well with a hole of .028-in. diameter. Furthermore, the gas pressures apparently do not have to be adjusted as critically as with the hotter flame. The illuminating gas is taken directly from the laboratory supply line, and only a single regulator and the needle valve at the torch are used to control the flow of oxygen.

Extensive experience with the apparatus during more than a year of operation has shown that a single operator can take care of from 1000 to 1200 eggs per hour.

13754

Toxicity of 3,3′-Methylenebis (4-hydroxycoumarin).

CHARLES L. ROSE, PAUL N. HARRIS AND K. K. CHEN.

From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis.

Dicumarol,* chemically 3,3′-methylenebis(4-hydroxycoumarin), is the hemorrhagic principle isolated by Link and his coworkers.[1, 2] It can now be prepared synthetically by improved methods.[3] The substance is responsible for the "sweet clover disease" of cattle,[4, 5] and lowers prothrombin level of blood plasma.[6–10] In view of the recent clinical interest in dicumarol,[11–16] a systematic study of its toxicity became highly desirable.

* Dicumarol is the collective trademark of the Wisconsin Alumni Research Foundation, which controls the use thereof.

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11 Butt, H. R., Allen, E. V., and Bollman, J. L., *Proc. Staff Meet., Mayo Clin.*, 1941, **16**, 388.

12 Bingham, J. B., Meyer, O. O., and Pohle, F. J., *Am. J. Med. Sc.*, 1941, **202**, 563.

13 Meyer, O. O., Bingham, J. B., and Pohle, F. J., *J. A. M. A.*, 1942, **118**, 1003.

Single Doses. Mice, rats, and guinea pigs were employed. For both intravenous and oral administration, a fresh stock solution of the disodium salt of Dicumarol was prepared according to Professor Link's directions.[17] All doses were calculated on the basis of Dicumarol.

Animals receiving lethal doses by intravenous injection died in 3-4 hours. Amounts greater than lethal doses caused practically immediate death preceded by a series of clonic convulsions. Most animals surviving sublethal doses showed a prolonged period of respiratory depression. The symptomatology after oral administration was similar to that after intravenous injection, but the period of intoxication was longer. Death usually occurred in 3-4 days, or complete recovery took place. Bleeding from the nose and anus was apparent in many animals. All surviving animals were observed for a week before the experiment was concluded. The median lethal doses (LD_{50} ± standard error) determined intravenously and orally in mice and rats, and intravenously in guinea pigs, are shown in Table I.

Prolonged Administration. Four groups of 5, 6, 5, and 5 dogs, each received daily doses of 50, 20, 10, and 5 mg per kg, respectively. Their weight varied from 5 to 13.8 kg, average 9.14 kg. Dicumarol dispensed in gelatin capsules was given by mouth. All animals died within 28 days. Careful necropsies were performed, and microscopic examinations of viscera routinely made. Hemorrhage into various tissues and organs (stomach, intestines, lungs, thymus, and serous cavities), and pulmonary edema were present in most of the dogs. Of the 21 animals, 5 presented microscopic evidence of central necrosis of the liver—moderate in 1 but slight in the remaining 4.

Five groups of 5 rabbits each were injected by the marginal ear

TABLE I.
Acute Toxicity of Dicumarol.

Animal	Avg wt, g	Administration	No. animals used	LD_{50} ± S.E. mg per kg
Mice	16.2	Intravenous	32	64.30 ± 6.11
		Oral	48	232.8 ± 46.56
Rats	84.5	Intravenous	32	52.13 ± 1.79
		Oral	48	541.6 ± 67.7
Guinea Pigs	251.3	Intravenous	32	58.60 ± 2.29

[14] Barker, N. W., Butt, H. R., Allen, E. V., and Bollman, J. L., *J. A. M. A.*, 1942, **118**, 1003.

[15] Townsend, S. R., and Mills, E. S., *Canad. Med. A. J.*, 1942, **46**, 214.

[16] Lehmann, J., *Lancet*, 1942, **1**, 318.

[17] Link, K. P., private communication.

vein with daily doses of 2, 1, 0.5, 0.25, 0.1 mg per kg, respectively. Their body weight ranged from 1.75 to 2.14 kg, average 1.906 kg. The experiment was intended to last 6 weeks. All rabbits on daily doses of 1 and 2 mg per kg died within 10 days except one receiving the smaller dose, which died on the 29th day. Two out of 5 animals on 0.5 mg per kg also died on the 29th day. One out of 5 animals died after the 15th dose of 0.25 mg per kg. The other animals survived the entire experiment, namely 42 days. Necropsies of the rabbits that died revealed a uniform occurrence of hemorrhage and pulmonary edema. Out of 13 animals, 6 showed central necrosis of the liver—4 moderate and 2 slight. All the animals surviving 42 doses of 0.5, 0.25, and 0.1 mg per kg were sacrificed for postmortem examination, and found normal.

Five groups of 5 mice each ingested various concentrations of Dicumarol in food—0.5, 0.1, 0.05, 0.01, and 0.005%, respectively. The majority of them died within 9 days; others within 23 days; and 2 survived 30 days on 0.005% of the drug in food. Of the 23 mice which died from Dicumarol, hemorrhage and pulmonary edema were the prominent features, while central necrosis of the liver occurred in 2 animals. The 2 surviving animals were normal.

Six groups of 5 rats each were similarly fed Dicumarol in food—1, 0.5, 0.1, 0.05, 0.01, and 0.005% respectively. Twenty-one animals died within 9 days; 4 within 18 days; and 1 on the thirty-first day. One rat survived 30 days on 0.01% of Dicumarol, and 3 on 0.005%. Hemorrhage into various tissues and organs, and pulmonary edema were observed in most rats. Of the 25 animals examined which died from the toxic action of Dicumarol, 13 showed central necrosis of the liver. An example is given in Fig. 1. No pathologic changes could be detected in the 4 rats surviving 30 days of medication.

Comment. The above data indicate very clearly that Dicumarol is a drug toxic to animals, particularly by prolonged administration. As in the "sweet clover disease" of cattle, hemorrhage into tissues and organs is the most prominent feature of those animals which die from the administration of this substance. Pulmonary edema is also frequently encountered. The rat's liver appears to be more susceptible to Dicumarol than that of other species of animals, often displaying central necrosis, although the same lesion may occasionally occur in mice, dogs, and rabbits—more so in the last. It must be realized, of course, that these experiments were carried out under the most severe conditions, and that the results recorded in the present work should not be used as arguments against further careful

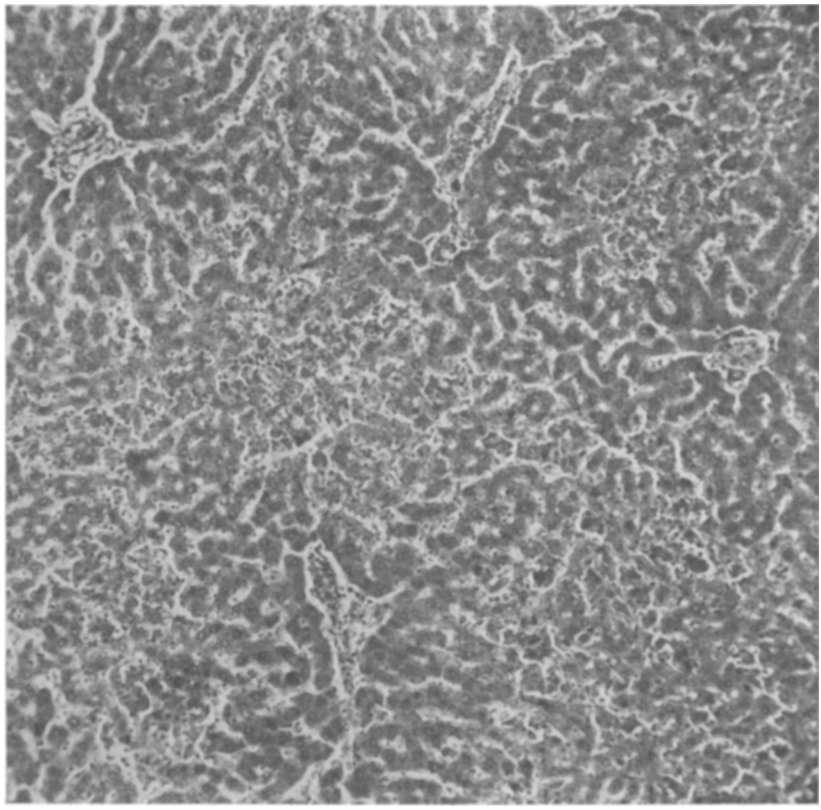

FIG. 1.
Action of Dicumarol on the Rat's Liver.
Rat numbered 16, female, weighing 100 g, was fed Dicumarol, 1% in food. It died on the sixth day. The daily food intake was recorded. The animal had ingested a total of 140 mg of the drug during the first 3 days, and it refused to eat thereafter. At autopsy the liver was found to be most affected. Note the central necrosis and leucocytic infiltration.

clinical trials. There is a possibility that amounts corresponding to therapeutic doses may be harmless. Our plea is the same as that of clinicians;[18, 19] that is, the administration of Dicumarol must be controlled by definite criteria such as the prothrombin determination and other appropriate tests.

Summary. (1) The median lethal doses of Dicumarol have been determined intravenously or by mouth in mice, rats, and guinea pigs. (2) Death uniformly occurs in rabbits with intravenous injection of daily doses of 1-2 mg per kg; in dogs with oral adminis-

[18] Meyer, O. O., Bingham, J. B., and Axelrod, V. H., *Am. J. Med. Sc.*, in press.
[19] Shapiro, S., Sherwin, B., and Gordimer, H., *Ann. Surg.*, in press.

tration of daily doses of 5-50 mg per kg; and in mice and rats with the feeding of 0.01-1% Dicumarol in food. The majority of rabbits can tolerate daily doses of 0.1-0.5 mg per kg by vein for 6 weeks, and a few mice and rats can survive 30 days on a diet containing 0.005% Dicumarol. (3) Most animals dying from Dicumarol develop hemorrhage into various tissues and organs, and pulmonary edema. Central necrosis of the liver has been observed in about one-half of the rats examined, and occasionally in rabbits, mice, and dogs.

13755

Anesthetic Activity of Some New Derivatives of Barbituric Acid.*

THOMAS C. BUTLER AND MILTON T. BUSH. (Introduced by P. D. Lamson.)

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tenn.

This is a study of 7 barbituric acids which, so far as we are aware, have not previously been referred to in the literature.

We have found all of these compounds to be anesthetic in mice. The anesthetic and lethal doses are shown in Table I. The corresponding data for two familiar drugs, pentobarbital and evipal, are included for comparison.

Methods. Animals. Male white mice were used in all of the experiments.

Administration of Drugs. All drugs were given as freshly prepared solutions of the sodium salts. (The doses shown in Table I are expressed in terms of the acid form.) The concentrations of the solutions were such that the mice received about 0.012 cc of solution per gram of body weight for the intravenous doses, about 0.017 cc per gram for the intraperitoneal anesthetic doses, and about 0.025 cc per gram for the intraperitoneal lethal doses. In the determination of the intravenous anesthetic doses, the injection occupied a period of ½ minute.

* This work was aided by a grant from Mallinckrodt Chemical Works. The first five compounds listed in Table I were kindly made for us by Mallinckrodt Chemical Works. The other two new compounds were synthesized in this laboratory.