on the central nervous system after such small doses of serum, as were obtained in the present research. In the opinion of the authors, pemphigus should be described in treatises on internal diseases with equal propriety as smallpox, leprosy, syphilis, pellagra, and other constitutional diseases are discussed in such text books.

Summary. 1. The neuro-muscular responses and general behavior of white rats were studied, (1) in a circular maze, (2) on animals trained to walk a tightly-stretched horizontal rope, and (3) on animals trained to climb a vertically-stretched rope.

2. Injections of small quantities of pemphigus sera in these rats produced a definite depression of performance in all 3 tests, while control experiments with normal sera did not affect the rats adversely.

3. These findings corroborate the authors' experiments with pemphigus sera on plants and indicate that this disease is a systemic, rather than a cutaneous one, as is indicated by the toxic effects exerted by the blood serum of patients with pemphigus.

15575

The Vitamin B_6 Group. IX. Comparative Growth and Anti-anemic Potencies of Pyridoxal, Pyridoxamine and Pyridoxine for Dogs.*

P. S. SARMA, E. E. SNELL, AND C. A. ELVEHJEM.

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

Fouts *et al.*[1,2] and subsequently others[3,4] showed that dogs, when kept on vitamin B_6 deficient diets, developed a characteristic microcytic hypochromic anemia which could be cured with pyridoxine. Anemia has also been produced in chicks,[5] ducks[6] and swine[7] by feeding diets low in vitamin B_6.

Since enzymatically effective derivatives of vitamin B_6 are related directly to pyridoxal[8,9] and pyridoxamine,[10] rather than to pyridoxine, and since the 3 compounds have equivalent growth-promoting activities when injected intraperitoneally into rats or chicks,[11] it seemed possible that the antianemic potencies of the former 2 compounds might surpass that of pyridoxine. Results reported below show that when injected intravenously, pyridoxal, pyridoxamine and pyridoxine are equally active in increasing hemoglobin levels and supporting growth of dogs.

Experimental. Littermate male pups 8 weeks old (No. 41 and 42) and one older female pup 14 weeks old (No. 40) were treated twice with an anthelmintic (tetrachlorethylene), freed of external parasites with rotenone and placed in individual cages. The vitamin B_6-deficient diet had the following

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For paper VIII of this series see reference 11.

1 Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., *J. Nutrition,* 1938, **16,** 197.

2 Fouts, P. J., Helmer, O. M., and Lepkovsky, S., *J. Nutrition,* 1940, **19,** 393.

3 Borson, H. J., and Mettier, S. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43,** 429.

4 McKibbin, J. M., Schaefer, A. E., Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.,* 1942, **142,** 77.

5 Luckey, T. D., Briggs, G. M., Jr., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1945, **58,** 340.

6 Hegsted, D. M., and Rao, M. N., *J. Nutrition,* 1945, **30,** 367.

7 Cartwright, G. E., Wintrobe, M. M., and Humphreys, S., *J. Biol. Chem.,* 1944, **153,** 171.

8 Bellamy, W. D., Umbreit, W. W., and Gunsalus, I. C., *J. Biol. Chem.,* 1945, **160,** 461.

9 Green, D. E., Leloir, L. F., and Nocito, V., *J. Biol. Chem.,* 1945, **161, 559.**

10 Umbreit, W. W., O'Kane, D. J., and Gunsalus, I. C., *J. Bact.,* 1946, **51,** 576.

11 Sarma, P. S., Snell, E. E., and Elvehjem, C. A., *J. Biol. Chem.,* 1946, **165,** 55.

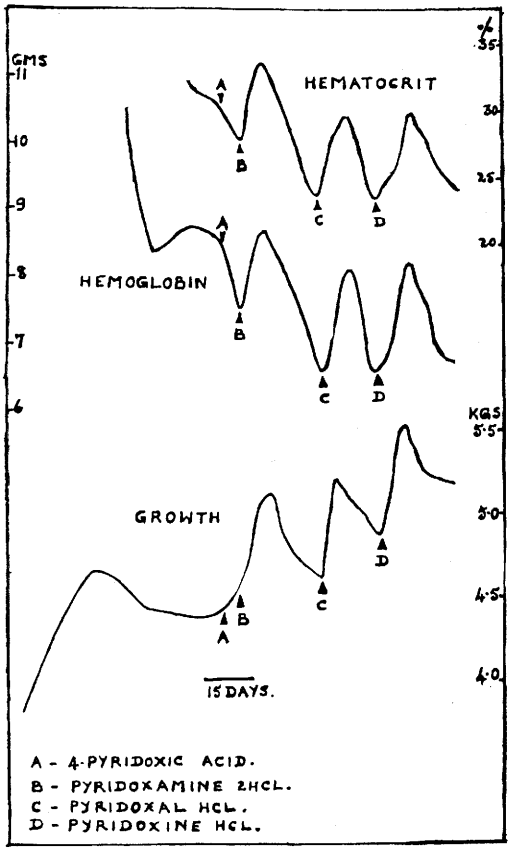

Fig. 1.
Growth, hemoglobin and hematocrit curves of Dog No. 40.

composition: sucrose 66, acid-washed alcohol extracted casein 19, cotton seed oil 11, salts IV[12] 4. All animals received food and water *ad libitum*. The animals also received an aqueous suspension of all the B vitamins except vitamin B_6, twice a week, the administration being based on the following daily requirement of the vitamins per kg body weight of the dog: thiamin 0.1 mg, riboflavin 0.1 mg, calcium pantothenate 0.5 mg, nicotinic acid 2.0 mg, choline 50 mg and biotin[13] 0.025 mg. Halibut liver oil was given twice a week to supply 1800 I.U. of vitamin A and 500 I.U. of vitamin D per dog per day. The dogs were weighed semiweekly during the depletion period and more frequently when the actual experiments were in progress. Hemoglobin determinations[14] and hematocrit readings were carried out on 3 ml samples of blood obtained from the radial vein of the dog before the morning feeding. These determinations were made twice weekly during the depletion period, but more often during the period of supplementation.

Results. The results obtained with dog No. 40 are shown in Fig. 1. After an initial increase, the rate of growth showed a plateau and then decreased slowly. After 2 months, the hemoglobin level of this dog reached a low figure of 8.48 g per 100 ml of blood. At this point (A) daily injections of 4-pyridoxic acid (40 μg per kg of body weight) were made into the radial vein for 4 consecutive days (total dose 704 μg). A slight

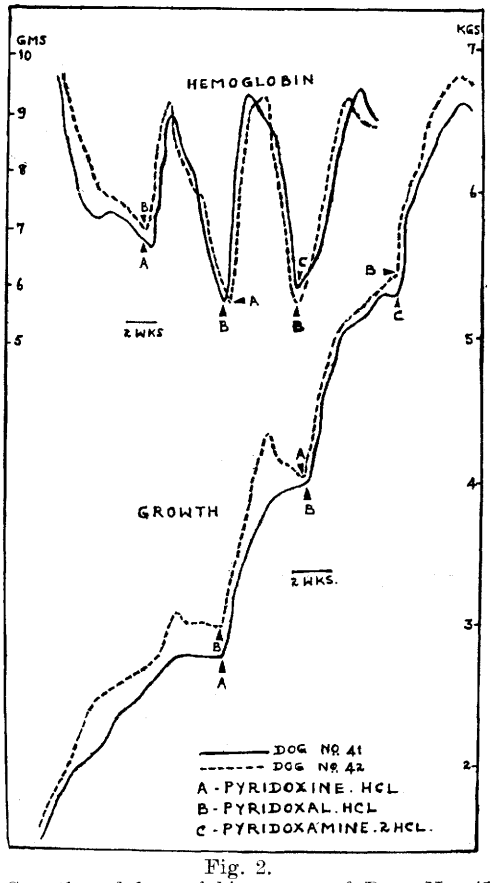

Fig. 2.
Growth and hemoglobin curves of Dogs No. 41 and 42.

[12] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

[13] Ruegamer, W. R., Michaud, L., Elvehjem, C. A., and Hart, E. B., *Am. J. Physiol.*, 1945, **145**, 23.

[14] Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.

gain in body weight accompanied by a marked decrease in hemoglobin and hematocrit readings occurred. At point B, pyridoxamine dihydrochloride was similarly injected. There was a prompt response in growth and in hemoglobin and hematocrit levels. A total of 860 μg of pyridoxamine dihydrochloride was injected over a period of 4 days. This amount was insufficient to raise the blood level to the maximum value. Subsequently, the dog soon began to lose weight and again became anemic. Following this second depletion period a very similar response resulted from the administration of an equimolar quantity of pyridoxal hydrochloride (point C) and finally from pyridoxine hydrochloride (point D). It is apparent from Fig. 1 that the 3 members of the vitamin B_6 group are equally active in promoting growth and blood regeneration.

These results were confirmed and extended in experiments with dogs No. 41 and 42 (Fig. 2). These 8-week-old pups showed lower hemoglobin and hematocrit values than the older dog No. 40, after they were kept for a period of 2 months on the vitamin B_6-deficient diet described earlier. Dog No. 41 received pyridoxine hydrochloride by injection at 40 μg/kg of body weight, while dog No. 42 was injected with an equimolar amount of pyridoxal hydrochloride. These injections were given daily for 5 consecutive days. Increases in weight and hemoglobin were approximately the same in both dogs. In contrast to the findings with the older dog No. 40, which, though heavier, received a relatively smaller amount of vitamin B_6, cessation of vitamin injections did not result in a rapid decrease in weight, though the hemoglobin values decreased in a short time. After 3 weeks, when the hemoglobin values were sufficiently reduced, dog No. 41 received pyridoxal hydrochloride while dog No. 42 received pyridoxine hydrochloride at the same level for a period of 7 days. This interchange in the administration of pyridoxine and pyridoxal was effected to eliminate possible biological variations in response of the 2 animals. Again there was a prompt response which was greater than in the previous case due to the longer period of administration. The weight gains as well as the increases in blood hemoglobin and hematocrit values were almost identical in both cases. As a result of the increased amounts of pyridoxine and pyridoxal which were injected, the dogs gained weight even after vitamin administration was discontinued. However, the hemoglobin values decreased gradually and this decrease was aided by the withdrawal of 100 ml of blood from the saphenous artery in both dogs. After 3 weeks, dog No. 41 was given pyridoxamine dihydrochloride while the other dog was given pyridoxal hydrochloride. Supplementation was at the same level (40 μg/kg of body weight) as in the preceding case and was again continued 7 days. Again, a prompt response in growth and hemoglobin values resulted (Fig. 2) and the responses were of equal magnitude in the 2 animals. In all these cases, the hematocrit values changed in a manner similar to the hemoglobin values.

Discussion. Pyridoxal, pyridoxamine and pyridoxine, when injected in equimolar amounts, are equally active in promoting growth in dogs. These results agree with earlier observations made on the growth-promoting property of these compounds in vitamin B_6-deficient rats and chicks.[11] When examined by their ability to promote blood regeneration in anemic dogs, the 3 compounds likewise appear to be equally active. Separate experiments, which it seems unnecessary to report in detail, showed that the antianemic potencies of the 3 compounds for chicks were likewise equal when the compounds were administered by injection. By analogy with the results obtained in rats and chicks,[11] administration of pyridoxal and pyridoxamine mixed in the ration of the dogs should decrease their apparent antianemic potency as compared with that of pyridoxine. This had not been tested experimentally. 4-Pyridoxic acid is inactive in promoting growth and blood regeneration in the vitamin B_6-deficient dog. It has also been found inactive for rats.[15]

Summary. Pyridoxal, pyridoxamine and pyridoxine were administered by intravenous injection to vitamin B_6-deficient dogs. The 3 compounds were equally active in promoting growth and blood regeneration in this species.

[15] Snell, E. E., and Rannefeld, A. N., *J. Biol. Chem.*, 1945, **157**, 475.