6951

Blood Regeneration in Anemic Rats on a Vitamin G-Deficient Ration.*

CHARLES J. STUCKY AND ERWIN BRAND.

From the Department of Chemistry, New York State Psychiatric Institute and Hospital, New York City.

Pellagra is frequently complicated by anemia during the advanced stages.[1] One investigator[2] suggested that the disease may be due to an iron-deficiency. However, Halliday failed to cure vitamin G deficiency in her rats by means of iron therapy.[3] Recently it was reported that an anemia could be produced in rats in which vitamin G deficiency was accompanied by skin lesions.[4] Accordingly, experiments were undertaken to determine the effect of a vitamin G deficient ration on hemoglobin regeneration in anemic animals.

A group of rats of the Wistar strain, reared as previously described,[5] was employed. When weaned (21 days) such animals have a lower Hb level (6.0 gm.) than our normally bred rats[6] (av. Hb = 8.0 gm.). The animals were housed individually in wire cages with false bottoms and fed the following vitamin G deficient, iron-containing ration: corn starch 61, casein (vitamin-free) 20, lard 10, cod liver oil (Squibb) 5 and salt mixture (Osborne and Mendel) 4%. Each animal received daily a supplement of 0.2 pigeon unit[7] of a vitamin B_1 (anti-neuritic) concentrate.† Hemoglobin was estimated on tail blood at weekly intervals.[6]

Results. None of the rats (8 animals from 2 litters) made significant gains in body weight and all declined before the end of the experimental period (approx. 1 month). No skin lesions were observed in these rats. In contrast to the failure in growth, there was a rapid increase in the Hb in all cases until normal levels (av.

* A preliminary report of this paper was presented before the Division of Biological Chemistry of the American Chemical Society, Washington, D. C., March 29, 1933.

1 Turner, R. H., *Am. J. Med. Sci.*, 1933, **185**, 381.

2 Bliss, S., *Science*, 1930, **72**, 577.

3 Halliday, N., *Science*, 1931, **74**, 312.

4 Sure, B., Kik, M. C., and Smith, M. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 498.

5 Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, xiii.

6 Stucky, C. J., and Brand, E., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 932.

† Kindly furnished by Dr. Richard J. Block.

7 Block, R. J., Cowgill, G. R., and Klotz, H. B., *J. Biol. Chem.*, 1932, **94**, 765.

Hb = 15.5 gm.) were reached. Our stock animals of the same age show similar Hb values[6] (av. = 15.0 gm.).

From these data it would appear that vitamin G deficiency is not due to an iron-deficiency. The results confirm the reports of other investigators.[3, 4]

Summary. Young anemic rats were fed a vitamin G deficient (iron-containing) ration for approximately one month. The animals failed to grow but recovered from their anemia in 3 to 4 weeks.

6952

Methods and Results of Barbital Research.

THEODORE KOPPANYI, WILLIAM S. MURPHY AND STEPHEN KROP.

From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine.

We described[1] a colorimetric test for barbiturates and the rate of excretion of barbital determined by this test. We here report on (a) urinary elimination, (b) fate in the blood, (c) presence in organs of barbiturates, and (d) on the clinical application of this test.

The progress in these studies was made possible by the colorimetric test and by improved methods of extraction of barbiturates, consisting of shaking urine (alkalinized), oxalated whole blood, plasma or spinal fluid with equal volumes of 10% copper sulphate solution, filtering, acidulating the filtrate with dilute sulphuric acid and shaking the filtrate with 10 volumes of chloroform. The chloroform extract may be concentrated on water bath. Ground organs, before they are shaken with copper sulphate must be liquefied, either by 3% HCl and pepsin, or by mixing thoroughly with 5% KOH. Heating must be excluded in these procedures. The acid-pepsin treated organs must be alkalinized before shaking with copper sulphate.

Results. (a) *Urinary Excretion.* The excretion of barbital was studied in normal dogs (70-300 mg. per kg., intravenously), in the cat (250 mg. per kg., intravenously), humans (30 grains total, by mouth) and in the fowl (225 mg. per kg., intravenously). Normal dogs (8), cat (1), and humans (2) excreted from 42% to 89% of barbital during 7 days (more than ½ of the dose usually in the

[1] Koppanyi, Murphy, and Krop, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 542.