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

produced severe hypochromic microcytic anemia which was rapidly relieved with a rice-bran eluate rich in B₆. They later² reproduced the result with crystalline B₆ isolated from natural sources.³ In the present report it is shown that synthetic B₆ (2-methyl, 3-hydroxy, 4,5-di-[hydroxy-methyl] pyridine)[†] will replace the natural material in the relief of this anemia.

Adult mongrel male dogs varying in weight from 7.5 to 21 kg were maintained on a purified diet of the following composition: casein (extracted for a week with acidulated water) 37%, sucrose 34.8%, cotton-seed oil 12%, lard 9%, cod-liver oil 3%, bone ash 2.4%, and salt mixture (Cowgill) 1.8%. The original salt mixture was modified by doubling the amount of ferric citrate. Supplements of various members of the vitamin B complex were given (per kg per day) as follows: thiamin chloride 50 µg, riboflavin[‡] 50 µg, nicotinic acid[‡] 1 mg, liver extract[§] 0.06 ml. The liver extract was prepared from a crude liver concentrate by repeated treatment with fuller's earth to remove the adsorbable components. It was shown by rat assay to be rich in Factor 2 (rat growth factor) but practically free of B₆. Diet and water were given *ad lib*.

Four dogs, Nos. 74, 164, 165, and 188, maintained on this regime for from 120 to 135 days, developed hypochromic microcytic anemia with red blood cell counts of 5.78, 5.10, 4.95, and 4.91 million; hemoglobins of 6.3, 7.5, 7.2, and 5.9 g; mean corpuscular volumes of 51, 47, 49.5 and 45.8 cubic micra respectively. When the diet was started the red counts were 6.71, 6.87, 6.43, and 6.44 million; hemoglobins were 15.6, 14.7, 15.3, and 14.4 g; M C V were 64, 72, 75, and 64 cubic micra. Dog 74 was then given natural B₆ (obtained from Dr. Samuel Lepkovsky) in doses of 60 µg per kg per day. A slight reticulocytosis occurred (4% on the fifth day) and on the thirtieth day the red count was 7.0 million and the hemoglobin 11.6g. Dog 164 was given synthetic B₆ in doses of 60 µg per kg per day. The reticulocytes increased on the second day and reached a peak of 6.8% in 6 days. In 14 days the red count had risen to 5.5 million, the hemoglobin to 10.2 g, and the M C V to 61.8 cubic micra. On the thirty-fifth day the red count was 6.19 million, hemoglobin 12.6 g and M C V 66.3 cubic micra. Dog 165 was given 60 µg of synthetic

[†] Supplied by Dr. D. F. Robertson, Merck and Co., Rahway, N.J.

² Fouts, P. J., Helmer, O. M., and Lepkovsky, S., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 4.

³ Lepkovsky, S., *J. Biol. Chem.*, 1938, **124**, 125.

[‡] Supplied by the Galen Co., Berkeley, Calif.

[§] Made and assayed by the Galen Co., Berkeley, Calif., and the Vitab Corp., Emeryville, Calif.

B₆ per kg per day. After a slight increase in reticulocytes to 4.6%, the red count and hemoglobin increased by the fourteenth day to 5.78 million and 9.5 g respectively. The M C V was 60.5 cubic micra. On the thirty-fifth day the red count was 6.24 million, hemoglobin 11.4 g and M C V 61.3 cubic micra. Dog 188 was given 60 μ g synthetic B₆ per kg per day and in 14 days the red count was 6.1 million and the hemoglobin 9.5 g.

A control animal was maintained on the same regime with the addition of vitamin B₆, first in the form of a rice-bran eluate§ (free of the "filtrate factors") and later as synthetic B₆ in the amount of 60 μ g per kg per day. He did not develop hypochromic anemia, but after several months he developed very mild normochromic normocytic anemia. This was found to be the result of a mild deficiency of the liver extract supplying the non-adsorbable "filtrate factors." Two animals maintained on the diet without addition of the filtrate but with 60 μ g per kg per day of B₆ developed moderate anemia of the normochromic normocytic type.

At the start of the experiment the requirement of the dog for the non-adsorbable factors other than nicotinic acid was not known and we later found that the amounts given were insufficient. This prevented the complete relief of the hypochromic anemia by the administration of B₆, as the following data show. Dog 74, as noted above, responded rapidly to crystalline B₆. After the thirty-first day of treatment, however, the hemoglobin failed to rise further and during the next 14 weeks it remained at about the same level, fluctuating between 10.7 and 11.6 g. The red count slowly dropped from 7.0 to 5.4 million. Substitution of synthetic for natural B₆ in both the same and double dosage had no effect. Addition of rice-bran eluate (containing only adsorbable factors) and increase of riboflavin had no effect. When finally the liver filtrate level was tripled, the hemoglobin rose within 28 days to 13.9 g, the red count to 6.28 million and the M C V from 51.5 to 73. An adequate supply of a factor (or factors) present in the filtrate after exhaustive adsorption with fuller's earth is apparently necessary for complete relief of the anemia resulting from deficiency of B₆. This interrelationship is being further investigated. The filtrate used is known to be a rich source of the chick anti-dermatitis factor and the rat growth vitamin, but it cannot be said that the factor required by the dog is identical with either of these.

In addition to the anemia, the dogs deficient in B₆ presented other manifestations. All except one lost weight and all exhibited marked listlessness and asthenia. The lassitude was not entirely the result of the anemia, since it improved remarkably after only a few days

of treatment with B₆ before the hemoglobin had appreciably increased.

Conclusions. Synthetic vitamin B₆ relieves the hypochromic microcytic anemia produced in dogs deficient in this factor. An adequate supply of the non-adsorbable fraction of the vitamin B complex is necessary for the complete disappearance of this anemia.

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Response of Hypophysectomized Rats to Highly Purified Extracts of Pregnant Mare Serum.

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Goss and Cole¹ have shown that extracts of mare serum can be prepared testing 4000 to 7000 R.U. per mg total solids (40,000 to 70,000 R.U. per mg nitrogen). The question arises regarding the nature of the hormone present in these extracts as compared to that in untreated mare serum. Evans, *et al.*,² and Hellbaum³ have reported that more than one gonadotropic hormone is present in mare serum though these reports have not been confirmed. In the first mentioned paper the male rats showing only a Leydig tissue response were possibly sacrificed too soon to expect an effect upon the seminiferous epithelium. Further, had the time of autopsy of the females been delayed beyond 72 hours corpora lutea might have been encountered following the treatment with so-called FSH fractions. The extracts prepared by these authors were relatively crude preparations. We were interested, therefore, in determining whether or not highly purified materials would give similar evidence of fractionation of gonadotropic hormones. A fraction giving pure follicular stimulation in the female with little or no effect on the interstitial tissue of the male would give evidence of a purified follicle stimulating hormone whereas a converse relationship would indicate the presence of a luteinizing hormone.

Experimental. Two extracts were used: one, No. 15-19-2, tested

¹ Goss, H., and Cole, H. H., *Endocrinology*, 1940, **26**, 244.

² Evans, H. M., Korpi, K., Simpson, M. E., and Pencharz, R. I., *Univ. of California Publication in Anatomy*, 1936, **1**, 275.

³ Hellbaum, A. A., *Am. J. Physiol.*, 1937, **119**, 331.