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Action of Sodium Salicylate on Prothrombin Time in Rabbits.*

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Link[1] has postulated, on the basis of the degradation of dicumarol to salicylic acid *in vitro*, that the hypoprothrombinemic action of dicumarol is due to its degradation to salicylic acid *in vivo*. Supporting this hypothesis was the finding of Link, Overman, Sullivan, Huebner, and Scheel[2] that single doses of salicylic acid administered either orally or intravenously to rats maintained on an artificial diet low in vitamin K, caused a temporary hypoprothrombinemia. The increase in prothrombin time after administration of salicylates has been confirmed clinically by Rapoport, Wing and Guest,[3] Shapiro[4] and others. However, Link's views on the relationship of the action of dicumarol and salicylate on prothrombin have not been supported. Link's data indicate that salicylates have a very weak action in lowering prothrombin compared with dicumarol. Lester[5] failed to find salicylates in the urine after the administration of dicumarol to rats. Stefanini and Petrillo[6] observed that the addition of sodium salicylate to human plasma *in vitro* in concentrations greater than 0.2% markedly lengthened the prothrombin time. Clark and Spitalny[7] have observed that other analgesic-antipyretic drugs (antipyrine, aminopyrine, acetanilid, acetophenetidin and cinchophen) have a similar action on prothrombin time, and that the prothrombinopenic action of salicylate was greatly augmented by hyperthermia and by an increased metabolism from other causes. This suggests that an important contributing factor to the action of salicylates on prothrombin clinically is the accompanying clinical syndrome, and that the effect observed by Link and co-workers is a non-specific effect, unrelated to the action of dicumarol.

Since dicumarol has been synthesized from salicylic acid and since dicumarol is presumably formed from the coumarins in spoiled sweet clover by bacterial action,[1] there remains as a further possible basis for the prothrombopenic action of salicylates, the conversion of these substances to dicumarol or related compounds. To test this possibility, a study has been made of the response to dicumarol and sodium salicylate in rabbits upon oral and intravenous administration.

Methods. Normal rabbits of 2 kg body weight and maintained on the normal colony diet were used. Prothrombin times were determined daily, on both undiluted plasma and also on 50, 25, 12.5 and 6.25% plasma. As reported by previous workers, the salicylate had little effect on the prothrombin time of undiluted plasma so that all the results reported are for the 12.5% plasma. Prothrombin times were determined by the Quick technique, using an acetone-dried horse brain powder for preparation of the thromboplastin.[8] We are indebted to Dr. L. A. Kazal of Sharp and Dohme, Inc., who kindly supplied this material. To control minor variations in thromboplastin, in all cases a normal rabbit with the same initial prothrombin time was included in each series and the prothrombin times are reported as experimental/(normal

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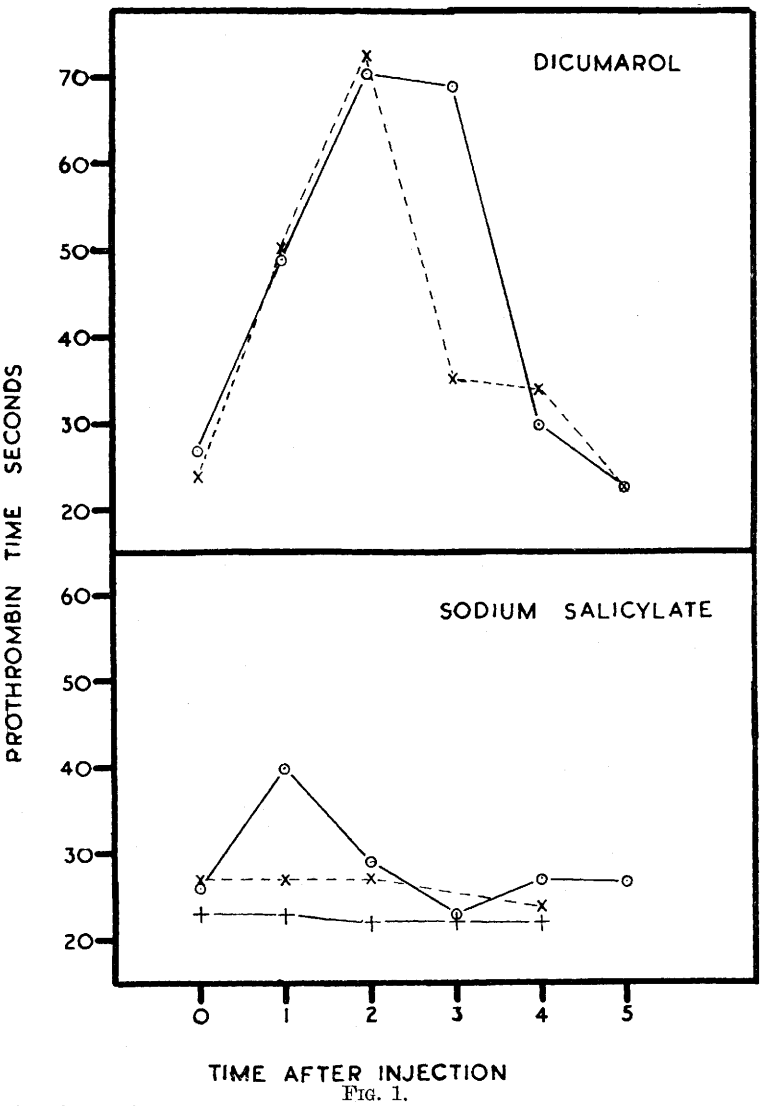

FIG. 1.

Effect of sodium salicylate and dicumarol on prothrombin time in rabbits. Rabbit 16. 6 mg of dicumarol, 0.8 g of sodium salicylate. —○—○— Oral; ——×——×—— Intravenous; —+—+— 1 g of sodium salicylate + 2 g of sodium sulfasuccidine orally.

control). The prothrombin response of each rabbit to 6 mg of dicumarol and 0.5 g/kg of sodium salicylate was determined both on oral and intravenous administration. The response was also determined after the oral administration of 2 g of sodium sulfasuccidine and of 2 g of sodium sulfasuccidine plus 0.5 g/kg of sodium salicylate. The sodium sulfasuccidine was given in 4 divided doses at 9 a.m. and 4 p.m. on 2 successive days, the sodium salicylate being given at 2 p.m. of the second day. After the experimental period of 5 to 6 days, a period of 4 days to several weeks was allowed before testing the next dose. The responsiveness of the animals was checked at intervals in the course of the experiment with the standard dose of dicumarol intravenously. A similar series was attempted using acetylsalicylic acid but it was found that the dose required orally to affect the prothrombin time was above the lethal dose when given intravenously.

TABLE I
Peak Prothrombin Times (12.5 % plasma) of Rabbits after Administration of Dicumarol and Sodium Salicylate.
secs.

	Salicylate			Dicumarol	
Rabbit	Oral	Intrav.	+ sulfa-succidine.	Oral	Intrav.
6	31.0 (26.5)	26.8 (26.7)		69.2 (24.8)	71.4 (26.3)
11	26.8 (25.0)	25.2 (26.3)	22.4 (21.8)	45.0 (24.8)	65.0 (26.2)
12	27.0 (24.8)	26.2 (26.3)	22.0 (21.8)	70.2 (25.9)	72.3 (22.6)
13	31.0 (27.2)	26.5 (26.4)		32.0 (25.0)	47.5 (26.3)
16	40.0 (27.2)	27.8 (27.3)	23.0 (21.8)	70.2 (25.9)	72.3 (22.6)

( ) = control prothrombin time on normal rabbit.

Results and Discussion. The prothrombin time response to the oral and intravenous administration of dicumarol and of sodium salicylate and to the oral administration of sodium salicylate + sodium sulfasuccidine are shown in Fig. 1. The peak prothrombin times obtained in 5 rabbits with these substances are reported in Table I. A definite increase in prothrombin time was observed after the oral administration of sodium salicylate. However, with the same dose administered to the same rabbit intravenously, no change in the prothrombin time was observed, although as previously reported by many investigators, the prothrombin response to dicumarol given intravenously to the same animal was of the same extent as after oral administration. Since Vitamin K was not withheld from the diet, the oral administration of sodium sulfasuccidine did not affect the prothrombin time. However, as shown in Table I, it did prevent the increase in prothrombin time after the administration of sodium salicylate.

The most direct explanation of the difference in the results on oral and intravenous administration of sodium salicylate is conversion of salicylate to dicumarol or a similarly acting compound in the intestinal tract. The action of sodium sulfasuccidine in abolishing the prothrombopenic action of salicylate suggests that this conversion is due to bacterial action. Attempts have been made to demonstrate such conversion with intestinal contents *in vitro,* by isolation of the products. This was unsuccessful. It may have been due to adverse conditions for bacterial synthesis or due to the small amounts of material involved. The prothrombopenic action of 1 g of sodium salicylate in the rabbits was equivalent to that of approximately 2 mg of dicumarol, so assuming formation of this compound, less than one per cent of the salicylate underwent conversion.

These results are somewhat at variance with those of Link[1] who reported that intravenous administration of sodium salicylate to rats did cause an increase in prothrombin time. This difference in results may be due to the use of a different animal species and also may be related to the vitamin K deficiency produced in the rats by Link. Further, Link does not give direct comparisons of oral and intravenous administration and his results are the average response of 6 rats. As originally shown by Link[1] on rabbits, marked differences occur in the response of individual animals to dicumarol (and also salicylate, Table I), and this is accentuated on a vitamin K-deficient diet. The results of Clark and Spitalny, of Stefanini and Petrillo, and the results reported here suggest that there are 3 possible mechanisms operative in the increased prothrombin time after the administration of salicylates, namely, a general action related to their analgesic-antipyretic properties, a direct effect on the clotting system of the blood, and lastly, conversion in the intestinal tract to dicumarol or a similarly acting compound.

Summary. The effect on the prothrombin

time of 12.5% plasma, of oral and intravenous administration of sodium salicylate, and of dicumarol has been compared in rabbits on a normal diet.

The oral and intravenous administration of dicumarol gave the same prolongation of the prothrombin time. However, while the oral administration of 0.5 g/kg of sodium salicylate definitely prolonged the prothrombin time, the intravenous administration of the same dose in the same animal did not result in any change in the prothrombin time. After the oral administration of sodium sulfasuccidine, the oral administration of sodium salicylate did not affect the prothrombin time.

It is suggested as an explanation of these results that salicylate may be converted to dicumarol or a substance with similar prothrombinopenic properties by bacterial action in the intestinal tract.

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Quantitative Aspects of the Inhibition of Anaphylactic Shock in Guinea Pigs.

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While the modifying action of the proprietary compounds β-dimethylaminoethyl benzhydryl ether hydrochloride (benadryl) and N′-pyridil-N′-benzyl-N-dimethylethylenediamine (Pyribenzamine) on histamine and anaphylactic shock has been vigorously investigated,[1] some conflicting data have appeared in the literature with regard to the action of these drugs on true anaphylactic shock in guinea pigs. Mayer, *et al.*,[2] demonstrated that 5 guinea pigs injected with horse serum 21 days before, were protected against an intracardial shock dose of 0.5 cc of horse serum when they were previously treated subcutaneously with 1.0 mg/kg of pyribenzamine. Campbell, *et al.*,[3] reported that although benadryl offered effective protection against histamine shock in the rabbit, it was ineffective in controlling anaphylaxis in actively sensitized (hen egg white) rabbits and guinea pigs. These latter workers used shock doses of 0.75 cc of the antigen for guinea pigs, given intraperitoneally. They conclude that their results seem at direct variance with those of Loew and Kaiser[4] and of Friedländer, *et al.*,[5] since these groups had found, similarly to Mayer's group,[2] that benadryl offers marked protection against anaphylaxis in the guinea pig.

Since in none of this previous work on benadryl and pyribenzamine cited was the attempt made to put protection against anaphylaxis in the actively sensitized animal on a quantitative basis, similar experiments were repeated with this view in mind.

Materials and Methods. The whites of 3 hen eggs were separated from the yolks, strained through cheesecloth into a beaker and then stored in a rubber stoppered vaccine bottle in the refrigerator. This material (Pro-

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