The crystals appear uniform and possess constant biological activity upon recrystallization. Their activity is destroyed by heat, acid, or alkali. This would indicate that the crystalline protein is identical with tetanal toxin.

Complete chemical, physical, and biological characterization of the crystalline toxin will have to await the accumulation of larger quantities of material. The detailed procedure for the isolation and crystallization of the toxin as well as its characterization will be presented at a later date.

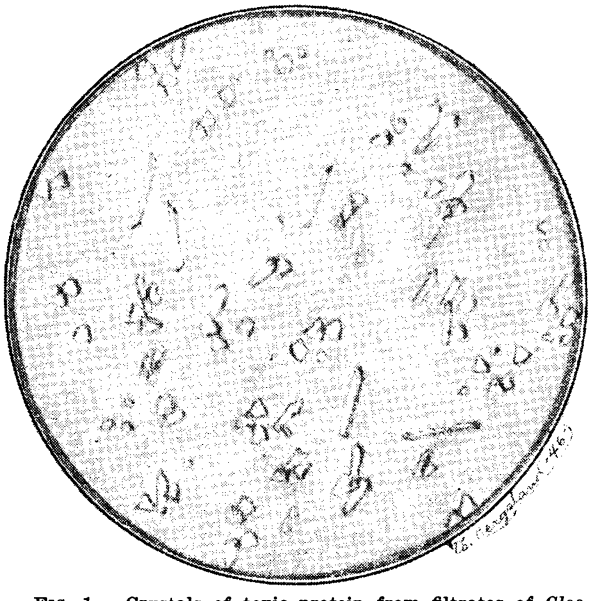

FIG. 1. Crystals of toxic protein from filtrates of *Clostridium tetani*, magnified 430 times.

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The Effect of the Prepartum Diet of the Cow on the Vitamin A Reserves of Her Newborn Offspring[1]

G. H. WISE, M. J. CALDWELL, and J. S. HUGHES

Kansas Agricultural Experiment Station, Manhattan

The importance of the prenatal nutrition of the calf is recognized, but the problem has been given scant consideration experimentally. Analyses of livers (*1, 3, 5, 6, 7, 8, 12*) and of blood (*14, 15*) from young calves (fetuses and newborn) revealed low vitamin A reserves. The general uniformity of these results in conjunction with observations of laboratory animals has led to the belief that the gestation diet of the dam has no appreciable effect on the vitamin A content of the fetal calf liver. Recently, however, Braun and Carle (*3*) noted that the vitamin A content of the fetal calf liver, though low, was in direct relationship to the diet of the mother. Unfortunately, conclusions from many of the data are vitiated by pathological complications in the experimental subjects.

In view of the foregoing evidence, steps were taken to ascertain the effects of the plane of carotene and vitamin A intake of the dam during the latter stages of gestation on the vitamin A reserves of the normal newborn offspring.

Procedure. The experimental subjects were healthy dairy animals of the Ayrshire, Holstein, and Jersey breeds, of which the latter constituted about 50 per cent. During the immediate prepartum period, the dams were placed under three dietary regimes with respect to the carotene and vitamin A intake: standard, high carotene, and high vitamin A. All the cows were fed a basal ration consisting of good-quality alfalfa hay, sorghum silage and a concentrate mixture. Group I (the standard, or control) was restricted to this ration, but Group II (high carotene) was grazed on pasture forage in addition, and Group III (high vitamin A) was fed a vitamin A supplement.[2] The carotene intake was undetermined, but the vitamin A consumption per cow was one million U.S.P. units daily. The prepartum period of pasture grazing ranged from 14 to 90 days, and of vitamin A supplementation from 8 to 45 days.

Samples of venous blood were drawn from the calves, usually within four hours after birth and always before colostrum ingestion. The collection of blood in the early postpartum stages was necessitated by the tendency of the vitamin A concentration in the serum to decrease in the fasted newborn (*9*). Subsequently several of the calves from dams in each group were sacrificed to obtain their livers for analysis.

The general analytical procedures adopted in the determinations of carotene and vitamin A were the Kimble (*11*) for blood and a modification of the Guilbert and Hart (*6*) for liver. The major deviation in the latter case was in the use of ether extract from a sample of dry tissue (dehydrated by grinding with anhydrous sodium sulfate) for saponification.

Results. The data in Tables 1 and 2 reveal no marked differences between the vitamin A reserves of calves from cows on the basal ration and those on the high carotene but a significantly higher storage in

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[2] "Dry vitamin A" supplied by Distillation Products, Inc., Rochester, N. Y.

the case of vitamin A supplementation. The results further indicate a positive relationship between the vitamin A of the blood and that of the liver. In accord with observations of other investigators carotene was usually present in the serum but undetectable in the liver.

TABLE 1

THE EFFECT OF THE PREPARTUM DIET OF THE DAMS ON THE VITAMIN A AND CAROTENE IN THE BLOOD SERUM OF THE NEWBORN CALVES

Group	Supplements to basal ration	No. of calves	Average values for serum	
			Vitamin A	Carotene
			γ/100 ml.	γ/100 ml.
I	None	4	2.90 ± 0.42*	0.65 ± 0.38*
II	Pasture	8	2.65 ± 0.49	1.39 ± 0.47
III	"Dry vitamin A"	4	9.40 ± 0.96	1.47 ± 0.70

* Standard error as determined by *Bessel's Formula* (*16*).

TABLE 2

THE EFFECT OF THE PREPARTUM DIET OF THE DAMS ON THE VITAMIN A AND CAROTENE IN THE LIVER OF THE NEWBORN CALVES

Group	Supplements to basal ration	No. of calves	Average values for liver	
			Vitamin A	Carotene
			γ/gram*	γ/gram
I	None	3	0.49 ± 0.19	0
II	Pasture	5	0.56 ± 0.12	0
III	"Dry vitamin A"	2	6.75 ± 1.15	0

* Wet basis.

The stage in the fetal development when vitamin A supplementation was most effective in augmenting the body reserves is undetermined, but the observations made in this study revealed no advantage for periods longer than the terminal two weeks of gestation.

Discussion. A basis for further appraisal of the magnitude of the vitamin A stores in the newborn offspring may be obtained by comparing the data presented in Tables 1 and 2 with those derived from older calves. The vitamin A concentration of 9.4γ/100 ml. of serum from the prenatally supplemented newborn was about the same as noted in the colostrum-fed calves 12 hours of age (*9, 15*) and near the lower level of the range reported to be normal for young calves (*13*), presumably beyond the colostrum ingestion stage. For the livers the average value of 6.75γ of vitamin A/gram, in the group from vitamin A-supplemented dams, was considerably greater than 2.2γ/gram found in a calf 12 hours after receiving colostrum, but markedly less than 19.5γ/gram, in a calf fed colostrum for four days (*9*). It should be emphasized, however, that the values from colostrum-fed calves are subject to wide variations, due to the variable amounts of the food ingested and to the extremes in vitamin A and carotene potency of the colostrum.

The low values for vitamin A normally found in the fetal calf liver seemingly are ample for development *in utero* but inadequate for continued postnatal health. A rapid increase of the vitamin A concentration in the newborn apparently is essential in protecting it from many calfhood disorders. The first primary source of nutrients is colostrum, the vitamin A potency of which depends on the prepartum diet of the dam (*9*) as well as on other factors. Recent studies (*9*) have revealed that parturient cows under standard feeding and managerial practices occasionally do not produce colostrum, a failure that is likely to be overlooked in the average herd. Thus it appears that prenatal supplementation is an expedient means of endowing the young calf with vitamin A and of aiding in the prevention of diarrhea in the newborn (*4*).

The effect of the prenatal diet on the vitamin A reserves of the offspring introduces the conjectural issue of placental transmission and fetal storage. From the low vitamin A values normally observed in the fetal liver two principal theories have emerged: either (1) the placenta permits only limited amounts of carotene and vitamin A to pass or (2) fetal metabolism inhibits extensive storage. Evidence gleaned from the literature indicates that the placenta plays the primary regulatory role. On this premise a *placental threshold* conceivably may serve in the control mechanism. Feeding massive amounts of vitamin A thus would increase the concentration in the maternal circulation to the extent that a portion of the excess passes into the fetus. This, however, is not in accord with the low values from the pasture-supplemented group. A more feasible explanation may be found in the recognized differential permeability of the placental membrane. Possibly the esterfied form of vitamin A, present in the fish liver oils and their distilled concentrates (*10*) and defected in the circulation of the "dry vitamin A"-supplemented cow (*9*), traverse the placenta more readily than the alcohol form which is suggested (*2*) as the principal form normally present in the circulation of the bovine.

The stability of vitamin A is another factor that perhaps plays a role in storage as well as in transmission. The ester form may be resistant to destructive processes in the placenta and to metabolic activity in the fetus. If this were true, however, the problem of utilization probably would tend to nullify the value of the reserves.

Factors involved in the prenatal nutrition of the bovine fetus are being explored further with the view of overcoming some of the difficulties to which the newborn calf is subject.

Summary. Feeding vitamin A at the rate of a million U.S.P. units daily to individual dairy cows in

the latter stages of gestation augmented significantly the vitamin A concentration in the blood and the livers of their newborn calves, but pasture grazing, providing an abundance of carotene in the prepartum diet of the dams, failed to effect an increase over that observed in calves from dams restricted to a standard winter ration. The explanation for these divergent results is obscure, but it is suggested that the placental membrane may be more permeable to the ester form of vitamin A than to the alcohol form. This high initial vitamin A reserve in the newborn calf should have practical value in the maintenance of its postnatal health.

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The Low Therapeutic Activity of Penicillin K Relative to That of Penicillins F, G, and X, and Its Pharmacological Basis

HARRY EAGLE and ARLYNE MUSSELMAN

The Johns Hopkins University and *U. S. Public Health Service Venereal Disease Research Laboratory and Postgraduate Training Center, Baltimore, Maryland*

Commercial penicillin contains at least four molecular species, identified as F, G, K, and X and differing from each other in the nature of the side group attached to a common nuclear structure (*8*). These are known to vary significantly in their bactericidal activity *in vitro*. Thus, referred to penicillin G as 100, the relative activities per milligram of penicillins F, G, K, and X against *Staphylococcus aureus* are reported to be 90, 100, 140, and 55, respectively (1,550, 1,667, 2,300, and 900 units/mg.) (*7*). In this laboratory, crystalline samples of F, G, K, and X were found to have relative gravimetric activities *in vitro* of 82, 100, 120, and 140 against the C-203 strain of hemolytic streptococcus, and 53, 100, 75, and 50, respectively, against a cultured strain of *Spirochaeta pallida* (Reiter) (*3*).

Subsequently, however, results obtained in the treatment of experimental syphilis indicated differences in the relative activity of the several penicillins *in vivo* far exceeding those hitherto observed *in vitro*. The curative dose (CD_{50}) of commercial penicillins, which probably consisted largely of penicillin G, had been found by Eagle, Magnuson, and Fleischman (*2*) to be 1,650 units/kg. when given every four hours for 20 injections. Almost identical results were obtained by Fleming (*4*), using both commercial penicillin and crystalline G. However, with penicillin K similarly administered preliminary data provided by Chesney (*1*) and confirmed by Mahoney and Arnold (*5*) indicated that even 16,000 units/kg. were not curative.

The obvious explanation for this marked discrepancy between the activities of penicillins G and K seemed to be that penicillin K, despite its definite activity against cultured, nonpathogenic spirochetes, was relatively inactive against pathogenic *S. pallida*. An alternative, if less likely, explanation was that penicillin K might be excreted or destroyed in the body more rapidly than the other penicillins and would therefore be relatively ineffective not only in the treatment of syphilis but in other infections as well. The data here presented indicate that this is in fact the case. Penicillin K disappears from the blood, and presumably the tissue fluids as well, far more rapidly than do the other penicillins; and the relatively small amount excreted in the urine suggests that it is inactivated *in vivo* to a greater degree than penicillins F, G, or X. Finally, corresponding to its pharmacologic behavior, and in complete agreement with the results in experimental syphilis (*5, 9*), it has proved approximately 9 to 15 per cent as active as penicillins F, G, or X in the treatment of experimental pneumococcus or streptococcus infections in white mice.

Preliminary data indicate that the rapid disappearance of penicillin K from rabbit blood and its relatively low recovery in the urine are due to its inactivation by the circulating blood. In rabbits the inactivating agent appears to be a relatively thermolabile, nondialyzable constituent of plasma. It is not present in any of the highly purified protein fractions of human plasma so far tested.

Blood levels and urinary excretion in rabbits. When rabbits were injected intramuscularly with crystalline penicillins F, G, K, and X[1] at equivalent dos-

[1] The following firms have made available samples of crystalline penicillin used in this study: Upjohn (F), Squibb (G), Lederle (X), and Abbott (K). Their cooperation is gratefully acknowledged.