Studies on Distribution of Dicumarol. (22506)

JACK PETER GREEN,* EBBE SONDERGAARD, AND HENRIK DAM.
Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark.

It has been shown that under certain experimental conditions between 60 and 80% of the dicumarol (3, 3'-methylenebis-(4-hydroxycoumarin)) in liver from dicumarol-treated rats is associated with the supernatant that remains after centrifugation of the mitochondria(1). As vit. K_1 effectively antagonizes dicumarol it seemed of interest to see whether the vitamin alters either the intracellular localization of the drug or its total concentration in liver or in blood. We have also measured the distribution of dicumarol in several tissues of the rat and chick.

Methods. Chicks and rats used in the experiment received a commercial chicken ration(2). The animals were fed dicumarol diluted in fructose for 2 successive days. Rats received 0.1 mg dicumarol per g body weight; chicks 0.7 mg per g body weight since they are relatively resistant to lower doses(3). Vit. K_1 was given at a level of 0.1 mg per g body weight in the form of a transparent colloidal solution in water(2). Forty-eight hours after the first treatment, prothrombin times were determined by the method of Dam and co-workers(4) on blood obtained from the jugular vein. The normal prothrombin times for chicks and rats are about 18-21 seconds by this method. The rats and chicks were then killed by decapitation (the rats after a blow on the head), and the tissues were homogenized in isotonic sucrose with Teflon homogenizers. Cellular fractionation was carried out as described by Schneider and Hogeboom (5), except that after separation of the mitochondria the remaining supernatant was not further fractionated. Dicumarol was determined by the method of Weiner and associates(6). Preliminary experiments showed that the apparent amount of "dicumarol" in livers of untreated chicks varied from 0.8 to 1.9 μg per g liver; rat liver had no blank dicumarol. The recovery of dicumarol added to liver homogenates from normal animals varied from 84 to 103%.

Results. The representative experiments shown in Table I demonstrate that vit. K_1, while markedly reducing prothrombin time, failed to influence concentration of dicumarol in either whole liver or its subcellular fractions. Dicumarol levels in chick liver were higher than in rat liver, which can probably be attributed to the higher amount administered to the chick. Mitochondria and supernatant fractions of chick and rat liver contained about the same amount of dicumarol: the nuclear fraction of chicken liver contained 2-3 times the quantity of dicumarol found in the same fraction of rat liver. Undoubtedly part of the dicumarol found in the supernatant represents intravascular dicumarol. Livers from chicks given dicumarol in doses of 0.1 mg per g body weight on two consecutive days and killed 24 hours after the last dose contained only insignificant amounts of dicumarol.

In Table II, it may be seen that vit. K_1 did not influence blood levels of dicumarol although it prevented prolongation of the prothrombin times by dicumarol.

Table III shows that liver, blood and kidney contain the highest concentration of dicumarol, as has been observed previously for dicumarol(6,7) and other coumarin anticoagulants(7-9). Part of the drug that appears to be in tissues is actually in blood: in one experiment, perfusing the liver decreased its dicumarol concentration from 24 to 15 μg per g.

Discussion. After one injection of the dose of vit. K_1 used in these experiments the liver contained about 50% of the injected amount (10,11). The deposition is assumed to take place mainly in reticulo-endothelial tissue (10). The level of vit. K_1 used (0.1 mg per g body weight) corresponds to about 1200 times the dose that would have produced normal prothrombin time in 20 hours when given

* Fellow of Life Insurance Medical Research Fund. Present address: Yale University School of Medicine, New Haven, Conn.

TABLE I. Intracellular Distribution of Dicumarol.

Species	Body wt, g	Treatment* 0 hr	24 hr	36 hr	48 hr	Prothrombin time	μg dicumarol/g liver: Whole homogenate	Nuclei	Mitochondria	Supernatant
Chick	344	D K_1^{iv}	D	K_1^{iv}	k	21″	72	31	9	28
	356	D K_1''	D K_1''	K_1''	k	2′ 10″	74	31	9	29
	346	D	D		k	10′ 00″	78	33	8	29
Rat	287	d K_1^{iv}	d K_1^{iv}		k	18″	56	14	9	34
	298	d	d		k	3′ 12″	44	9	6	32

* D = Dicumarol, .7 mg/g body wt, orally. d = Dicumarol, .1 mg/g body wt, orally. K_1^{iv} = Vit. K_1, .1 mg/g body wt, intravenously. K_1'' = Vit. K_1, .1 mg/g body wt, orally. k = killed.

orally to vit. K deficient chicks(4). We have given 2 such injections and yet, while preventing dicumarol-induced hypoprothrombinemia, the vitamin did not change either the liver content of dicumarol or its distribution among the liver fractions. Conversely, other experiments suggest that coumarin-anticoagulants may cause a prolonged prothrombin time even when the liver contains large stores of vit. K_1

TABLE II. Blood Levels of Dicumarol.

Chick body wt, g	Treatment* 0 hr	24 hr	48 hr	Prothrombin time	μg dicumarol/ml blood
359	D K_1^{iv}	D K_1^{iv}	k	18″	59
383	D K_1^{iv}	D K_1^{iv}	k	18″	62
390	D	D	k	1′ 43″	30
387	D	D	k	2′ 04″	53

* D = Dicumarol, .7 mg/g body wt, orally. K_1^{iv} = Vit. K_1, .1 mg/g body wt, intravenously. k = killed.

(11). Correction of a dicumarol-induced hypoprothrombinemia by vit. K_1 does not appear to depend on gross displacement of the drug from either whole liver or its subcellular fractions. Nor does the vitamin appear to affect the blood levels or metabolism of the drug.

Lee and co-workers(12) have shown that injection of vit. K in the form of 2-methyl-1, 4-naphthohydroquinone phosphoric acid ester favored more rapid displacement of dicumarol from the livers of rabbits and mice. As they administered dicumarol intravenously and determined dicumarol at shorter intervals, the results cannot be directly compared. Jaques (13-15) has emphasized that there exists a correlation between the time dicumarol remains in the liver and the duration of hypoprothrombinemia. Others(16-18) have noted that, in general, plasma levels of dicumarol and related drugs are correlated with duration of the prothrombin response. Our experiments indicate that dicumarol levels in both liver and blood may remain elevated at the same time that prothrombin times become normal.

Summary. Vit. K_1 given intravenously in massive doses to dicumarol-treated rats and chicks did not influence the concentration of dicumarol in whole liver, the subcellular fractions (nuclei, mitochondria and supernatant) of liver, or whole blood, while the usual dicumarol-induced hypoprothrombinemia was prevented. The tissue distribution of di-

TABLE III. Tissue Distribution of Dicumarol.

Species	Body wt, g	Prothrombin time	μg dicumarol/g or ml of: Liver	Kidney	Lung	Spleen	Brain	Heart	Skeletal muscle	Blood	Plasma
Chick*	365	4′ 42″	55	48	21	36	16	32	7.2	49	73
	340	8′ 32″	65		16	30	13	28	7.8	42	68
Rat*	266	2′ 25″	24	15	7.9	2.3	1.8	5.3	2.2	11	
	286	4′ 38″	18	9.8	6.8	.8	1.4	2.5	1.9		
	244	4′ 40″	45	21	20	1.4	5.6	14	5.7	35	
	286	5′ 50″	40	18	19	1.7	2.3	12	2.4	23	

* Chicks and rats were treated with dicumarol given in doses of 0.7 mg and 0.1 mg/g body wt, respectively, on 2 consecutive days and killed 24 hr after the last dose.

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Increase in Induced Pulmonary Tumors in Mice Associated with Exposure To High Concentration of Oxygen. (22507)

W. E. HESTON AND A. W. PRATT.

National Cancer Institute, N. I. H., Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

Reports on the effect of concentration of oxygen upon mutation rate have been of considerable interest to those investigating relationships between mutagenesis and carcinogenesis. The original report was that of Thoday and Read(1) who observed that bean root cells X-irradiated in nitrogen contained a lower proportion of chromosomal aberrations than those irradiated in oxygen. Subsequent work(2-4) has shown that the concentration of oxygen affects the rate of gene mutations as well as that of chromosomal changes, and has also shown that the effect is not limited to irradiated cells. Increased interest in possible relationships of these observations to carcinogenesis arose when Plaine and Glass(3) reported an increase in occurrence of melanotic tumors in *Drosophila* larvae irradiated in the presence of increased concentrations of oxygen. Recently Plaine (5) has reported an increase in the melanotic tumors with increase in concentration of oxygen without radiation.

Comparison of these melanotic tumors in *Drosophila* with neoplasms in mammals morphologically is limited, but genetically similarities between the two exist. This fact together with the observations that many agents known to increase mutation rate in lower organisms are carcinogenic when tested in mice made it highly desirable to test the concentration of oxygen on the occurrence of neoplasms in mice. The fact that the effects of oxygen in the lower organisms was not limited to irradiated cells increased the possibilities for a study of the effect of concentration of oxygen on carcinogenesis. The purpose of this report is to record an increase in occurrence of pulmonary tumors in mice exposed to a high concentration of oxygen.

Materials and methods. Pulmonary tumors in the genetically highly susceptible strain A