5</sup> György, P., *J. Am. Chem. Soc.*, 1938, **60**, 983.

<sup>6</sup> Harris, S. A., and Folkers, K., *Science*, 1939, **89**, 347.

<sup>7</sup> Kuhn, R., Wendt, G., Westphahl, O., and Westphahl, K., *Naturwissenschaften*, 1939, **27**, 469.

<sup>8</sup> Möller, Zima, Jung and Moll, *Naturwissenschaften*, 1939, **27**, 288.

<sup>9</sup> Moll, Th., and Schnittspahn, M., *E. Merck's Jahresber.*, 1938, **52**, 10.

\* We recognize that this diet is deficient in other factors of the vitamin B complex but since the cure of rat acrodynia is the criterion in this test these other deficiencies do not interfere, as shown by the histopathological studies of Antopol and Unna (*Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 126).

TABLE I.

Substance	Dose in $\gamma$	No. animals	No. complete cures	No. partial cures	No. no cures	Avg wt gain
Natural B <sub>6</sub>	100	10	10	0	0	19.4
	50	6	5	1	0	13.3
	25	10	3	2	5	6.3
Synthetic B <sub>6</sub>	100	11	11	0	0	18.8
	50	16	12	4	0	12.3
	25	7	3	3	1	9.0

and 25  $\gamma$  will produce some improvement in several of the animals but very few complete cures. These data are in good agreement with those published by Moll<sup>9</sup> for the natural vitamin B<sub>6</sub>.

In the rat prophylactic method 21-day-old male rats were placed on the same vitamin B complex-free diet as used in the curative test.

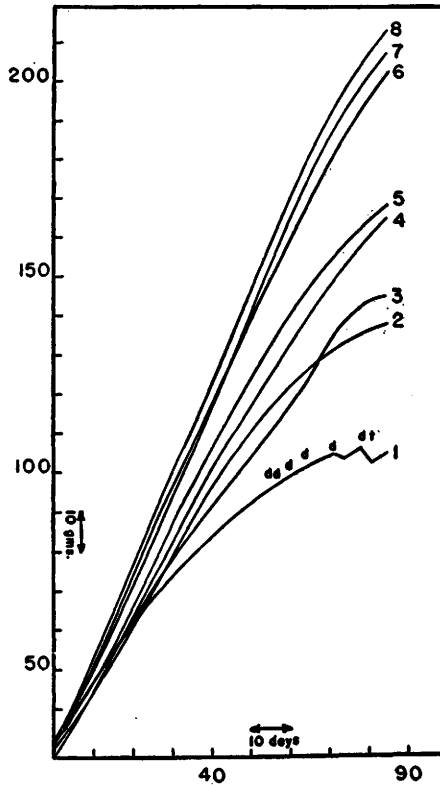


FIG. 1.

Growth response of rats to daily feeding of graded doses of natural and synthetic vitamin B<sub>6</sub>. 1—No B<sub>6</sub>; 2—2.5  $\gamma$  synthetic B<sub>6</sub>; 3—2.5  $\gamma$  natural B<sub>6</sub>; 4—5  $\gamma$  natural B<sub>6</sub>; 5—5  $\gamma$  synthetic B<sub>6</sub>; 6—12.5  $\gamma$  synthetic B<sub>6</sub>; 7—10  $\gamma$  synthetic B<sub>6</sub>; 8—25  $\gamma$  synthetic B<sub>6</sub>; d = dermatitis; + = death.

From the beginning of the test, each rat received a daily supplement of 40  $\gamma$  each of thiamin chloride and riboflavin, 0.5 mg nicotinic acid and 0.3 cc of a highly potent liver concentrate of Factor II (Lepkovsky, Jukes and Krause<sup>10</sup>) kindly prepared by Dr. J. C. Keresztesy. Eight groups of 10 rats each were started on this regimen. The doses administered and the weight responses of the animals are shown in Fig. 1. The negative control group grew slowly for 7 weeks and most of the animals developed typical dermatitis within the 12 weeks' period of the test. The rats receiving 2.5  $\gamma$  each of the natural and the synthetic vitamin B<sub>6</sub> grew at a somewhat more rapid rate but within 12 weeks their weight had plateaued, although no symptoms of dermatitis were evidenced macroscopically. The animals in these groups were sacrificed at this point. A histological examination of their tissues revealed no evidence of B<sub>6</sub> deficiency. The groups receiving 5 micrograms each of natural and synthetic vitamin grew more rapidly and were still gaining weight when the experiment was terminated. The rats receiving 10 micrograms, 12.5 micrograms and 25 micrograms of synthetic B<sub>6</sub> respectively grew at practically normal rates and there was no significant difference in growth rate among these 3 groups.

The close parallel in the rate of growth of the rats receiving 2.5  $\gamma$  each of natural and synthetic B<sub>6</sub> and in the groups receiving 5  $\gamma$  each of the natural and synthetic B<sub>6</sub> is further evidence as to the biological identity of these two forms of the vitamin. It is also apparent that doses greater than 10 micrograms B<sub>6</sub> per rat per day have no significant effect on the rate of growth. This latter observation confirms the findings of Dimick and Schreffler<sup>11</sup> who worked with the natural vitamin B<sub>6</sub> from rice polishings.

*Summary.* 1. Rat curative tests and rat prophylactic tests show that natural and synthetic B<sub>6</sub> are identical in their physiological action. 2. A single dose of 50 micrograms of either natural or synthetic B<sub>6</sub> is sufficient to cure severe symptoms of vitamin B<sub>6</sub> deficiency in 70-80% of the test animals within 14 days. 3. Ten micrograms of vitamin B<sub>6</sub> per rat per day is sufficient to produce practically normal growth.

We wish to acknowledge the technical assistance of Miss Josephine Dawson, Miss Evelyn Ruddy and Miss Saramae Woodford.

<sup>10</sup> Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

<sup>11</sup> Dimick, M. K., and Schreffler, C. B., *J. Nutrition*, 1939, **17**, 23.