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Dicumarol (3,3'-Methylene-Bis-(4-Hydroxy-Coumarin)) in Rats with Impaired Liver or Kidney Function.

R. K. Richards and F. R. Steggerda.*
From the Department of Pharmacology, Abbott Laboratories, North Chicago, Ill.

Extensive research has been carried on in recent years concerning the use of the anticoagulating agent, Dicumarol, as a substitute for Heparin.[1,2] Of the numerous problems to be investigated before such a substance can be used with safety in clinical medicine, not least in importance is the effect of its use in pathological cases. It is the purpose of this paper to report the effects of administering Dicumarol to rats with impaired liver or kidney function.

While our work was in progress, Bollman and Preston[3] reported that dogs in which liver damage had been induced by the administration of carbon tetrachloride, or in which kidney impairment was produced by subcutaneous injections of uranium acetate showed a markedly increased sensitivity to Dicumarol.

In this paper we report similar experiments, done on rats instead of dogs, since there is a difference between dogs and rats in susceptibility to Dicumarol.[4] Furthermore, our experiments were done over relatively long periods of time on a large number of animals in an attempt to show quantitative differences in response to Dicumarol at different stages of impairment of these organs.

Procedure. In all cases albino rats weighing 125-175 g were kept on a special diet recommended by Overman, Field, Baumann and Link[5] because of the more consistent results obtained in their observations with Dicumarol over those of ordinary stock diets. Blood samples were collected by cardiac puncture, and the plasma diluted with saline to a 12.5% concentration. A modification[6] of the Quick method was used in making the prothrombin determinations. The dosage of Dicumarol was 2.5 mg given by stomach tube in a suspension of .5% gum acacia. In nearly all cases blood collections were made 24 hours after the administration of Dicumarol, this time interval being used because in the rat it has been demonstrated to be the point of maximal effect.[4]

Liver damage was produced by subcutaneous injection of .2 cc/kg or .5 cc/kg carbon tetrachloride. These injections were made daily in a large number of rats. A part of these animals were used as controls and their blood prothrombin time determined in intervals of one or more days. The other part was fed 2.5 mg Dicumarol once after having been treated for several days with carbon tetrachloride. Prothrombin time was determined 24 hours later.

Fig. 1 shows the result in the form of a graph. Every point on the curve represents the average prothrombin time of groups of 6 to 15 animals except the last 2 determinations at 14 days, with .5 cc/kg carbon tetrachloride, where only 2 and 5 animals were still alive. The graph clearly demonstrates that the injections of carbon tetrachloride alone produce only a mild, if any, elevation of the prothrombin time, even after administration is continued for as long as 14 days.

The observations of previous investigators[7,8]

* Department of Physiology, University of Illinois, Urbana, Illinois.

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2 Allen, E. V., Barker, N. W., and Waugh, J. M., *J. A. M. A.*, 1942, **120**, p. 1009.

3 Bollman, J. L., and Preston, F. W., *J. A. M. A.*, 1942, **120**, 1021.

4 Overman, R. S., Stahmann, M. A., Sullivan, W. R., Huebner, C. F., Campbell, H. A., and Link, K. P., *J. Biol. Chem.*, 1942, **142**, 941.

5 Overman, R. S., Field, J. B., Baumann, C. A., and Link, K. P., *J. Nutrition*, 1942, **23**, 589.

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7 Cameron, G. R., and De Sarem, *J. Path. and Bact.*, 1939, **48**, 49.

8 Cameron, G. R., and Karumaratue, W. H. E., *J. Path. and Bact.*, 1936, **42**, 1.

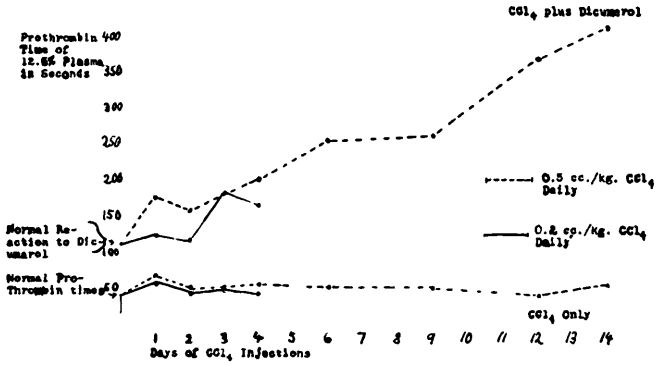

FIG. 1.
Effect of One Dose of 2.5 mg Dicumarol in Rats after Repeated Subcutaneous Injections of CCl_4.

as well as our own histological examinations prove that only a few injections of this dose were adequate to produce definite liver damage. Our findings, as represented in the graph, confirm those of others[9,10] in that the injured liver alone, unless such injury is very extensive, does not appreciably change the prothrombin time. The upper curves of the graph demonstrate how animals treated with carbon tetrachloride react to Dicumarol with a much more intensive depression of prothrombin level than with animals without liver damage. This increased sensitivity occurs earlier with higher doses of carbon tetrachloride and becomes more pronounced with the increasing number of carbon tetrachloride injections.

One notices on all 4 curves that the prothrombin time on the second day is slightly below the value on the first day. One may speculate whether this is due to a transitory stimulation of prothrombin formation.

It is also interesting that carbon tetrachloride-treated rats show an increased response to Dicumarol at a time when their prothrombin level is normal or only slightly elevated.

In another series of experiments, we tested the effect of kidney impairment upon the action of Dicumarol. Unilateral or bilateral nephrectomy was performed on rats. In the control group we found that the nephrectomy caused no change from normal in prothrombin time over a period of 4 days, at the end of which the rats died. Unilateral nephrectomy failed to change the reaction of such animals to Dicumarol. The bilaterally nephrectomized group which received Dicumarol immediately after the operation showed the same degree of prothrombin time elevation within 24 hours as normal animals do. However, 48 hours after the administration of Dicumarol, instead of going down as usual, the prothrombin time had increased from an average of 120 seconds to 229 seconds. In 72 hours it had reached 270 seconds. (See Table I.) These results would indicate that complete loss of kidney function greatly increases the effect of Dicumarol.

It might be argued that the accumulation of urea could affect the Dicumarol action on prothrombin time. In a limited number of experiments we found that 1.5 g of urea administered simultaneously with Dicumarol had no greater influence on prothrombin time than Dicumarol alone would have.

Since the exact mode of action and the fate of Dicumarol in the body is not entirely known, no complete explanation of the above-described findings can be given. However, it appears that liver damage interferes with the reserve power of the liver to produce needed prothrombin. As long as that damage is mild, the prothrombin level can obviously be sustained at about normal values. But the additional depressing effect of Dicumarol results in a marked lowering of the blood prothrombin content.

The results in nephrectomized animals would indicate that the kidney apparently is

[9] De Lor, C. J., and Reinhart, H. L., *Am. J. Clin. Path.*, 1940, **10**, 617.

[10] Steggerda, F. R., and Richards, R. K., *Anesthesia and Analgesia*, 1943, **22**, 1.

TABLE I.
Effects of Double Nephrectomy on Prothrombin Time in the Rat Before and After Administration of Dicumarol by Stomach Tube.
Prothrombin Time in Seconds.

Without Dicumarol				After Dicumarol (2.5 mg)				
No. of animals	Days after nephrectomy	Range, sec.	Avg, sec.	No. of animals	Days after nephrectomy	Time after Dicumarol (hrs)	Range, sec.	Avg, sec.
8	2	33-43	38	4	1	24	85-175	117
3	4	39-43	41	6	1	24	62-225	120
			—	10	1	24	60-188	130
		Avg	40	6	3	24	82-145	107
				7	1	24	100-154	128
							Avg	120
				7	3	48	98-367	197
				7	2	48	150-348	234
				10	2	48	103-540	256
							Avg	229
				6	3	72	107-465	270
							Avg	270

not predominantly concerned with the metabolic effect of Dicumarol, since the depression of prothrombin level after 24 hours is the same in normal and nephrectomized animals. However, it must be assumed that Dicumarol or an active split product leaves the body by way of the kidney. This would explain the continued and increased depression of the prothrombin level in nephrectomized animals beyond the time in which a return to normal occurs in intact rats.

Summary. Experiments in rats are described in which the effect of gradually increasing liver damage upon the action of Dicumarol was studied. Injections of carbon tetrachloride which do not significantly influence the prothrombin level greatly sensitize rats to Dicumarol. Bilateral nephrectomy results in a prolonged and increased effect of this drug. These findings indicate the necessity of care in the administration of this drug to patients having even a mild degree of liver damage or suffering from impairment of the kidney function.