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Antagonistic and Antidotal Action of Vitamins K_1 and K_3 *Versus* Dicumarol in Dogs.* (30385)

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Clinically, it has been found that vit. K_3 is not an antidote to the indirect anticoagulant drugs, only K_1 being effective(1). In contrast to this is the observation by Johnson (2) of the equal effectiveness of K_1 and K_3 in preventing hemorrhage from dicumarol in rats and the observation of the antagonist action of K_3 as well as K_1 to dicumarol in dogs by Jaques and Dunlop(3). The differences between the antagonistic and antidotal effects of vit. K_1 and K_3 against dicumarol may be partially explained in terms of the marked difference in rates of absorption and metabolism of vit. K_1 and vit. K_3(4,5). On the other hand, the K-vitamins may have a hemostatic action independent of effects on the coagulation system, possibly through the effects of quinones on capillary resistance(6). To assist in clarification of this problem, a study has been made of the relative effectiveness of vit. K_1 and K_3 given orally, or intravenously, before, after and together with dicumarol, on the prothrombopenic effect of dicumarol in dogs.

Subjects, materials and methods. Mongrel dogs (mean weight 10.9 kg, range 5.2 to 20.4 kg) were fed a commercial dog ration (Early's Sunlight Fox Cubes). Direct spectrophotometric analysis of chromatographed extracts of the ration by Dr. R. Losito in our laboratory showed this ration contained $<1:3 \times 10^6$ parts of vitamins-K or an average daily intake of <30 μg of K-vitamins.

We are indebted to Abbott Laboratories Ltd., Montreal, for a supply of dicumarol. Vit. K_1 (2 methyl, 3 phytyl 1:4 naphthoquinone) and vit. K (2 methyl 1:4 naphthoquinone) were purchased from Nutritional Biochemicals Corp., Cleveland. The drug and vit. K_1 and K_3 were administered in single doses of 5.0, 13.05 and 5.0 mg/kg respectively. For oral administration of dicumarol and vit. K_3 the required amount was prepared in individual gelatin capsules. For intravenous and oral administration of vit. K_1, 1 ampoule

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(1000 mg) of vit. K_1 was dissolved with approximately 2 ml of Tween 80, diluted to 25 ml with distilled water. For intravenous administration of K_3, 250 mg of menadione was dissolved in 15 ml 95% alcohol, diluted to 25 ml with distilled water or dissolved in 25 ml of olive oil.

Blood samples were collected by syringe from the antibrachial or saphenous veins of the dog into 1/10 volume of 3.8% sodium citrate solution to give plasma for coagulation tests.

Prothrombin times. A modified Quick method was followed using 0.02 M calcium chloride and commercial acetone-dried rabbit brain thromboplastin (Cappel Laboratories). Extracts were discarded if the prothrombin time of normal dogs was longer than 10.5 seconds, but this rarely occurred.

Prothrombin concentration was determined by a modification of Owren's method described by Tocantins(7). 0.1 ml of 1/10 plasma in veronal buffer plus 0.1 ml prothrombin-free reagent plus 0.1 ml of thromboplastin were incubated for 30 seconds at 37°C. 0.1 ml calcium chloride was added and fibrin formation timed.

Factor X was determined in a similar manner(8) using Seitz-filtered plasma as substrate, and Russel Viper Venom (Burroughs-Wellcome Co.) plus vegetable lecithin instead of thromboplastin.

Factor VII + X was determined(9) using Seitz-filtered plasma plus thromboplastin.

Results and discussion. Dogs were given dicumarol, and assays of prothrombin time, prothrombin concentration, Factor VII and Factor X were performed for five days. Determinations were made at 24 and 48 hours and more frequently on the third day as the values reached a peak. Three weeks to a month later, the same dogs were given dicumarol again and in addition, vit. K_1 or K_3, intravenously or orally, simultaneously or on the third day after dicumarol. In view of the storage which occurs with vit. K_1(10), the experiment with K_1 was the last experiment. Typical responses in the prothrombin time to combinations of treatments are shown in Fig. 1. Prothrombin times have been recorded on a logarithmic scale(11). To compare the results with K_1 and K_3 for various schedules and factors, the mean values of the area under the coagulation time response curve(12) has been calculated and is shown in Table I. Each group contained 14-15 dogs. To assess the reproducibility of the response to dicumarol, the responses to dicumarol up to the third day were calculated for all dogs not receiving K-vitamins until the third day, and compared to the response to the initial dose of dicumarol. The results of this analysis suggested a reduced prothrombin time response after the initial dose of dicumarol. Consequently, 5 dogs not previously on any experiment were given 5 mg dicumarol/kg 5 times with an interval of 3 weeks between doses. The mean area values for the prothrombin time on successive doses were: 48.0, 41.6, 39.2, 42.0, 41.5. These are within the normal range of variability of response but when the values on each day were averaged, there was a significantly faster return of the prothrombin time to normal values following the later doses. Similar results were obtained with prothrombin concentration, Factor VII and X.

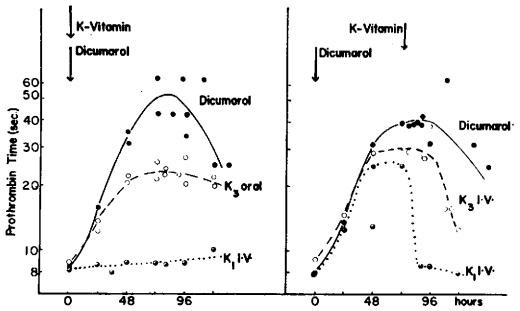

FIG. 1. Effect of K-vitamins on prothrombin time with dicumarol. Left side—antagonistic effect. Right side—antidotal effect.

The results reported in Table I for the prothrombin time response of the dogs show: (1) Vit. K_1 and K_3 given orally with dicumarol on Day 0, significantly reduced the dicumarol effect ($P<0.01$). There was no significant difference between the actions of the two vitamins ($P=0.7$). Administration of the vitamin 3 hours before dicumarol or in repeated doses on Days 0, 1, 2, 3, resulted in the same prothrombin time response with K_3 but possibly a greater antagonistic effect with K_1 ($P=0.07$)(2). Vit. K_1 given intravenous-

TABLE I. Mean Response Areas of Groups of Dogs Receiving Dicumarol and K Vitamins.

Day vitamin given	No vitamin	K_1 I.V.	K_1 oral	K_3 I.V.	K_3 oral	No vitamin	K_1 I.V.	K_1 oral	K_3 I.V.	K_3 oral
	Based on prothrombin times					Based on prothrombin concentration				
0	55.1 (12.2)	2.5 (3.6)	40.9 (15.5)	43.7 (14.8)	42.5 (10.4)	21.2 (11.2)	1.1 (.9)	10.5 (8.0)	19.0 (10.0)	18.5 (8.0)
0, 1, 2, 3					37.9 (9.4)					19.2 (7.7)
0 (−3 hr)			30.1 (15.4)		40.5 (15.3)			14.0 (5.1)		20.9 (8.6)
3		21.4 (5.6)	25.9 (4.6)	40.1 (10.8)	35.8 (13.9)		12.7 (5.6)	9.8 (4.8)	16.7 (7.8)	19.2 (7.7)
3*	27.6 (10.6)	− .2 (−1.2)	3.4 (2.8)	16.4 (10.7)	15.6 (10.9)	12.0 (7.2)	4.0 (2.0)	4.1 (2.4)	7.2 (5.0)	9.8 (4.3)
	Based on Factor VII					Based on Factor X				
0	78.3 (12.1)	11.3 (23.5)	56.5 (12.1)	63.3 (26.5)	70.0 (21.1)	19.2 (9.1)	.2 (.5)	14.5 (8.2)	22.3 (10.5)	5.7 (5.3)
0, 1, 2, 3					66.1 (15.8)					13.5 (5.7)
0 (−3 hr)			58.1 (22.4)		74.4 (19.2)			15.5 (7.6)		24.9 (6.4)
3		40.8 (10.1)	46.5 (7.2)	58.0 (19.9)	60.4 (25.2)			12.0 (3.3)	16.5 (2.1)	14.5 (7.1)
3*	31.6 (16.1)	2.2 (3.7)	6.9 (4.5)	22.5 (14.4)	26.8 (19.4)	7.6 (5.2)		3.2 (9.6)	10.8 (1.0)	8.7 (4.9)

Mean response areas calculated by formula in text for daily determinations on days 0 to 5.
* Mean response areas calculated from determinations on day 3, 4 and 5.
Figures in parentheses = standard deviations.

ly on Day 0 completely antagonized the dicumarol, while K_3 had the same effect as when given orally(3). Vit. K_1 and K_3 given on Day 3 displayed significant antidotal properties ($P<0.001$ and <0.01 respectively). By either route, vit. K_1 was much more effective than vit. K_3, and the intravenous route was superior to the oral route when vit. K_1 was used ($P=0.03$). The antidotal effect was demonstrated more clearly by measuring the area under the response curve after Day 3, when the K vitamin was given. Vit. K_3 hastened the return of the prothrombin time to normal values but the effect of vit. K_1 was more dramatic(4). The results for the responses of specific coagulation factors were similar to those found for the prothrombin time, but not as marked. This would be expected since the prothrombin time response is a sum of these responses.

Vit. K_3 and vit. K_1 appear to be interchangeable in correcting the coagulation deficiency in the K-deficient animal(13). In fact, the 2-methyl naphthoquinone diphosphate appears to act more rapidly in the dog (14). With the discovery of dicumarol, it was immediately appreciated(15) that the K-vitamins were antagonists to the indirect anticoagulants but there was considerable confusion before it was realized that there is a marked difference in the ability of the two forms of the vitamin to act as an antidote (correct the prolonged prothrombin time) to dicumarol(16). Many investigators have described the antagonism between dicumarol and the K-vitamins, but no distinction has been made between antagonistic and antidotal effects. The present investigations make a direct comparison of antagonism and antidote against an indirect anticoagulant. It is evident that as an antidote, vit. K_1 in the dosage used is completely effective while the equimolar dose of vit. K_3 is only 50% effective. Since vit. K_3 is rapidly absorbed into the portal system(4) and metabolized very rapidly, repeated oral doses of K_3 would be expected to produce blood levels similar to a single intravenous dose of vit. K_1, yet did not result in a marked antagonistic effect. In view of the universal opinion today that in-

travenous forms of K_1 are required for a clinically effective antidote the efficacy of oral vit. K_1 was surprising. We must then explain why oral vit. K_1 is no better as an antagonist than vit. K_3.

There appear to be three possible explanations. First, intestinal absorption of vit. K_1 may depend directly or indirectly on the level of prothrombin time, so that absorption is greater on the third day than on Day 0. Second, there may be a difference in the fate of vit. K_1. It has been shown(10) that when vit. K_1 is given intravenously, much is fixed in the tissues; when given orally, much is metabolized. Martius(18) and Billeter, Bolliger and Martius(19) have recently reported that in pigeons on oral administration, the side-chain of vit. K_1 is largely removed by intestinal bacteria and the 2-methyl naphthoquinone nucleus (K_3) reappears in tissues as vit. K_2 (with C_{20} side-chain). Our results do not contradict Martius' view that vit. K_2 is the active form of the vitamin, but why then is vit. K_3 on oral ingestion not as effective a material for vit. K_2 synthesis as is vit. K_3 formed from vit. K_1? Is this due to the different route of absorption, since with ingestion of vit. K_3-C^{14} the radioactive label is found in the portal blood, while with ingestion of vit. K_1-C^{14}, the label is found in the lymph? The third possibility is that there are 2 different actions of the K-vitamins in opposing the action of the indirect anticoagulant in these animals. Lowenthal and MacFarlane(17) in experiments on rats, have shown paradoxical differences in the effect of an indirect anticoagulant on the action of vit. K_1. They found that warfarin inhibited the Factor VII response to vit. K_1 in the vit. K-deficient rat but not in the anticoagulant-pretreated rat, indicating a difference in antidotal effects of the vitamin in vitamin deficiency *vs* anticoagulant treatment and explain this in terms of an alternate site or mechanism for the vitamin.

No evidence of spontaneous hemorrhage or deaths from hemorrhage or other causes occurred during the period of study with the animals receiving dicumarol alone or with the vit. K_1, or oral vit. K_3. Two dogs receiving intravenous vit. K_3 developed hemolysis. This did not occur in any other animals, although all blood samples were checked for hemolysis. One of the 2 showed extreme hemolysis in the blood sample on the first day. The hemolysis decreased but the animal died 3 days after treatment. On postmortem examination, moderate lung hemorrhage was found with free blood in the pleural cavity. The kidneys, heart and gall bladder were enlarged. Histologically, marked acute congestion of the lungs and liver was observed with early glomerulonephritis, and infiltration of the gall bladder wall with exudate. Mysliveček(20) observed that 6 mg of synkavite (vit. K_3 diphosphoric ester) intramuscularly in rats caused extensive discharge of mast cell granules in connective tissue and wall of the aorta, indicating a toxic effect of vit. K_3. This combined with the vasotoxic and anticoagulant actions of dicumarol discussed by Millar *et al*(12) was probably the cause of hemorrhage in the dog.

Summary. Dogs received 5 mg dicumarol/kg orally and 13.05 mg vit. K_1 or 5 mg of vit. K_3/kg, orally or intravenously on Day 0, Day 3, or Days 1, 2, 3 after dicumarol. Values for prothrombin time, prothrombin concentration, Factor VII, Factor X were determined. Vit. K_1 and K_3 were equally effective oral antagonists when given with dicumarol, but vit. K_1 was much more effective on intravenous administration. When given on the third day after dicumarol, both vit. K_1 and K_3 had an antidotal effect but vit. K_1 was much more effective.

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Utilization of CO_2 and Acetate in Amino Acid Synthesis by *Streptococcus bovis*.* (30386)

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Carbon dioxide exerts a marked influence on the metabolic activities and nutritional requirements of *Streptococcus bovis*. A requirement for CO_2 exists when some strains are grown in media containing simple nitrogen sources(1-4); in other strains of *S. bovis*, CO_2 is involved in the production of polysaccharides(5-6), and of destransucrase(7). Acetate also affects the metabolism of *S. bovis*. Bailey and Oxford found that it was required for dextran synthesis(6), and we demonstrated its ability to stimulate growth and to be incorporated into cells growing in an ammonium medium(3).

This paper reports the utilization of CO_2 and acetate in amino acid synthesis by *S. bovis* growing on different nitrogen sources, and elaborates further some of the nutritional requirements for growth of this organism in the absence of CO_2.

Materials and methods. Microbiological. The organism used was *Streptococcus bovis* strain P 10. Procedures for maintenance of cultures, performance of growth tests, and preparation of inocula were identical to those previously described(8). The basal medium was that of Prescott and Stutts(1), supplemented as indicated below with nitrogen sources consisting of (i) *L*-arginine, (ii) ammonium acetate, or (iii), the complex amino acid mixture of Henderson and Snell(9). Some growth experiments requiring removal of CO_2 were performed with special flasks, (10), but usually, cultures were grown in 50 ml Erlenmeyer flasks, stoppered with porous plastic plugs, and incubated over 20% KOH in large vacuum desiccators. Shredded filter paper was immersed in the KOH solution to increase surface area, and the desiccator was evacuated to approximately 40 mm. In some experiments, cultures were incubated over the KOH *in vacuo*; in others, air was readmitted through a trap containing 20% KOH. The CO_2 was supplied to cultures either as a filter-sterilized $NaHCO_3$ solution, or as a gaseous atmosphere. The latter was accomplished by evacuating a desiccator to approximately 40 mm, then slowly admitting filtered CO_2 from a cylinder until a pressure about 10 mm below atmospheric was reached as indicated by an open limb manometer.

C^{14} tracer experiments. Preliminary experi-

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