Technical Papers

Factors Influencing Blood Clotting Time in the Chick

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It is well known that vitamin K, which is necessary for normal blood clotting, is synthesized in the intestinal tract of the chick (*1, 2*). It has been suggested, that in order to produce a deficiency of this vitamin experimentally, the chicks must be fed a ration devoid of vitamin K, the feed must not be moistened, the water containers must be kept scrupulously clean to avoid bacterial synthesis of the vitamin, and coprophagy must be avoided (*1*). The symptoms can be hastened in chicks by depleting the parent stock.

Notwithstanding these views, hemorrhages occurred in this laboratory in chicks originating from parent stock fed a diet containing 10% alfalfa meal and having access to green vegetation as well. The chicks did not have access to their excreta. They were fed a diet that was low, although not entirely deficient, in vitamin K. The subcutaneous hemorrhages occurred mainly in the legs and sometimes in the wings and the breast, whereas intramuscular hemorrhages were frequently observed in the thigh and breast. Hemorrhages were observed as early as the 3rd wk after hatching. This indicated that the residual vitamin K in the chick at time of hatching had been rapidly depleted. The symptoms simulated those recently observed under field conditions and those generally associated with vitamin K deficiency.

The appearance of the syndrome under practical conditions, where management practices would preclude the conditions that normally lead to a deficiency of vitamin K, might be explained on the basis (*a*) that in some instances rations currently in use are marginal with respect to this vitamin and (*b*) that a lesser amount was being synthesized in the gastrointestinal tract than formerly. The use of greater amounts of solvent extracted soybean meal and lesser amounts of alfalfa meal would reduce the vitamin K level in the ration, whereas the addition of growth stimulators (arsenicals and antibiotics) and coccidiostatic drugs, or combinations thereof, could conceivably influence the intestinal synthesis of vitamin K that normally covers a part of the chick's requirement.

TABLE 1

Composition of the Basal Diet

Ground yellow corn	66.35 lb
Soybean meal*—50% protein	30.00
Bonemeal, steamed	2.00
Limestone	1.00
Iodized salt	0.50
$MnSO_4$, feed grade	0.05
A & D oil (600D–3000A/g)	0.10
B_{12}, 1 mg	+
Riboflavin, 200 mg	+
Niacin, 1 g	+
Total	100 lb

* Hexane extracted commercial meal subjected to adequate heat treatment.

TABLE 2

Experimental Design and Results

Supplement to basal	Mg/kg basal diet	Av. Clotting Time (min) A: At 28 days	B: Chicks surviving at 31 days, Before Addition of Menadione	B: Chicks surviving at 31 days, After Addition of Menadione
None	—	9.8 (25)*	9.1 (23)*	1.9
Vitamin K (Menadione)	5	1.9 (25)	—	—
p-Aminophenylarsonic acid (arsanilic)	100	15.8 (23)	16.0 (15)	2.1
3-Nitro, 4-hydroxyphenylarsonic acid (arsonic)	50	16.2 (20)	13.3 (13)	1.3
Nitrophenide	125	6.3 (25)	5.2 (23)	2.1
Sulfaquinoxaline	125	8.5 (24)	9.1 (19)	1.6
Procaine penicillin G	15	9.2 (25)	8.7 (19)	1.8
Terramycin	15	16.1 (24)	10.9 (20)	2.1
Aureomycin	15	4.7 (23)	4.4 (21)	1.6
Bacitracin	15	5.9 (25)	5.9 (22)	2.4
2,5-Ditertiary-butyl-hydroxy-quinone	75	5.0 (25)	4.7 (21)	2.6
6-Ethoxy-2,2,4-trimethyl-1,2-dihydroxyquinoline	75	4.5 (23)	4.4 (15)	1.8

* Number of chicks.

Analysis of Variance

	DF	SS	MS	F
Treatment	11	6,275.9046	570.54	4.99†
Error	275	31,429.5960	114.29	
Total	286	37,705.5006		

Least significant difference ($p = 0.05$) . . . 6.1

† Highly significant.

After a number of preliminary trials, a series of experiments was initiated to examine the extent to which certain supplements, when added to a practical ration at accepted levels, would influence blood clotting time in the young chicken. The experiment reported herein involved 12 groups of 25 female crossbred day-old chicks (New Hampshire ♂ × Columbian ♀) from nondepleted stock. They were fed a ration of the same type as fed when the syndrome was encountered for the first time in this laboratory. Its com-

position is given in Table 1. The chicks were reared in an electrically heated battery, equipped with raised wire floors. The water was changed daily and the containers were cleaned by brush. At 4 wk of age, 1 ml of blood was drawn by heart puncture and the clotting time for whole blood was recorded. The results are shown in Table 2, column A.

Statistical analysis of the data showed that terramycin and arsonic acid prolonged the blood clotting time significantly at $P < 0.05$, whereas arsanilic acid was almost significant at the 0.05 level, as compared with the nonsupplemented diet. Vitamin K (Menadione), on the other hand, significantly reduced the blood clotting time.

To test the hypothesis that the action of these supplements on the clotting time was due to a lack of available vitamin K, a curative level of Menadione (20 mg/kg diet) was added to each ration and the clotting time was again determined after 72 hr. These results are shown in Table 2, column B. It appears that the addition of Menadione reduced the blood clotting time to normal in every case. The difference in the number of birds in columns A and B resulted from two causes: (*a*) two individuals from each lot were removed for histological studies, and (*b*) a number of birds died after the heart puncture. For the histological examination, birds with a relatively long clotting time were selected.

Although it is clear that certain of the supplements prolonged blood clotting, the exact mechanism remains to be determined. The supplements could affect the metabolism of bacteria synthesizing the vitamin, or exert their influence by altering number and/or type of bacteria in the intestinal tract. The possibility of these supplements functioning as antagonists is not excluded.

References

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The Preparation of Latex Casts of Soil Cavities for the Study of Tunneling Activities of Animals

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As part of a study of the roles of soil animals in relation to soil structure and fertility, it became necessary to obtain a clearer picture of the tunnels made by these animals. It was found that by pouring liquid latex into such openings as the tunnels of earthworms, ants, and spiders, and into shrinkage cracks in soil, casts could be made and preserved. These casts have revealed much about the tunneling and feeding habits that would have been difficult to discern otherwise.

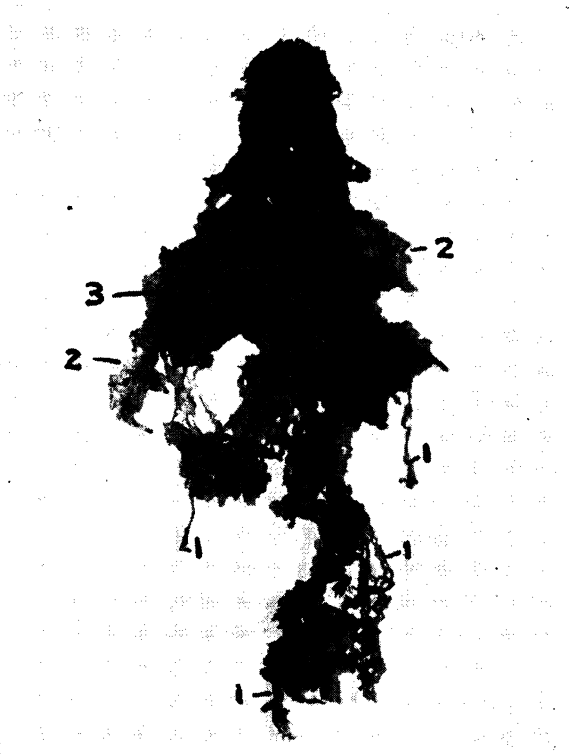

FIG. 1. Latex model of large interconnected soil voids, made by pouring latex into a surface opening in garden soil at Richmond, Indiana: (1) earthworm tunnels; (2) "drought cracks"; (3) walls roughened by "nibbling" of ants. About one-half natural size.

The simplest technique is carried out as follows. Liquid latex is thinned to a suitable consistency with commercial ammonia, diluted 1 to 8 with distilled water. The thinned material is poured into open holes until they are filled. The latex is allowed to harden for 2 or 3 days. The cast is then dug up and cleaned by washing under a stream of water.

The size of openings penetrated is controlled by the consistency of the latex. In some experiments the latex has been considerably diluted by ammonia water to which small amounts of the detergent and wetting agent (Joy) have been added. Also, some of the soil samples were partially evacuated in order to enhance penetration. For special purposes the latex may be colored with fat-soluble dyes.

The nature of the tunneling activities of animals has been revealed more effectively by this means. The casts show that in good garden soil near Richmond, Indiana, earthworms make a complex set of horizontal tunnels an inch or so beneath the surface of the ground. These connect by vertical shafts with other complex sets of horizontal tunnels in the decaying litter at the bottom of the furrow, and with other vertical shafts going 2 or 3 ft into C-horizon of the soil. The tunnels of earthworms are recognizable by the

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