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.

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Methods and Results of Barbital Research.

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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.

first 48 hr.), from 13% to 16% of phenobarbital (2 dogs, 100 mg. per kg., intravenously) and about 40% each of neonal (1 dog, 60 mg. per kg., intravenously) and dial (1 dog, 80 mg. per kg., intravenously) and only small amounts of pernoston and amytal (3 dogs). Diuretic treatment (intravenous injections of 50 cc. of 10% glucose solution per kg. of body weight) did not increase the percentage excretion of barbiturates (from 50% to 92% for barbital (5 dogs), 16% for phenobarbital (1 dog), and 31% dial (1 dog, 100 mg. per kg., intravenously). One chicken behaved atypically, it excreted only 28.4% of barbital and never recovered completely.

One dog with the left kidney removed, excreted 69% of barbital (225 mg. per kg., by vein) during a period of one week.

(b) *Fate in Blood.* All doses of barbital in the animal experiments were administered intravenously in the form of the soluble sodium salt, which changes to diethyl-barbituric acid in the blood, even if Na Barbital is added to drawn blood in the test tube. This fact was demonstrated by recovering all of the hypnotic from the blood without acidulation. The ratio of the amount of barbital taken up by the plasma to that taken up by the corpuscles is approximately 3 to 1.

The concentration of barbital in the blood was studied in normal and bilaterally nephrectomized dogs. Following the intravenous injection of the minimum anesthetic dose (225 mg. per kg.) in normal dogs, the following determinations were made (typical experiment): 1 minute, 0.4 mg. per cc. blood; 3 minutes, 0.375 mg.; 5 minutes, 0.3 mg.; 10 minutes, 0.25 mg.; 30 minutes, 0.225 mg.; 1 hr., 0.15 mg.; 4 hr., 0.12 mg.; 6 hr., 0.075 mg.; 24 hr., 0.018 mg. Following the intravenous administration of the same dose in bilaterally nephrectomized dogs, the following figures were obtained: 2 hr., 0.15 mg. per cc. of blood; 3 hr., 0.15 mg.; 4 hr., 0.15 mg.; 20 hr., 0.15 mg.; 40 hr., 0.15 mg. Other barbiturates were detected in the blood in far smaller amounts. The bilaterally nephrectomized dogs maintaining a constant barbital level in the blood never recovered from the anesthesia and eventually died. However, when bilaterally nephrectomized dogs were anesthetized with neonal, nembutal, or pernoson, they recovered from the anesthesia.

(c) *Presence in Organs.* The presence and concentration of barbital in organs was studied in dogs and cats. We submit 2 typical cases:

Dog, 8.23 kg., 225 mg. sodium barbital per kg. intravenously. Twenty-four hours after injection.

Liver (145.0 gm.)—75.0 mg. total
Muscle (138.0 gm.)—22.5 mg. total
Brain (71.0 gm.)—24.88 mg. total
Heart (65.2 gm.)—27.0 mg. total
Saliva (45.0 cc.)—20.0 mg. total

Kitten, 0.670 kg. 400 mg. sodium barbital per kg. intravenously. One hour after injection:

Heart (2.13 gm.)—1.0 mg. total
Kidneys (2.8 gm.)—3.37 mg. total
Liver (24.75 gm.)—14.4 mg. total
Brain (19.13 gm.)—3.75 mg. total
Spleen and pancreas (8.3 gm.)—2.25 mg. total
Muscle (33.2 gm.)—21.41 mg. total

Barbital was also recovered in the cerebro-spinal fluid and in the amniotic fluid and embryos of pregnant dogs.

In conclusion we might say that barbital is almost evenly distributed in the different organs without concentrating in any particular tissue.

(d) *Clinical Applications.* Poisoning with barbital is frequently encountered clinically, and during the brief span of 2 months, 5 cases were referred to us. In each case we were able to diagnose the poisoning by urine analysis. For example, 95 cc. of urine of Mr. H. G. (Gallinger Municipal Hospital) contained 383.0 mg. barbiturate. Subsequent investigations revealed that the patient had taken 6 tubes(?) of veronal tablets. Mr. P. (Gallinger Municipal Hospital) was transferred to the hospital in comatose condition. Two catheterized specimens of urine contained a total of 298.0 mg. barbiturate. At the same time, 0.15 mg. of barbiturate was found per cc. of blood.

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Inhibition of Diuresis by Hypnotics.

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The depression of diuretic function by anesthetics or hypnotics has often been attributed to certain effects peculiar to the type of anesthetic under consideration. Dooley and Wells[1] have suggested that ether anuria may be explained as a reflex arising from pulmonary irritation. Pick and collaborators[2, 3, 4, 5] have reported widely

[1] Dooley and Wells, *Am. J. Physiol.*, 1929, **90**, 330.