nuclear inclusion-producing agent resembling measles virus. No clear cut effect was seen with 6 other Coxsackie strains, 3 influenza prototype viruses, or hemagglutinating virus of Japan. 3) Simultaneous titration of several adenovirus, poliomyelitis, and Coxsackie virus strains in tonsil and HeLa cells gave high and comparable titers.

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On Inhibition of Vit. K Activity by Sulfaquinoxaline in Chicks.*† (23600)

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Supplementation of poultry rations with sulfaquinoxaline is known to increase the requirement of chicks for vit. K(1,2). Mushett and Seeler(3) have shown that the action of this sulfonamide is not likely to be related to curtailment of the biosynthesis of vit. K in the intestinal tract, or to histological changes in the liver. A quantitative relationship between sulfaquinoxaline and vit. K has been indicated by preliminary work done at the Ill. Agric. Exp. Station. It is the purpose of the present report to investigate some aspects of this relationship.

Methods. Female crossbred chicks were kept on a purified diet deficient in vit. K to the age of 2 weeks, when the chicks were distributed into groups of 10, and graded levels of sulfaquinoxaline (0.025—0.4%) added to their diet. The basal diet is given in Table I. After 6 days graded levels of menadione sodium bisulfite complex (MSBC) dissolved in water, and containing 33% 2-methyl-1,4-naphthoquinone, were force-fed to the chicks. As the feed, and therefore the intake of sulfaquinoxaline, were influenced by the size of the chick, the doses of MSBC were adjusted to the body weight of each chick and were expressed in μg/100 g of body weight. Twenty-four hours later blood was drawn by heart puncture and plasma prothrombin times determined by a modification of the onestage method of Quick(4), using sodium citrate as an anticoagulant and acetone-dehydrated chick brain as a source of thromboplastin.§ Due to considerable

TABLE I. Composition of Basal Diet.

Ingredient	%
Drackett protein	30
Cerelose	30
Starch	29.36
Salt mixture*	5.34
Solka floc†	3
Refined corn oil	1
Glycine	.50
DL-methionine	.40
Choline-Cl	.20
Total‡	100

* Griminger *et al.*(6).

† Non-nutritive fiber, containing 99.5% cellulose.

‡ Plus the following vitamins (mg/kg diet): Thiamine HCl 25; riboflavin 16; niacin 100; calcium pantothenate 20; pyridoxine HCl 6; folic acid 4; biotin 0.6; cyanocobalamine 0.02; ascorbic acid 200; alpha-tocopherol acetate 20; 10,000 I.U. vit. A and 600 I.U. vit. D_3.

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§ An outline of the method employed will be sent on request.

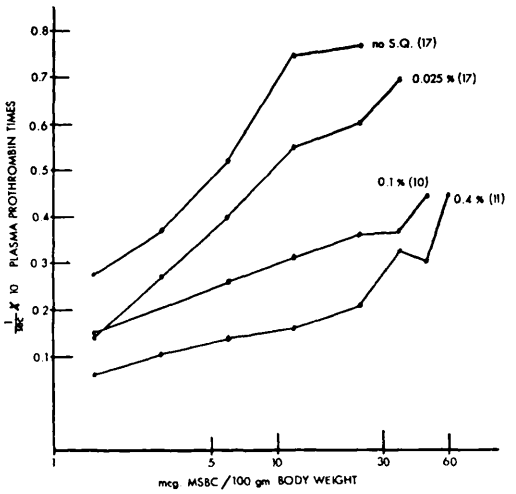

FIG. 1. Effect of single doses of menadione sodium bisulfite complex (MSBC) on plasma prothrombin times of chicks fed various levels of sulfaquinoxaline. Figures in parentheses refer to number of chicks on each ration finishing the experiment.

mortality in the lots getting higher levels of sulfaquinoxaline, the experiment was repeated with 12 chicks lot. Fig. 1 shows the averages of all the prothrombin values obtained for 4 levels of the drug with different doses of MSBC. Average prothrombin times of the chicks not receiving doses of MSBC were 116 sec. without sulfaquinoxaline, 154 sec. for the 0.025% level, and over 180 sec. for the 0.1 and the 0.4% levels of the drug. These control values were not included in the log-dose-response presentation (Fig. 1).

In another experiment, 3-week-old K-deficient chicks were given a single dose of

TABLE II. Effect of Sulfaquinoxaline on Prothrombin Times of Chicks after Receiving 2 μg Menadione/100 g Body Weight.

Days after menadione dose	Avg plasma prothrombin times in sec.	
	Control diet	0.2% sulfaquinoxaline diet
1*	17 (578 ± 35)†	20 (502 ± 19)
2	24 (411 ± 59)	40 (251 ± 11)
3	37 (271 ± 45)	102 (98 ± 18)
4	49 (206 ± 18)	180 (56)‡
5	58 (172 ± 11)	

* Four chicks from each group bled each day.

† Figures in parentheses represent reciprocal prothrombin time avg and stand. errors $\sqrt{\frac{(\Sigma d^2)}{n(n-1)}}$, both × 10^4.

‡ None of the samples clotted within 180 sec.

TABLE III. Influence of Single Doses of Sulfaquinoxaline and Menadione on Prothrombin Times.

Sulfaquinoxaline	μg menadione/mg sulfaquinoxaline	Individ. plasma prothrombin times in sec.	Avg plasma prothrombin times, sec.*
—	—	40, 90	56
+	—	115, 119, 180+	132
+	.1	19, 28, 32	25
+	.2	19, 21, 24	21
+	.6	14, 15, 17	15
+	1.0	13, 13, 17	14
+	2.0	13, 14, 16	14

* Mean values on the basis of reciprocals of individual plasma prothrombin times.

2 μg menadione, dissolved in refined corn oil, /100 g body weight. Half of the group of 36 chicks were left on the control diet, the other half were given the diet supplemented with 0.2% sulfaquinoxaline. Four chicks were taken from each group every 24 hours for plasma prothrombin time determinations. The results of this trial are shown in Table II.

In a third experiment each of a group of 20 chicks, selected for similar body weight (mean weight = 269 g, std. error = 1.6 g) received a dose of approximately 50 mg sulfaquinoxaline in a gelatine capsule (range 46.8-56.1 mg): the capsule was followed within 20 minutes with graded doses of menadione, dissolved in refined corn oil (10 μg/ml), except for a number of controls. The levels of menadione that were fed were defined in μg mg of sulfaquinoxaline given to each individual chick. Plasma prothrombin times were determined after 24 hours. The results are given in Table III.

In all trials, the chicks were raised in electrically heated batteries equipped with thermostatic temperature controls and raised wire floors. Feed and water were kept before the birds at all times; to minimize bacterial synthesis of vit. K, the water was changed daily and the containers cleaned carefully.

Prothrombin times were averaged on the basis of the reciprocals of the individual clotting times(5); the averages were then reconverted into seconds. Observations were discontinued if no clot was observed after 180 seconds, and "180" used in the calculations. The use of reciprocals helps to mini-

mize inaccuracies in this range.

Results. Fig. 1 indicates that when higher levels of sulfaquinoxaline are incorporated into the feed, larger single doses of MSBC have to be supplied to obtain a given level of prothrombin. Although it might be an empirical relation, it has been shown that over the sensitive range a plot of the mean reciprocal prothrombin times against the logarithm of the vit. K dosage yields practically a straight line(5). The data presented in Fig. 1 seem to approach such lines, within the normal range of error, for each of the levels of sulfaquinoxaline in the feed. There appears to be a tendency for a decrease in slope with increasing sulfaquinoxaline levels. If this decrease is real, it would mean that with higher levels of the inhibiting compound, more of the vitamin per unit of inhibitor is required to overcome the inhibition. Experiments to investigate this proposition are presently under consideration.

The role of vit. K in the synthesis of prothrombin has not been clarified. Therefore, hypotheses about the mode and type of inhibition of this synthesis must, for the present time, remain in the realm of speculation. So far it can only be stated that the oral administration of sulfaquinoxaline will inhibit the formation of prothrombin, as measured by the one-stage method, that this inhibition can be reversed by the administration of vit. K-active compounds over a wide range, and that there appears to be a relationship between the quantities of inhibitor and of vitamin that can overcome the inhibition.

A single dose of 2 μg of menadione/100 g body weight, given to vit. K-deficient chicks, will lower prothrombin times appreciably, without, however, increasing prothrombin levels to normal. Decreases in prothrombin, as manifested by increased prothrombin times, were observed in Exp. 2 from day to day. After 5 days prothrombin times were approximately as long as before the dose of menadione had been given. The "residual" level of prothrombin found at that time was probably due to amounts of vit. K in the feed ingredients, or to intestinal synthesis, or both, and was probably less than 5% of the normal level. When 0.2% sulfaquinoxaline was included in the ration, this level of depletion was reached in half the time, and nearly complete depletion was achieved within 4 days, as shown in Table II.

An attempt was made in Exp. 3 to estimate the amount of menadione necessary to overcome a certain amount of sulfaquinoxaline, when both were given in the form of a single dose to vit. K-deficient chicks. With chicks of this age, the individual dose of 50 mg corresponds approximately to the daily intake of chicks receiving 0.15% of sulfaquinoxaline in their ration. The prothrombin times of each of the 20 chicks involved in this test, as measured after 24 hours, are given in Table III. Trial 1 of this series, and other experiments have shown that for depleted chicks of this weight, and in the absence of inhibitors, a single dose of 16-22 μg of menadione will suffice to restore normal prothrombin times. Although it appears that there is a certain amount of individual variation in the sensitivity of chicks to sulfaquinoxaline, an overall ratio of menadione to sulfaquinoxaline can be estimated for this range of the drug used. From the data shown in Table III it can be calculated that in the range studied, and after subtracting the menadione required for normal prothrombin levels in the absence of inhibitor, about 1.2 μg of menadione were required to overcome the inhibitory effect of 2 mg of sulfaquinoxaline. As the drug has about twice the molecular weight of menadione, over 800 moles of sulfaquinoxaline were required in this trial to inhibit the prothrombin-forming activity of 1 mole of menadione. It has to be stressed that these data were calculated on the basis of the amount of vitamin and drug ingested; rate and degree of absorption could change materially the ratio of the compounds interacting at the site of prothrombin formation.

Conclusion. Increasing doses of vit. K-active compounds are necessary to overcome the inhibition of prothrombin formation in chicks fed increasing levels of sulfaquinoxaline. The drug will hasten depletion of prothrombin in chicks kept on a K-deficient ration. When a single dose of about 50 mg

of sulfaquinoxaline was administered orally with varying levels of menadione, one mole of menadione appeared to overcome the inhibitory effect of more than 800 moles of sulfaquinoxaline.

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Non-Transfer of *Trans* Fatty Acids from Mother to Young.*† (23601)

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Bertram(1) first reported the presence of traces of *trans* Δ11 octadecenoic acid in oxen, sheep and butterfat. More recently Swern *et al.*(2) reported that beef fat contained not only traces but between 5 and 10% *trans* fatty acids. Hartman and his coworkers extended these investigations to the depot fats of various animals(3) and reported that whereas ruminants contained considerable amounts of *trans* fatty acids, non-ruminants and birds contained little or none. Reiser(4) has suggested that the occurrence of *trans* fatty acids in ruminants is due to the activity of the intestinal flora. Although *trans* fatty acids do not normally appear in the depot fats of non-ruminants *trans* fatty acids are deposited in the tissues whenever they are fed as a dietary component(5-7). It therefore, appeared of interest to note whether *trans* fatty acids were transferred to the tissues of non-ruminants by other than dietary means.

In the present study considerable amounts of *trans* fatty acids were deposited in the tissues of female rats by feeding these acids in the form of hydrogenated margarine stock. The rats were then mated and both mother and young analyzed for their *trans* fatty acid content.

Methods. One kg of the basal diet was prepared from glucose 640 g, casein 310 g, and Wesson salt mix(8) 50 g. Four g of a water soluble vitamin mix was added to each kg of feed(9) and the fat soluble vitamins were given by dropper twice each week.‡ Fifteen % of hydrogenated margarine stock containing 40.7% *trans* fatty acids was added to the feed at the expense of the glucose. Two % of corn oil was also added at the expense of glucose to serve as a source of essential fatty acids. Three rats were sacrificed at the beginning of the experiment, and 10 rats were placed on the experimental diet for 73 days and then mated. Eight mothers and their litters were sacrificed by anesthetisation with ether immediately after birth of the young. The remaining 2 litters were allowed to suckle the maternal milk for 9

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‡ One drop/rat of the following vitamin mixture administered twice each week. Five g vit. A (200,000 U.S.P. units, courtesy Distillation Products), .0054 g vit. D_2 and 2.535 g vit. E (mixed tocopherols) in 100 ml of olive oil.