TABLE I.

Group of 6 rats	Dosage per rat	Treatment of rats during 8-day assay period	Degree of healing observed
I	0.1 ml daily, subcutaneously, of propylene-glycol	Kept in darkness	0
II	Same	Irradiated daily	0.1
III	Same, orally	" "	0.2
IV	0.2 ml every other day subcutan. of 7-dehydro-cholesterol in propylene-glycol	" "	1.86
V	Same of *activated* 7-dehydro-cholesterol	Kept in darkness	3.21
VI	Same but 0.1 ml daily orally	" " "	3.43
VII	Same but 0.2 ml every other day subcutan.	" " "	0

The experiments demonstrate the following:

1—Activated 7-dehydro-cholesterol, as expected, shows a strong curative effect on rachitic rats when it is injected subcutaneously.

2—The subcutaneous injection of provitamin D to rachitic rats kept in darkness is totally ineffective.

3—The subcutaneous injection of provitamin D to depleted rats is effective only if the rats receive ample irradiation.

4—Irradiation of rachitic rats alone under the conditions of our experiments does not prove curative.

5—There is no essential difference in the curative effect of activated 7-dehydro-cholesterol whether it is given the rat subcutaneously or *per os*.

The failure of irradiation to produce any healing effect in the rachitic rat makes it doubtful whether the mammalian organism can produce any appreciable amount of provitamin D. It seems possible, therefore, that the 7-dehydro-cholesterol isolated by several authors from mammalian tissue was not synthesized in the body but was present as a result of previously ingested food containing it or was perhaps produced by oxidation of the cholesterol in the course of its preparation.[6]

[6] Wintersteiner, O., and Bergstrom, S., *J. Biol. Chem.*, 1941, **137**, 785.

14003

Prothrombin Studies: III. Effect of Vitamin K upon Hypoprothrombinemia Induced by Dicumarol* in Man.

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The recent work of Overman, Field, Baumann and Link[1] and of Overman, Stahmann and Link[2] has established that in animals vitamin K administered in adequate dosage will counteract the hypoprothrombinemic effect of Dicumarol (3, 3′ methylene-bis[4-hydroxycoumarin]).

In the work reported in man this has not

* Dicumarol is the collective trade-mark of the Wisconsin Alumni Research Foundation which controls the use thereof.

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

[2] Overman, R. S., Stahmann, M. A., and Link, K. P., *J. Biol. Chem.*, 1942, **145**, 155.

been demonstrated.[3,4,5] In our own clinical experience it has likewise been observed that synthetic vitamin K failed to alter the prolonged prothrombin time induced by Dicumarol administered for therapeutic purposes. An examination of the published data and of our own records revealed the fact that the quantities of synthetic vitamin K given were relatively small although within the limits recommended for treatment of hypoprothrombinemia as it occurs clinically and that at the same time the dosages of Dicumarol administered were relatively high. In view of the findings of the Wisconsin workers[1,2] it appeared desirable, in order to determine whether a similar relationship between vitamin K substitute and Dicumarol can be demonstrated in man, to use larger doses of synthetic vitamin K and the smallest effective quantities of the hypoprothrombinemic agent.

Methods. In a previous study we found the smallest single dose of Dicumarol adequate to prolong the diluted (12.5%) plasma prothrombin time in individuals with initial normal prothrombin time to be 100 mg. Six subjects showing this response were selected. Two additional cases were studied: In one the minimal effectual dose was 150 mg and in the other 200 mg. Five were males. The ages varied between 24 and 72 years. The diets of these subjects were not controlled. Since the diet was in all cases adequate it was felt that variations in the food intake did not significantly alter the results obtained in studies of such short duration. In each case the initial diluted plasma prothrombin time was normal or only slightly prolonged.

The technic used for prothrombin estimation was an extension of that described by Link and his associates.[6] In our procedure the prothrombin time of both whole and diluted (12.5%) plasma are estimated. The rationale has been set forth in a previous communication.[7] The thromboplastin used in this study was prepared from fresh rabbit lung. This yields a somewhat shorter prothrombin time than that found with rabbit brain.[8]

After the smallest effectual single dose was determined, the prothrombin time was estimated daily. On the fourth or fifth day the single dose of Dicumarol was given by mouth in sealed capsules of 50 mg each. Daily estimation of the prothrombin time was continued until the values returned to normal. After a lapse of several days the same quantity of Dicumarol was repeated and simultaneously and at regular intervals thereafter for the 2 succeeding days, synthetic vitamin K was given perorally and intramuscularly. The prothrombin time was followed for 3 days. In one case the sequence was reversed.

Results. The prothrombin times were within the normal range during the period prior to administration of Dicumarol except in Cases 4 and 5 in which there was slight initial prolongation of the diluted plasma prothrombin time.

The single dose of Dicumarol induced prolongation of the diluted plasma prothrombin time in each instance (Fig. 1). In Cases 3‡ and 5 the whole plasma prothrombin time increased also.

After simultaneous feeding of Dicumarol and synthetic vitamin K and continuation of the latter on the succeeding 2 days the

[3] Butt, H. R., Allen, E. V., and Bollman, J. L., *Proc. Staff Meet. Mayo Clin.*, 1941, **16**, 388.

[4] Meyer, O. O., Bingham, J. B., and Axelrod, V. H., *Am. J. Med. Soc.*, 1942, **204**, 11.

[5] Lehmann, J., *The Lancet*, 1942, **1**, 318.§

§ In a subsequent communication which appeared after this paper was prepared Lehmann reports that he observed correction of the Dicumarol-induced hypoprothrombinemia by synthetic vitamin K. *Science*, 1942, **96**, 345.

[6] Campbell, H. A., Smith, W. K., Roberts, W. L., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 1.

[7] Shapiro, S., Sherwin, B., Redish, M. H., and Campbell, H. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 85.

[8] Shapiro, S., Redish, M. H., and Campbell, H. A., manuscript in preparation.

‡ Attention is drawn to the fact that in Case 3, in which the minimal effectual dose was 200 mg, the diluted plasma prothrombin time and the difference between the values of the prothrombin times of whole and 12.5% plasma were less than normal. A state of hyperprothrombinemia is believed to be a factor responsible for this.[7]

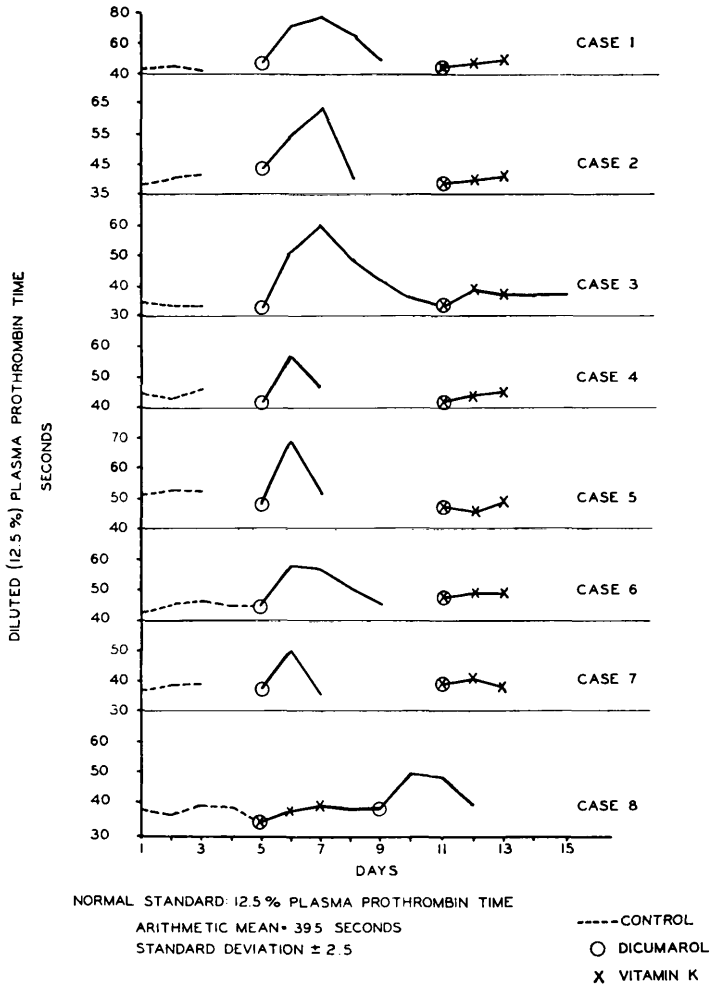

FIG. 1.

100 mg amounts of Dicumarol were given in all instances except Cases 3 and 8 where 200 mg and 150 mg respectively were administered. All doses were given orally.

Synthetic vitamin K was given as follows: 15 mg every 6 hours by mouth and 5 mg every 8 hours by intramuscular injection, making a total of 75 mg per day. In Case 3 the injections were given every 6 hours.

prothrombin times remained approximately within the range observed during the control period.

Discussion. The single dose of Dicumarol induced prolongation of the diluted plasma prothrombin time in each case. When this dose and the complementary synthetic vitamin K were administered practically no prolongation of the prothrombin time followed. This indicates that in the high levels used here synthetic vitamin K inactivated or neutralized the single minimal effectual dose of Dicumarol. That this action was not caused by the initial feedings of the hypoprothrombinemic agent was shown in Case 8 (Fig. 1) in which the order in which the experiment was conducted was reversed. This is in agreement with previous observations which show no development of sensitivity or immunity following administration of Dicumarol.

It is important to note the particulars in which the present study differs from the previous work in man referred to above.

First, much smaller amounts of the hypoprothrombinemic agent were used; and second, much larger doses of synthetic vitamin K were administered. That these were the significant differences was shown further by repeating the experiment in Case 3 using the same dosage of synthetic vitamin K and a larger dose (300 mg) of Dicumarol. Prolongation of the prothrombin time was not prevented at these respective levels.

It appears that the Dicumarol-induced hypoprothrombinemia involves a mechanism separate from that of a prothrombin deficiency consequent to a nutritional (vitamin K) lack. This is suggested by the fact that the doses ordinarily adequate for therapeutic effect do not detectably alter the prolonged prothrombin time caused by the anticoagulant although the extent of prothrombin time prolongation might be comparable.

If a quantitative relationship can be established between the activity of Dicumarol and of vitamin K it might be utilized as a means of biologically standardizing preparations of compounds exhibiting vitamin K properties. Work is now in progress to determine this.

Our findings are in accord with those of Link and his associates.[1,2]

Conclusion. In man, as has been demonstrated in animals, synthetic vitamin K administered in relatively large amounts has been found to counteract or neutralize the hypoprothrombinemia induced by a smallest single effectual dose of Dicumarol.

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14004

Modification of the Yeast-Growth Assay Method for Biotin.

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We wish to report a modification of the yeast-growth method of Snell *et al.*[1] for the assay of biotin in tissues and body fluids. This alteration was considered desirable since the existing method was found to be useful over a very limited range of biotin concentrations. Moreover, we encountered many instances of non-specific stimulation of yeast growth, particularly by human and canine urine samples. Our experience suggested that the medium previously employed was somewhat deficient for maximal growth of *S. cerevisiae.*

Mitchell and Williams[2] have demonstrated that certain amino-acids as well as a casein digest greatly enhance the growth of several strains of *S. cerevisiae* in a synthetic medium. These findings suggest that the addition of a biotin-free casein digest to the medium of Snell *et al.* may afford more nearly maximal growth and eliminate the occurrence of non-specific stimulation. We have accordingly developed a new medium containing an optimal amount of biotin-free casein hydrolysate.

Since our performance of the assay differs in many details from that of the earlier workers it is advisable to describe our procedure in some detail.

Method. The medium requires the following ingredients of highest purity:

[1] Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

[2] Mitchell, H. K., and Williams, R. J., *Biochem. J.*, 1940, **34**, 1532.