or amino acids. The combination of nicotinamide and biotin supports slow growth and under certain conditions nicotinamide alone may suffice.

Tests with the 4 vitamins singly and in various combinations show that the response to the vitamins depends to some extent upon the basal medium used in the tests. The results emphasize the importance of the composition of the basal medium in studies of vitamin requirements.

14270

Studies on Prothrombin: IV. The Prothrombinopenic Effect of Salicylate in Man.

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Following the demonstration[1] of the quantitative degradation of 3,3'methylenebis(4-hydroxycoumarin) to 2 mols of salicylic acid, Link and his students[2] demonstrated that in the rat salicylic acid induces a prothrombinopenia preventable by natural or synthetic vitamin K.

The purpose of this paper is to report that the fundamental observations made on the rat also apply to man.†

Experimental. The action of sodium salicylate, of acetyl salicylic acid with and without dicumarol (3,3'methylenebis(4-hydroxycoumarin)), and the effect of synthetic vitamin K were studied on individuals representing types in which salicylate therapy is commonly used. Since there was variation in age, nutritional state and diet, the salient facts can best be illustrated by representative cases and with dosages comparable to those used therapeutically.[3]

Twenty-seven adults varying in ages between 24 and 80 years were studied. Eight were apparently normal and ambulatory; 12 were chronically ill patients; 6 were cases of Laennec's cirrhosis of the liver; 1 was a case of acute rheumatic endocarditis.

The method used to estimate the prothrombin level (or activity) included a measure of the prothrombin time of whole and diluted (12.5%) plasma.‡ The rationale and clinical applications have been set forth in previous communications.[4-7] By estimation of 12.5% plasma prothrombin time the initial prolongation has usually been detected between 8 and 24 hours after adequate dosage of salicylate. It is obvious from Fig. 1 that detection of a change in prothrombin time on whole plasma is restricted to a very slight shift in seconds, but with 12.5% plasma a more practical working range is afforded.

Action of Sodium Salicylate. Fig. 2, curve 1 shows the response in a patient who received 8 g of sodium salicylate on 3 consecutive days. Two others receiving the same

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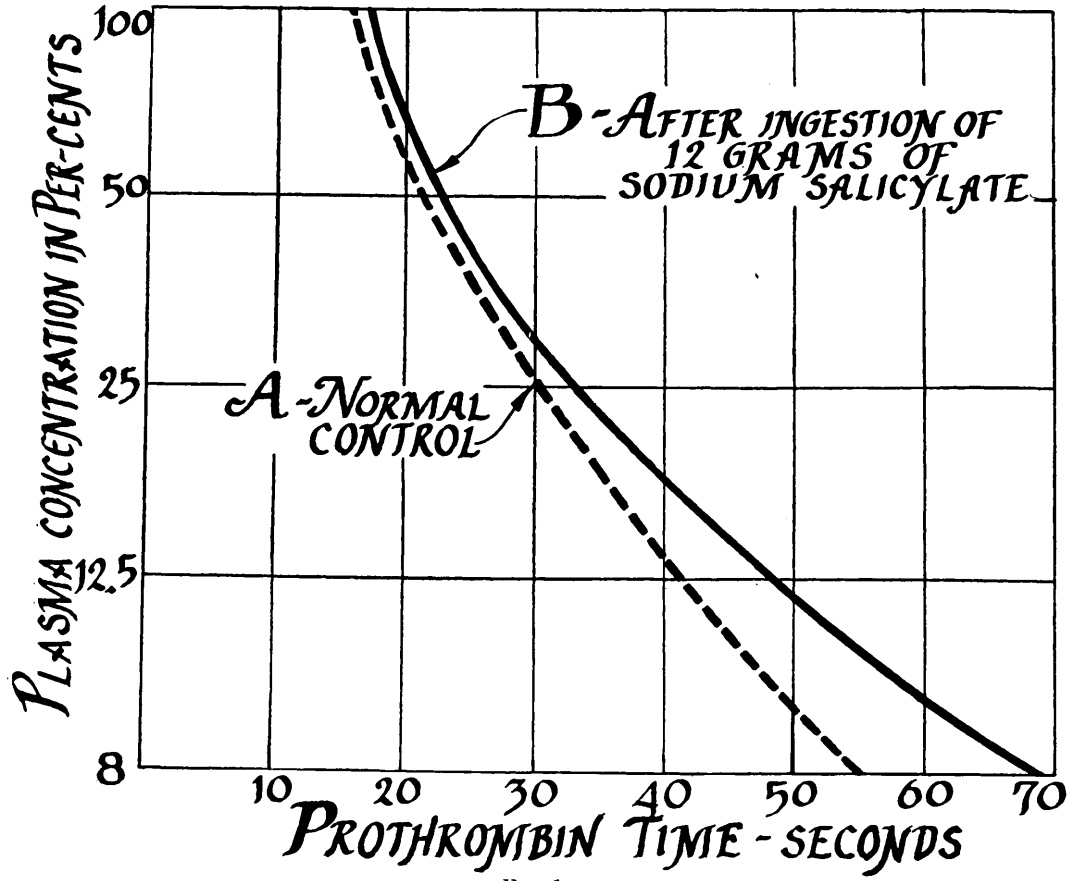

FIG. 1.
Representative dilution curve of normal and sodium salicylate-prothrombinopenic plasma.

dose for one day only showed increases of 6 and 9 seconds and 8 who were given 8 g daily for 2 successive days showed prolongation of 7 to 12 seconds (average 10). Five cases of Laennec's cirrhosis§ exhibited more pronounced prolongation after 16 g in 2 days. Four of these revealed increases between 13 and 23 seconds (average 17) and one yielded an extension from an initial value of 56 to 99 seconds.‖

Two cases in which the drug had to be withdrawn during the first day because of vomiting showed no increase in the prothrombin time. In one case of acute thrombophlebitis which initially revealed a reduction below normal of the difference between whole and 12.5% plasma prothrombin time (hyperprothrombinemia),[4] no alteration in the prothrombin time was detected after 24 g of sodium salicylate had been given in 3 days.

Action of Acetyl Salicylic Acid. Four subjects showed prolongation varying from 5 to 31 seconds in the 12.5% plasma prothrombin time after ingestion of 6 g of the drug in one day. Increases of 8, 12, and 14 seconds after 8 g in one day were found in 3 subjects, and in 1 an extension of 10 seconds after ingesting 16 g in 2 days.

Protective Action of Synthetic Vitamin K. Fig. 2, curve 2, shows the response of an apparently normal man, weighing 70 kg, to 8 g of sodium salicylate on each of 2 successive days, and then the protective action obtained with a very high dosage of synthetic vitamin K (20 mg orally and 2 mg intramuscularly 3

§ All had moderate prothrombinopenia: 12.5% plasma prothrombin time was 1½-2 times the normal value.[5]

‖ These findings are similar to those obtained in cirrhotics with dicumarol.[5]

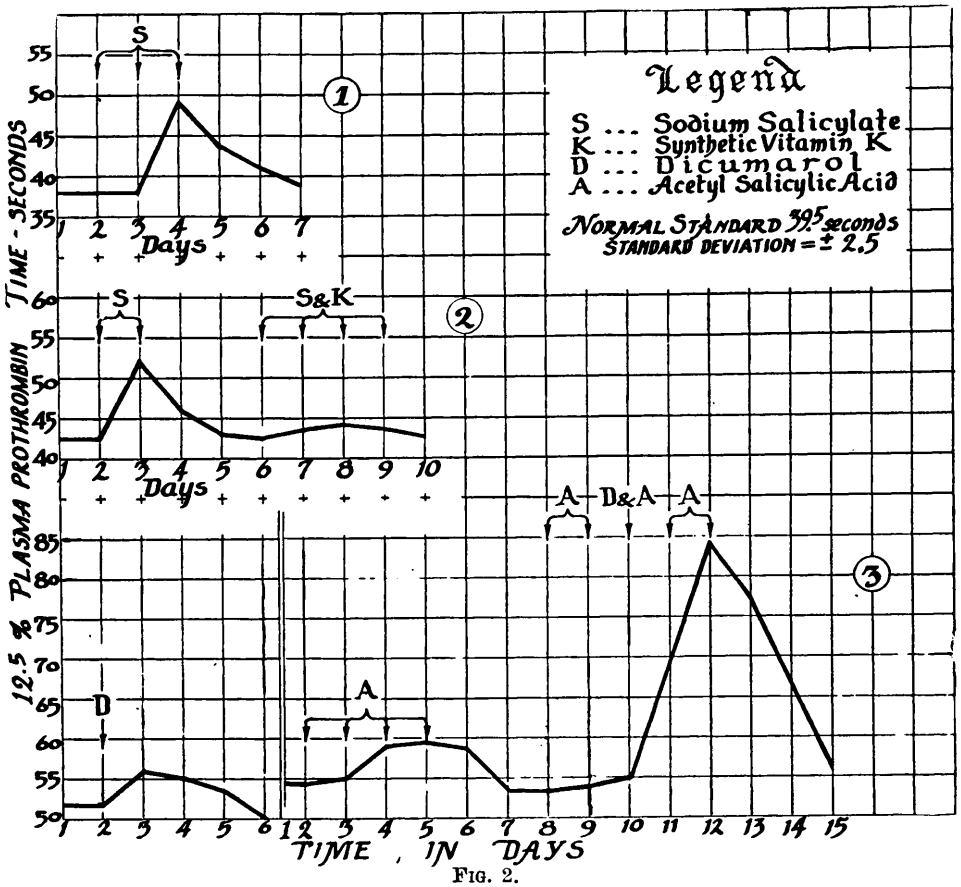

FIG. 2.
Representative effects of salicylate, protective action of synthetic vitamin K, and the combined action of acetyl salicylic acid and dicumarol.

times daily) on 4 successive days, with the same dose of salicylate. Three additional normals and 1 cirrhotic were treated similarly. In the normals the prothrombin time remained substantially the same, while the case of cirrhosis showed approximately the same degree of prolongation as when salicylate alone was given. At the outset these very high levels of synthetic vitamin K were used to insure adequate protection. Subsequent studies indicate that in normals 1 mg synthetic vitamin K intramuscularly can counteract the prothrombinopenia induced by 6 g acetyl salicylic acid given for 1 day.

Combined Effect of Acetyl Salicylic Acid and Dicumarol. Fig. 2, curve 3, illustrates the response in a case of Laennec's cirrhosis with preëxisting prothrombinopenia. The response to a single dose of 50 mg dicumarol is shown first (a comparable increase in a normal subject requires 100-150 mg dicumarol). One week later 4 g acetyl salicylic acid were given for 4 consecutive days. On the seventh day the prothrombin time was at the control level. On the eighth to the twelfth day inclusive 4 g acetyl salicylic acid were given daily. On the tenth day 50 mg dicumarol was also administered. The prothrombin time became prolonged to a maximum of 84 seconds, the control value being restored in 3 days.

The identical procedure was followed in 2 normal subjects. In neither instance did the 50 mg dicumarol or the acetyl salicylic acid alone increase the prothrombin time. Administered together the 2 drugs effected a prolongation in 1 case of 16 and in the other of 18 seconds.

Discussion. The significant prothrombinopenic action of salicylates raises a series

of problems for clinical workers. The diet and nutritional state (vitamin K-prothrombin reserves) of subjects being treated with salicylates merit close attention.[8-12] The fact that these vary in different individuals accounts for the inequalities in response noted above and shown in Fig. 2. This is also true of responses obtained with dicumarol.[5]

Cirrhosis of the liver and pre-existing prothrombinopenia which are known to augment the action of dicumarol[5] exert the same influence when salicylate is administered; both drugs may act through the same enzyme system in the liver.[13-14]

The different salicylates vary in potency as prothrombinopenia-inducing agents. In man as well as in the rat¶ acetyl salicylic acid seems to be more potent than the sodium salt. Additional data on this question as well as minimum effective dosage levels of vitamin K will be reported later.

The complementary action of salicylate and dicumarol may be important in the clinical use of the latter. Their accidental concomitant administration may be the basis for some of the extraordinary responses observed in man.** Both drugs might under certain conditions be given simultaneously to advantage. Work in Link's laboratory¶ (unpublished) indicates that animals can be kept mildly prothrombinopenic without manifestations of bleeding by first giving a moderate dose of dicumarol followed by smaller dicumarol-salicylate dosage.

Based on the fact that the prothrombinopenia can be restored to normal by a preparation of purified prothrombin,[1] fresh blood transfusion and vitamin K should be effective adjuncts in counteracting acute salicylate poisoning. The warning of Sir Arthur Hurst[15] that clinicians should be alert to the possibility of aspirin producing gastric hemorrhage when other known causes of bleeding have been excluded, is well sustained.

Summary and Conclusions. In man as in the rat, salicylate in adequate dosage induces prothrombinopenia which can be prevented by vitamin K compounds, if liver function is adequate. The effect on the prothrombin level (or activity) is detected most readily by the use of 12.5% plasma. The dietary intake particularly of vitamin K appears to play a significant role in determining the extent and duration of the prothrombinopenia. Cirrhosis of the liver and pre-existing prothrombinopenia augment the effect of salicylate. Acetyl salicylic acid appears to be a more potent agent than sodium salicylate. The action of salicylate is apparently identical with that of the anticoagulant dicumarol, but less effective. The two drugs can complement each other. This may be useful in the clinical application of dicumarol and should be realized to avoid excess prothrombinopenia.

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