of the streptococcus C203, it was necessary to use 8-hour cultures, to terminate the incubation after 16 hours, to employ 0.5% glucose broth, and to use inocula of higher counts. The experiments were otherwise carried out essentially as described above. Sterility tests were done after the incubation period, by inoculating 1 cc of the solutions into blood agar pour plates.

The results in Table III, pertaining to the first 4 substances, were obtained in the course of the experiment using inocula of 27,000 streptococci. Inocula of 153,000 organisms were used in the experiment which gave the data with respect to "Synkamin" and "Hykinone." It will be noted that several of these substances appear to exert a considerably greater antibacterial action against the C203 streptococcus than was observed in the case of the staphylococcus. None of the substances named in Table III in a concentration as high as 29.0×10^{-6} mols per 100 cc exerted any bacteriostatic or bactericidal effect against a strain of *Escherichia coli.*

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Production of Hypoprothrombinemia and Hypocoagulability of the Blood with Salicylates.*

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It has recently been reported by Link, Overman, Sullivan, Huebner and Scheel[1] that salicylic acid and sodium salicylate administered repeatedly, or in single doses, orally or intravenously, are capable of producing hypoprothrombinemia in rats. It had already been established[2,3] that salicylic acid is a degradation product of Dicumarol (3,3′ methylenebis (4-hydroxycoumarin)), which is now recognized to be a hypoprothrombinemic agent. In August of 1942 the observations of Link *et al.*[1] were made known to us, and shortly thereafter clinical investigations were begun in an attempt to reproduce the animal experiments. At the same time Dr. Shepard Shapiro of New York City Welfare Hospital, New York University, Division III, began similar studies independently.

The experimental group consisted of 31 adults, males and females, about a fourth of whom were normal individuals—nurses, technicians, and medical students—and the rest patients in the State of Wisconsin General Hospital who were afflicted with a wide variety of illnesses, arthritis of various types being the most common. These subjects received salicylates, in the form of acetylsalicylic acid or sodium salicylate in daily doses ranging from 20 to 80 grains (1.3 g to 5.3 g) for periods of 3 to 11 days.

Methods. The prothrombin time was determined on undiluted plasma with Quick's method as modified by Pohle and Stewart.[4] On each day that tests were made, one or more untreated patients was tested to serve as a control upon the technic used and the potency of the thromboplastin. Ordinarily the thromboplastin was of such potency as to result in a normal prothrombin time of 10 to 11 seconds for this group of experiments. The approximate percentages of prothrombin in human plasma as derived from the chart of Pohle and Stewart are as follows: A time of 12.5 seconds indicates a concentration of

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1 Link, K. P., Overman, R. S., Sullivan, W. R., Huebner, C. F., and Scheel, L. D., *J. Biol. Chem.*, 1943, **147**, 463.

2 Stahmann, M. A., Huebner, C. F., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 513.

3 Huebner, C. F., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 529.

4 Pohle, F. J., and Stewart, J. K., *Am. J. Med. Sc.*, 1939, **198**, 622.

TABLE I.
Effect of Administration of Acetylsalicylic Acid in 8 Individuals upon the Prothrombin Time and Coagulation Time.

	Days Control*	1*	2*	3*	4*	5*	6*	7	8
Prothrombin time in sec, avg	10.4	11.6	11.4	11.9	11.6	11.7	11.9	11.0	10.8
Coagulation time in min, avg	16.6	18.3	17.6	18.1	21.3	18.5	17.7	14.5 (4 cases)	17.6 (5 cases)

* 5 grains (0.3 g) acetylsalicylic acid q.i.d. for seven days.

about 50% of normal; 19 seconds, 25% of normal; and 30 seconds, 12.5% of normal.

The coagulation time was measured at room temperature by the two-tube method of Lee and White.[5] The precautions that were employed to insure accuracy have already been described. The liver function in many but not all was established as normal by the intravenous hippuric acid test of Quick.[6]

Materials. In all but one group of experiments the salicylates were administered orally, in tablet form, in 4 or more equally divided doses. In one series of experiments single doses of 30 to 40 grs. (2 to 2.6 g) were given.

Results. Thirteen subjects received 80 grains (5.3 g) of acetylsalicylic acid daily for periods of 3 to 11 days. For all but one of these both the prothrombin time and coagulation time varied significantly. The increase in prothrombin time ranged from a minimum of 1 second, which is significant under the controlled conditions set up, to a maximum of 4.5 seconds. In most cases the individual maximal prolongation was 2 or 3 seconds, a reduction of prothrombin to about 50% of normal; the coagulation time increased from an average control level of 18 minutes to 25 minutes. Usually an effect was discernible the day after the first administration of the drug, but in most instances the maximal hypoprothrombinemia and hypocoagulability were not observed until after three or four days or more of drug administration. They were not always exactly synchronous in their appearance, although there was, as might be anticipated, some rough parallelism. After administration of the drug had ceased the prothrombin level and coagulability of the blood usually returned to normal in from 2 to 4 days.

Smaller doses of acetylsalicylic acid, 20 grs. (1.3 g), were given to 8 subjects, 7 of whom were normal individuals. In every one of these the prothrombin time increased significantly, and in 7 of the 8 the coagulation time of the blood also increased significantly. The averages for the group are shown in Table I.

An individual experiment of this group is shown in Chart I.

Acetylsalicylic acid was administered in single doses of 30 grains (2 g) to 6 patients and in single doses of 40 grains (2.6 g) to 6

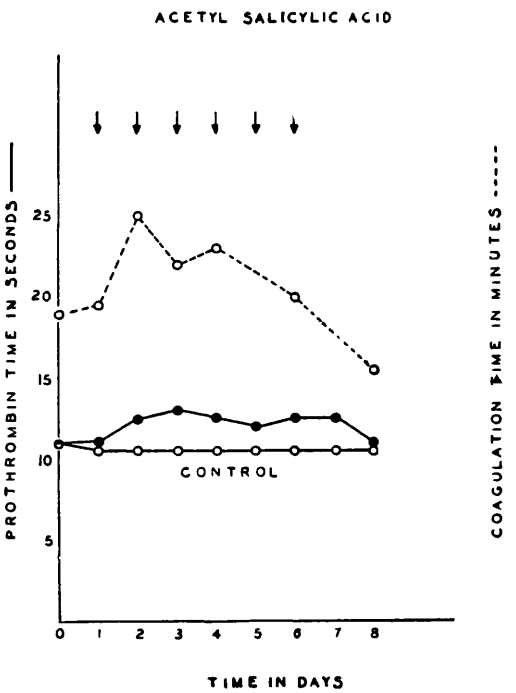

CHART 1.
Effect of administration of 20 grains (1.3 g) of acetylsalicylic acid in 4 divided doses daily to a male patient, T.U. Arrows indicate days that drug was administered.

[5] Lee, R. I., and White, P. D., *Am. J. Med. Sc.*, 1913, **145**, 495.

[6] Quick, A. J., *Am. J. Med. Sci.*, 1933, **185**, 630.

TABLE II.
Effect of Administration of Acetylsalicylic Acid and Vitamin K in 6 Individuals upon the Prothrombin Time and Coagulation Tme.

	Days control P	1 *P	2 *P	3 *P	4 *P	5 *P	6 *P
Prothrombin time, sec, avg	10.9	10.8	11.1	11	11	11.3	11.1
Coagulation time, min, avg	14.5	13.6	14.7	14.6	15.5	14.7	15 4 cases

P—2 mg Proklot 3 times a day.
*P—2 mg Proklot 3 times a day, 2-methyl-1,4-naphthaquinone (Proklot), and 5 grs (0.3 g) acetylsalicylic acid 4 times a day.

others, but no very significant increase in the prothrombin or blood coagulation time resulted. To consistently produce alterations in man with ordinary doses, repeated administration of a salicylate is usually necessary. There is a similarity here to the results in animals.[1]

Sodium salicylate was administered to 6 patients in divided doses of 75 to 80 grs. (5 to 5.3 g) for periods of 7 and 8 days, and in divided doses of 40 grs. (2.6 g) to three others for periods ranging from 3 to 8 days. The effects with the smaller doses were similar to those with the larger and were similar in all respects to those with acetylsalicylic acid. Significant hypothrombinemia and hypocoagulability occurred in 7 of the 9 patients. Prothrombin time increased from a control level of 11 seconds to a maximum of 15 seconds. The minimum prolongation in these 7 patients was 1.0 second. In one patient the coagulation time was increased from 19 to 32 minutes. This great increase was exceptional, but significant prolongation in the coagulation time appeared to be the rule.

Salicylates and Vitamin K. It is now well known that vitamin K will usually correct the hypoprothrombinemia associated with obstructive jaundice or that of the newborn, and that it will fail, save with minimal effective doses of Dicumarol and very large doses of vitamin K,[7] to correct the hypoprothrombinemia resulting from Dicumarol administration. Hence it was a matter of interest to determine whether or not the combined administration of acetylsalicylic acid and vitamin K would prevent the hypoprothrombinemia. It has already been shown by Link *et al.*[1] that vitamin K was completely effective in preventing the salicylate hypoprothrombinemia in animals.

Six subjects, 2 of whom were the same persons used in the study reported in Table I, were given, after control determinations of prothrombin time and coagulation time, 2 mg of 2-methyl-1,4-naphthaquinone (Proklot) three times a day and, beginning a day later, 0.3 g (5 grs.) of acetylsalicylic acid 4 times a day. Both drugs were continued daily for 4 days thereafter. In not one of the 6 individuals did a significant change in the prothrombin time or coagulability of the blood occur. This was particularly striking in the 2 individuals, B.H. and M.S,, who participated in the previous study, since in both of them striking changes in prothrombin and in coagulation time had occurred before.

The averages of the results are shown in Table II and they can be compared with Table I.

Discussion. These studies in man confirm the observations of Link, Overman, Sullivan, Huebner, and Scheel.[1] The basis for the investigations lies in the recognition by Huebner[2,3] that Dicumarol, which is capable of producing hypoprothrombinemia, might conceivably be degraded in the body into salicylic acid, and the salicylic acid or a product arising therefrom causes the hypoprothrombinemia.[1] This matter has been discussed in detail in an excellent paper by Link and his associates.[1]

The earlier studies in animals and the observations here recorded explain certain long-known and recorded observations, which have received little or no attention, upon the hazard of hemorrhage that attends the ex-

[7] Shapiro, S., Redish, M. J., and Campbell, H. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 12.

hibition of large doses of salicylates. Binz[8] stated that in some persons salicylic acid produced hemorrhages in the mucous membranes, and frequent or excessive menstruation in some females. Though the mechanism was not explained, he urged caution. Purpura following the administration of a salicylate was reported by Ramond[9]. Hurst and Lintott[10] reported a case wherein hematemesis attended the administration of aspirin.

It has been widely accepted that epistaxis and other hemorrhagic symptoms, such as hematuria, are not unusual manifestations of acute rheumatic fever. Rinehart, Connor, and Mettier[11] have suggested that a scorbutic state might be the basis of the hemorrhagic manifestations in these patients. This and other readily conceivable factors might furnish the explanation. But the possibility still remains that in some of these cases the administration of large doses of salicylates, a very common practice, might be responsible. Pertinent to these considerations, however, are the reports of Daniels and Everson,[12] Samuels, Ritz and Poyet[13] and Ritz, Samuels and Addiss[14] on the increased excretion of vitamin C in human beings and animals, and the tissue depletion of ascorbic acid in animals following the administration of salicylates.

Despite the observations here recorded, one is not justified in concluding that salicylate administration is a common or important cause of hemorrhage in human beings. Salicylates are used widely in large doses in many types of arthritis and other conditions, with and without medical supervision, without hemorrhage being anything but a rare and insignificant complication.

Several attractive hypotheses might suggest themselves to the reader to explain the mechanism whereby salicylates produce hypoprothrombinemia and hypocoagulability of the blood. For the present, however, it seems wiser to withhold speculation since the mode of operation is not understood.

Conclusions. 1. The oral administration of acetylsalicylic acid and sodium salicylate to human beings in daily doses of 20 to 80 grains (1.3 to 5.3 g) consistently produced hypoprothrombinemia and hypocoagulability of the blood. 2. The administration of vitamin K with the salicylate prevented the development of hypoprothrombinemia and prolongation of the coagulation time. 3. It is theoretically possible that the not unusual hemorrhagic manifestations of acute rheumatic fever may be due in some cases, at least in part, to the large doses of salicylates so commonly administered.

8 Binz, C., *Vorlesungen über Pharmakologie*, 2nd ed., Berlin, 1891; *Berlin Klin. Wchnschr.*, 1893, **30**, 985.

9 Ramond, *Le Progres Med.*, 1904, **33**, 471.

10 Hurst, A. Sir, and Lintott, G. A. M., *Guy's Hosp. Rep.*, 1939, **89**, 173.

11 Rinehart, J. F., Connor, C. L., and Mettier, S. R., *J. Exp. Med.*, 1934, **59**, 97.

12 Daniels, A. L., and Everson, G. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 20.

13 Samuels, L. T., Ritz, N. D., and Poyet, E. B., *J. Pharm. and Exp. Therap.*, 1940, **68**, 465.

14 Ritz, N. D., Samuels, L. T., and Addiss, G., *J. Pharm. and Exp. Therap.*, 1940, **70**, 362.