even 5.0 mg % was not appreciably inhibitory to them. There seems to be no parallelism between the susceptibilities of *Brucella* organisms to the two therapeutic agents tested.

It is beyond the scope of this experiment to estimate how much of the antibacterial action of the penicillin preparation used was due to penicillin itself and how much to the so-called penatin.[9,10,11] However, it may be pointed out that the penicillin was harvested at pH 7.5 and that it was extracted with an organic solvent. Moreover, neither the presence nor absence of a small amount of dextrose in the medium for the tests appreciably affected the antibacterial action of this material. These facts suggest that it was the penicillin rather than penatin that was chiefly responsible for the antibacterial action.

Conclusion. Penicillin exerted a considerable antibacterial action on 8 out of 15 strains of *Brucella in vitro*. This action was enhanced by the combination of penicillin with a small amount of sodium sulfathiazole.

9 Kocholaty, Walter, *J. Bact.*, 1942, **44**, 469.

10 Kocholaty, Walter, *Arch. Biochemistry*, 1943, **2**, 73.

11 Kocholaty, Walter, *Science*, 1943, **97**, 186.

14570

Absence of Liver Damage in Chicks Hypoprothrombinemic Due to Vitamin K Deficiency or Ingestion of 3,3'-methylenebis (4-hydroxycoumarin)*

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It is generally assumed that the hypoprothrombinemia resulting from vitamin K deficiency is due to the inability of the liver to synthesize prothrombin in the absence of an adequate amount of vitamin K. In chicks reared on an artificial vitamin K-free diet which produced clinical signs of the deficiency disease within 12 to 14 days, one group of investigators[1] observed fatty degeneration and necrosis in the liver and suggested that the absence of vitamin K may cause liver damage which may in turn be responsible for the failure of prothrombin formation. However, patients suffering from hypoprothrombinemia which can be corrected by vitamin K do not as a rule show an abnormal response in the usual tests of liver function.

The first description of the hemorrhagic disease in cattle caused by the feeding of spoiled sweet clover hay also mentioned liver damage accompanying the hypoprothrombinemia.[2] However, investigations of the sweet clover disease carried out since the isolation and synthesis of the causative agent, 3,3'-methylenebis (4-hydroxycoumarin), commonly referred to as dicumarol, have left undecided the question of the association of liver damage with this form of hypoprothrombinemia.[3,4]

The modes of action of vitamin K and dicumarol in the body remain unexplained; but in view of the possibility that their action might be expressed in morphologic as well as functional alterations in the liver, it seemed desirable to undertake a more detailed exam-

* Aided by a grant from the Josiah Macy, Jr., Foundation. We are indebted to Dr. K. P. Link and to Eli Lilly and Co. for supplying the dicumarol; and to Hoffman-LaRoche, Inc., for supplying the *d,l*-alpha-tocopherol acetate and Synkavite (tetra sodium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid) used in these experiments.

1 Hepding, L., and Moll, T., *E. Merck's Jahresbericht*, 1939, **53**, 5.

2 Schofield, F. W., *Can. Vet. Rec.*, 1922, **3**, 74.

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

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

TABLE I.
Chicks Fed Commercial Diet.

No. of animals	Age, days	Clotting power, % normal	Liver fat	Treatment prior to sacrifice
7	36-63	100	0	Fasted 18-24 hr.
5	37-62	100	0	Fed

TABLE II.
Chicks Fed Commercial Diet. Dicumarol Given by Mouth.

Animal	Age, da.	Clotting power, % normal	Liver fat	Dicumarol mg/da./g.b.w.	Dicumarol Days treated	Treatment prior to sacrifice
573	54	9.1	0	0.25	3*	Fasted 18 hr.
574	63	10	0	0.35	5*	" 25 "
575	56	5.2	0	0.25	5*	" 24 "
583	49	16	0	0.23	2*	" 27 "
2378	58	1	0	1.06	1†	" 24 "
2459	58	2	0	1.06	1†	" 24 "

* Killed 24 hr. after last dose of dicumarol.
† Killed 48 hr. after receiving dicumarol.

ination of the liver in a series of hypoprothrombinemic animals. The results of a study of 40 chicks subjected to various experimental procedures have shown that on a proper dietary regimen marked hypoprothrombinemia can be produced in these animals with no morphologic evidence of liver damage demonstrable by the methods employed.

Methods. White leghorn chicks were used. The animals weighed from 300 to 500 g at the time of the prothrombin determinations. Vitamin K deficiency was produced by feeding the following diet: alcohol-extracted casein 150 g, dried brewer's yeast (ether extracted) 100 g, salt mixture No. 3† 20 g, gelatin 80 g, gum arabic 50 g, sucrose 598 g, cystine 1 g, choline chloride 1 g, cod liver oil 50 g, *d,l*-alpha-tocopherol acetate 100 mg. Normal chicks were given a commercial chicken diet or the vitamin K-free diet plus 10 mg vitamin K substitute per kilogram. Dicumarol was fed as a powder or as tablets to chicks receiving the commercial diet. The clotting power of the plasma, $(K_n/K)100$, was determined as described by Dam and Glavind[5] (K_n = concentration of thromboplastin which clots plasma of normal chicks in 3 minutes under standard conditions; K = concentration of thromboplastin which clots the plasma to be tested in the same length of time.) For histologic studies tissues were fixed in Bouin's solution followed by Mallory-azan staining, and in formol-saline followed by staining of frozen sections with Sudan IV for fat. Tissues were also fixed and stained by the method of Regaud for demonstrating mitochondria.

Observations and Comments. Post-mortem examinations revealed no gross pathologic changes in the liver of any of the animals. Mitochondrial shape and abundance as well as other general histologic features of the livers from the experimental animals showed some variation, but the variations within the experimental groups did not distinguish the latter from the corresponding control groups.

The liver specimens were classified according to the abundance of fat estimated by inspection of sections stained with Sudan IV. The most fatty livers contained fat in practically every hepatic cell. In some livers the fat was more abundant around the central veins, while in others it was uniformly distributed or more abundant in the portal areas. Sudanofil fat was not present in the livers of chicks receiving the commercial diet (Tables I and II), but did occur in more than 50%

†$CaCO_3$ 400, $MgCO_3$ 20, NaCl 88, ferric citrate 32, $CuSO_4 \cdot 5H_2O$ 3, $MnSO_4 \cdot 4H_2O$ 6, di-iodotyrosine 0.005.

5 Dam, H., and Glavind, J., *Biochem. J.*, 1938, **32**, 1018.

TABLE III.
Chicks Fed Vitamin K-free Synthetic Diet.

Animal	Initial age, da.	On diet, da.	Clotting power, % normal	Liver fat	Treatment prior to sacrifice
325	19	27	20	++++	Fed
326	19	41	16	+	"
327	19	16	11	++	"
551	29	29	2.5	0	Fasted 26 hr.
553	29	29	2	0	" 28 "
554	29	27	14	—	" 26 "
555	29	27	30	++	" 24 "
2491	7	30	0	+	Fed
2493	7	29	4	0	"
2494	7	34	0	0	"
2498	7	21	<1	+	"
2560	7	16	—	++	Fasted 24 hr.
328	19	16	5	—	Fed*
552	29	31	3	0	Fasted 26 hr.†

*, † Killed 19 and 25 hours respectively after receiving 95 γ of 2-methyl-1,4-naphthohydroquinone. Prothrombin then 100%.

TABLE IV.
Chicks Fed Synthetic Diet with Added Vitamin K (Synkavite, 10 mg/kg).

Animal	Initial age, da.	On diet, da.	Clotting power, % normal	Liver fat	Treatment prior to sacrifice
330	19	41	100	++	Fasted 14 hr.
331	19	10	100	0	Fed
332	19	16	100	+++	"
333	19	16	100	0	"
334	19	41	100	+	Fasted 15 hr.
335	19	27	100	+	Fed
2455	7	58	100	+	"
2457	7	58	100	0	"

of the animals receiving the synthetic diet (Tables III and IV). However, neither the incidence nor the severity of the fatty change was increased by vitamin K deficiency. Furthermore, severe hypoprothrombinemia was present in animals whose livers contained no sudanofil fat (Table III). The observed fat accumulation in the liver thus appears unrelated to the intake of vitamin K, and probably should not be interpreted as a pathologic degeneration but rather as a metabolic variation in individual response to the synthetic diet. Dietary abnormalities other than the absence of vitamin K might explain the marked fatty degeneration and liver damage reported by Hepding and Moll.[1]

The incidence of fatty change in the liver was the same in chicks fasted as in those fed prior to sacrifice. This is in contrast to observations on the mouse liver,[6] in which fasting for 24 hours produces a marked increase in sudanofil fat.

Schofield[2] distinguishes between a hemorrhagic and an anemic type of the sweet clover disease. Both types are characterized by anemia and hypoprothrombinemia, but in the first type extensive subcutaneous and internal hemorrhages are a prominent feature; while in the second type hemorrhagic manifestations are limited to petechiæ and ecchymoses which may be present in any of the serous membranes. Marked pathologic changes including intralobular hemorrhage, cloudy swelling, and fatty degeneration were found in the livers of cattle dying with the hemorrhagic form of the disease. We are not in a position to decide on the validity of this distinction, but the hypoprothrombinemia produced in chicks by dicumarol feeding is comparable to that described in cattle by Schofield; although as previously mentioned no gross or microscopic

[6] Hodge, H. C., MacLachlan, P. L., Bloor, W. R., Stoneburg, C. A., Oleson, M. C., and Whitehead, R., *J. Biol. Chem.*, 1941, **139**, 897.

abnormality was observed in the livers of these chicks.

Since the completion of these experiments, Topelberg and Honorato[7] have reported that a vitamin K-free diet increases the total extractable fat of the chick liver, and that addition of vitamin K to the diet reduces the value toward that found in chicks fed a standard commercial diet. They also observed that injection of choline in the amount of 10 γ per animal per day would depress the fat content of the livers of chicks fed the artificial K-free diet. Our diet supplied much more choline than 10 γ per animal per day.

[7] Topelberg, G. S., and Honorato, C. R., *Rev. Soc. Argentina Biol.*, 1943, **19**, 409.

Summary. 1. Hypoprothrombinemia was produced in chicks by feeding a vitamin K-free diet, or by adding dicumarol to a standard commercial diet. 2. The livers of these animals showed no gross or microscopic changes which would distinguish them from corresponding groups of control animals. 3. Fatty infiltration of the liver which occurred in some of the chicks was related to the synthetic diet employed; but was not related to the lack of vitamin K or choline, or to the degree of hypoprothrombinemia. 4. In chicks there can be produced severe hypoprothrombinemia unassociated with histologic evidence of liver damage demonstrable by the methods employed in this study.

14571 P

Influence of Body Movement on Shock Due to Repeated Hemorrhage.*

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In the present experiments bodily movement is shown to have a beneficial effect on the mortality from repeated hourly hemorrhages in the dog. In a recent publication[1] a similarly beneficial effect was observed after severe trauma to both hind legs in animals allowed to move about as compared with those which were kept immobile in the supine position.

The present experiments were carried out as previously described except that instead of assuming the immobile supine position the animals were allowed to move freely about between each hemorrhage. Blood was removed (10 cc per kilo of body weight per hour) by puncture of the femoral artery through the intact skin except that it was often necessary after the initial hemorrhage to expose the contracted vessel. For this local anesthesia was employed when necessary. Hematocrit determinations were made from a portion of the removed blood. Twenty-five experiments were performed in all as described in Table I, of which 7 were controls. Of the 18 experiments permitted voluntary body movement, 6 were allowed nothing by mouth, 6 were allowed water *ad libitum*, and 6 were given a solution containing 5% hydrolyzed casein and pork pancreas (Amigen) and 5% glucose by gavage equal in amount to the blood which had been removed; it so happened that the volume of water voluntarily taken averaged the same as the amount given by gavage.

Experimental Findings. Hematocrit determinations revealed a drop in red cell volume of 20 to 30% below the initial value in all groups. The greatest drop occurred in those allowed water by mouth *ad libitum*, or given a solution of Amigen and water by gavage; of these 2, the drop was less rapid in the

* Aided by a grant from the Commonwealth Fund.

[1] Eversole, W. J., Kleinberg, W., Overman, R. R., Remington, J. W., and Swingle, W. W., *Am. J. Physiol.*, 1944, **140**, 490.