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INTRODUCTION

For a long time after the discovery of vitamins and the subsequent advance in the knowledge of mineral metabolism, the livestock industry tended to regard these findings as largely technical matter with little or no practical application. Evidence in recent years has shown, however, that certain conditions of very great economic importance, spontaneously appearing in livestock, can be explained only by a knowledge of the role of vitamins and minerals in maintaining the well-being of domestic animals. The energy and protein values of feedstuffs, based on digestible nutrients, are an entirely satisfactory means of evaluating feeds when the rations happen to contain the additional essentials of an adequate diet in sufficient amounts, and account for the importance placed on variety by the husbandman. Unfortunately, however, these additional feed essentials are liable not to be present in adequate amounts in the semiarid southwestern parts of the United States during the dry season when animals are kept entirely on the natural vegetation of the range.

Experiments with small laboratory animals show that the delicate physiological phenomena of reproduction may be more or less seriously affected by general undernutrition or by specific deficiencies. The reproductive failure may vary in character with the nature and degree of the deficiency, and there are four general types of its manifestations:

1. Failure of the females to come into estrum.
2. More or less regular occurrence of estrum and mating, with failure in fertilization.
3. Mating and fertilization followed by death of the fetus and abortion or resorption at various stages of gestation.
4. Failure in lactation.

Reproductive failure in cattle induced by lack of phosphorus has been clearly demonstrated in different parts of the world. The evidence on

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[2] The experimental work reported in this paper became cooperative with the United States Department of Agriculture Bureau of Animal Industry, July 1, 1929.

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phosphorus and protein deficiencies and on general undernutrition as related to reproduction under range conditions in California has been discussed by the authors in previous publications.[1, 2]

This bulletin summarizes information on the nature and occurrence of vitamin A, the experimental evidence on the relation of vitamin A to reproduction in cattle, data on the symptoms and differential diagnosis of vitamin-A deficiency, and evidence that this deficiency contributes to reproductive failure in range cattle.

NATURE AND OCCURRENCE OF VITAMIN A

In 1914 McCollum and Davis[3] found that vitamin A was contained in the unsaponifiable fraction of milk fat—that is, the portion that does not form soaps when heated with alcoholic potash. This evidence that the essential substance was not a fatty acid glyceride but something dissolved in the fat formed the basis of the terminology, "fat-soluble vitamin A."

Subsequent investigations by several workers demonstrated that the vitamin could be extracted from plant sources by fat solvents, such as ether and alcohol.

The occurrence of the vitamin associated with yellow pigmentation, especially in yellow corn, and its absence in white corn, led to research on the possible relation between the vitamin and these yellow pigments. As early as 1826 Wachenroder[4] had extracted and described the yellow-orange pigment of carrots. In 1864 Stokes[5] discovered a method of separating the yellow pigments of plants from the accompanying green chlorophyll, and in 1866 Piccalo and Lieben[6] extracted and crystallized the yellow pigment from corpora lutea (yellow body of the ovary) of cows.

In 1911 Tswett[7] proposed the name "carotenoids" for all pigments related chemically to carotene, the pigment of carrots. Escher[8] in 1913 demonstrated that the pigment of the corpora lutea of cows' ovaries was identical with carotene from carrots.

During the years 1914 to 1920 Palmer[9] and coworkers found carotene to be the principal pigment in the blood serum, body fat, and milk fat of cattle, and also in the skin secretion of Guernsey cattle. In chickens, on the other hand, the principal pigment of skin, shanks, body fat, and egg yolk proved to be xanthophyll, a closely related substance. Goats, swine, and sheep have little or none of either pigment, while human adipose tissue and blood serum contain both pigments in amounts tending to vary with the quantity of each in the diet. Palmer further established that the source of these pigments in normally pigmented species was the

feed. Chickens of breeds which are normally pigmented were raised devoid of yellow color, and they produced eggs devoid of yellow yolk color on carotenoid-free rations. Mead and Regan,[10] experimenting with Jersey and Holstein cattle, have produced body and milk fat without yellow pigment on a ration consisting solely of concentrates.

Steenbock and his associates, early impressed with the relation of vitamin-A potency to yellow pigment in plants, published a series of articles showing the close correlation between carotenoid pigment and vitamin A in roots,[11] maize,[12] leaves,[13] and peas,[14] as determined by feeding pigmented and colorless varieties to albino rats. About the same time, however, Drummond[15] failed to improve the condition of rats suffering from vitamin-A deficiency by feeding pure crystalline carotene. Later experiments by Steenbock, Sell, and Buell[16] with milk fats of high and low color, practically colorless cod-liver oil, and light-colored egg yolk failed to show any relation between vitamin-A potency and the amount of pigment present.

Meanwhile, workers were following a new line of investigation that has led to the present advance in knowledge. In 1920 Rosenheim and Drummond[17] in England stated that the well-known color reaction of sulfuric acid with cod-liver oil applied also to liver fats of other animals, and suggested a relation of the color-producing factor to vitamin A. Drummond and Watson,[18] after three years, were convinced that the relation was more than a casual one but, because the color produced was transient, could not obtain quantitative results. In 1925 Drummond, Rosenheim, and Coward[19] found that arsenic chloride gave, with cod-liver oil, a more stable blue color, the intensity of which indicated a relation to the vitamin-A content. Later that year Rosenheim and Drummond[20] gave the first definite technique for a quantitative chemical assay of vitamin A in oils. In 1926 Carr and Price[21] substituted antimony trichloride for the arsenic chloride and otherwise improved the technique for estimating vitamin A.

These discoveries, presenting a simple, rapid method of assaying oils for their vitamin-A content, in contrast to the tedious, expensive, time-consuming feeding tests with animals, stimulated widespread and intensive research concerning its validity. Spectrographic studies were made on solutions containing the vitamin and carotenoids, and there was renewed interest in the relation of the vitamin to the carotenoid pigments.

In 1928 Euler, Euler, and Hellström[22] reported that feeding of carotene to vitamin-A deficient rats restored growth. Their findings were confirmed by Moore[23] and others in 1929. Dulière, Morton, and Drummond,[24] however, using a technique similar to that employed in Drummond's 1919 experiments, failed to induce improvement in vita-

min-A deficient rats by carotene feeding. Their failure was subsequently shown to result from the employment of a solvent (ethyl oleate) in which carotene rapidly deteriorates.

In 1930 Moore[25] fed rats on a vitamin-A deficient diet until they showed signs of the deficiency; at autopsy the livers of a number of them were shown to be devoid of vitamin A by means of the color test with antimony trichloride. The diet of the remaining animals was supplemented with purified carotene. Deficiency symptoms disappeared, growth was resumed, and the livers of these animals were subsequently shown to be rich in vitamin A. Thus it was demonstrated that the pigment carotene is converted within the animal to vitamin A, which is practically colorless.

It is now clear that such species as swine, sheep, goats, and rats almost completely convert these carotenoid pigments to colorless substances and store in their bodies or secrete in their milk the practically colorless vitamin A. Cattle, on the other hand, convert part of the carotene in the feed to vitamin A and store part unchanged in the liver and adipose tissue or secrete it in the milk. Breed differences in this regard are typified by the highly pigmented milk fat and body fat of Jersey and Guernsey cattle as compared with that of Holstein cattle under the same feeding conditions. The biological value of the milk fat is therefore not necessarily correlated with the degree of color.

Whatever the origin of the widespread prejudice against yellow fat in the beef trade, it does not appear justified in light of present knowledge of nutrition. Aside from hereditary variations, the degree of pigmentation is governed largely by the quantity of carotenoids ingested with the feed and by a tendency to accumulate pigment with advancing age. Since carotene, the yellow pigment in beef fat, is a source of vitamin A in the human diet, discrimination against it appears inconsistent. The common appearance of yellow color associated with grass-fed beef lacking in finish and with dairy cattle has led to the use of color as an index of quality. Cattle fed heavily on grains containing little or none of this pigment, will eat relatively small amounts of forage normally rich in pigment, thus producing carcasses with light-colored fat. That color is not necessarily associated with low quality is shown by the fact that animals raised at the University Farm and awarded high honors at the Chicago International Livestock exposition were found on slaughter to have yellow fat. The grading of beef based upon conformation of the carcass, quality, smoothness, and degree of finish or covering with fat is sufficient to place all carcasses, including excessively colored carcasses of Jersey and Guernsey cows, in their proper place without regard to the color of the fat. This fat color may be the one point of superiority of

the carcasses of these breeds from the standpoint of human nutrition, though the concentration of the vitamin or its precursors even in highly colored fat is relatively low as compared with that in liver or green plant tissue.

Occurrence of Vitamin A or Its Precursors.—Vitamin A or its precursors are widely distributed in nature. Carotene is present in all green plant tissues, in carrots, and in other yellow roots. Seeds in general contain little vitamin A. Yellow corn contains considerable amounts, but white corn contains very little, while barley, wheat, rye, oats, and sorghum grains contain only small amounts, entirely inadequate for the nutritional requirements of animals. In animal tissues vitamin A is much more highly concentrated in the internal organs and glands than in the lean tissue or body fat. Liver tissues of most animals are so rich in it that the liver has come to be looked upon as its principal storehouse. The liver oil of cattle which have been fed rations rich in vitamin A has a much higher concentration than does cod-liver oil.

Conditions Which Destroy Vitamin A or Its Precursors.—Vitamin A and carotene are easily oxidized in the presence of air and light, and the rate of oxidation is accelerated by heat. The curing of hay under conditions of exposure to sunlight, which involves bleaching of the green chlorophyll, rapidly diminishes its vitamin-A potency.[26] Coward[27] found that vitamin A is completely destroyed when the leaves of plants dry up, become brown, and die. It is relatively stable to heat in the absence of oxygen.[28] According to Dann[29] it is resistant to oxidation in ethyl alcohol, alcoholic potash solutions, and ethyl acetate; and its potency is not diminished by aeration at 98° Centigrade for one hour in alcoholic potash solution. In this connection the influence of antioxygens in stabilizing vitamin A must not be overlooked.

Because of wide variations occurring in naturally cured hays according to the degree of exposure, sweeping generalities regarding the difference in vitamin-A potency of field-cured and artificially dried hays should not be made on the basis of experiments involving a few samples. Likewise the widely quoted statement of Jones, Eckles, and Palmer[30] that straw is a good source of vitamin A for cattle requires qualification. In the midwestern and eastern United States grains are cut and bound or headed as soon as possible after seed maturity. The plants may not be completely dead when harvested, some stems are partly green, and other green plants may be included with the straw. In California, grains ripen after the close of the rainy season, little or no green growth of any kind is present in some areas when the grain is ripe, the plants die, and the standing grain may be exposed to intense heat and sunlight

for two to eight weeks before harvesting. The straw consequently may be very deficient in vitamin A.

Corpus Luteum and Carotene.—The presence of carotene in the corpus luteum is of historical interest, since this was the first animal tissue from which the pigment was extracted in crystalline form by Piccalo and Lieben.[6] Since the corpus luteum is so intimately associated with the rhythmic functioning of the ovaries and with pregnancy, one may logically inquire into the possible function of carotene in this body. Moore[31] recently stated, in connection with an investigation on the presence of carotene and vitamin A in corpora lutea of cows, "the presence of such considerable amounts of carotene in the corpus luteum, apparently unaccompanied by vitamin A, must present an interesting problem in the mechanism of reproduction." Definite proof that carotene is the corpus-luteum pigment is available only for cattle and sheep. Bergh, Muller, and Broekmeyer,[32] and Palmer[9] found no carotenoids at all in the corpora lutea of swine—an argument against any specific reproductive function. We have examined an ovary carrying the corpus luteum of pregnancy from an animal in the experiment of Mead and Regan[10] on rations devoid of roughage for dairy cattle. The body and milk fat of these animals, as previously stated, were devoid of yellow pigment. Neither carotene nor vitamin A could be demonstrated in the corpus luteum from this particular cow. Others fed similarly have completed normal reproduction. Cod-liver oil was practically the sole source of vitamin A in these rations. Carotene would therefore appear to be present in the corpora lutea of cattle rather because of an affinity for the fatty substances in these bodies than because of a specific function in the mechanism of reproduction.

REVIEW OF LITERATURE ON RELATION OF VITAMIN A TO REPRODUCTION IN CATTLE

Definite disease syndromes have been described as caused by lack of vitamin A in certain species of farm animals. Thus Beach[33] in 1924 demonstrated that nutritional roup in chickens results from this deficiency. In areas where yellow corn is not available, its incidence is rather high unless a green feed is regularly supplied. Hughes, Aubel, and Lienhardt[34] in 1928 described typical symptoms in hogs which failed to get sufficient vitamin A in the diet. Their elaborate experiments covered a period of seven years.

Evidence regarding the necessity of this vitamin in reproduction in mammals has been definitely established, but the findings become particularly confused in the case of the bovine species.

Hart, McCollum, Steenbock, and Humphrey[35] in 1911 published their original work on the physiological effect on growth and reproduction in cattle of well-balanced rations from the wheat, corn, and oat plants respectively, and from mixtures of the three. Reproduction was most seriously interfered with in the animals on the wheat plant, next in those on the oat plant; while those on the corn plant produced young of normal weight and vigor. At the time no definite solution of the observations made was given; but the possibility of toxic bodies being carried in the rations or produced in the intestinal tract was thought to deserve consideration, as was also the poor mineral content of the diet.

In 1917 these authors[36] published further work along the same line. In the interim between the two publications, knowledge about the essentials of a ration had been expanded by their own work and that of others to include the fat-soluble A and water-soluble B vitamins.

In their effort to improve the unsatisfactory results with the wheat plant, milk fat was added at the rate of 2 pounds per 100 of the grain mixture. The ration, composed of 6.7 pounds of wheat grain, 0.3 pounds of wheat gluten, plus the milk fat, and 7 pounds of corn stover was given to each of two cows. One of these produced a 46-day premature weak calf that lived 10 hours; the other produced from the first gestation, a healthy calf 13 days premature; from the second gestation a weak calf at term which grew strong. These findings were somewhat confusing to interpret at the time and it was concluded that in the previous experiments the absence of vitamin A had not been the causal factor in reproductive failure. It was considered that the ration was so much improved by the mineral addition through the better roughage used (corn stover in place of wheat straw) and by a more abundant supply of fat-soluble A that successful resistance to the real factor, the toxicity of the wheat kernel, was maintained by the second cow during the first gestation. The toxicity was considered to be accumulative and to show its effect somewhat on the offspring from the second gestation.

This work also showed that rations restricted to the wheat plant did not sustain the growth of Holstein heifers. They failed to come into estrum and could not be bred. They showed pathological conditions such as blindness, feebleness, and emaciation, and abnormal excitability followed by collapse.

In 1920 Hart, McCollum, Steenbock, and Humphrey[37] published further work on the influence of rations restricted to the oat plant on reproduction in cattle. At this time they showed that breeding cows were inadequately nourished on this ration and that their offspring were born prematurely, very weak, or dead. In the earlier experiments the oat

straw had been grown on an alkaline soil and contained 0.84 per cent calcium oxide, and with this roughage better offspring were produced. In the later work the oat straw contained but 0.47 per cent calcium oxide, and the calves were far inferior.

Addition of vitamin A to this diet in the form of 2 pounds of milk fat per 100 pounds of grain mixture, or of improved protein by the addition of casein separately or combined did not help reproduction. When calcium salts were added in the form of carbonate, phosphate, or acetate, offspring of fair vigor were produced, even without the addition of a better protein or more vitamin A.

Although this much was accomplished with the experimental ration, the offspring were inferior to those produced from cows fed natural roughage such as corn stover, clover, alfalfa, or even marsh hay grown on an alkaline marsh. It was concluded from these data than an otherwise complete ration for a dry breeding cow should contain at least 0.45 per cent of calcium oxide, but that this may not apply when the ration contains some fresh green materials.

Little was mentioned in this publication regarding toxicity of the plant although just why low calcium intake should be the determining factor in normal or abnormal reproduction was not clear to the workers. They suggested that "on low-calcium rations there can be especially favorable conditions for continual absorption of products of intestinal origin, among which may be bacterial toxins or amines."

In 1924 Hart, Steenbock, Humphrey, and Hulce[38] reported new observations with reinterpretations of their previous experiments on the nutritive value of the wheat plant. They showed that when yellow corn grain was substituted for the wheat grain on the wheat-plant ration, reproductive failure continued. When the ration of yellow corn, wheat gluten, and wheat straw was supplemented by a salt mixture containing calcium, reproduction was normal. If, however, the original wheat plant ration (wheat grain, wheat gluten, wheat straw) was supplemented by the salt mixture containing calcium, nutrition was interfered with and there was a disturbance of the reproductive cycle. At the time of these earlier experiments the investigators had not known that yellow corn is liberally supplied with vitamin A, whereas wheat grain contains a very small quantity. They state: "In the light of our modern views of nutrition it is perfectly clear that the wheat ration was deficient in vitamin A and in calcium." They conclude that the addition of these substances makes the wheat-plant ration complete for growth and reproduction and that therefore the presence of an inherent toxic factor need not be assumed.

While the above classical experiments were being carried out on nutrition and reproduction, a very large amount of bacteriological investigation was conducted on bovine infectious abortion. This latter condition was conclusively demonstrated to be an infectious disease. Naturally, however, some conflict of opinion arose between the workers in nutrition and bacteriology on the interrelation of nutritive regimens and specific bacterial infection in reproduction in cattle.

The discovery by Evans and Bishop[39] of vitamin E, a hitherto unrecognized dietary factor essential for reproduction, the first note on which appeared in 1922, increased interest in the importance of nutrition in reproduction.

Working on this new information the United States Bureau of Dairy Industry conducted a limited series of experiments in which slight evidence was secured that sprouted oats overcame sterility in cows. These animals had been examined previously and showed no evidence of pathological or diseased condition of the genital tract. The work was published by Winters[40] in 1926 and by Graves and Miller[41] in 1927. The latter attributed success to the presence of vitamin E in the sprouted oats. Further data on the subject were included by Miller and Graves[42] in a history of reproduction of the Beltsville Herd of the Bureau of Dairy Industry. These meager experiments did not justify the assumption made regarding vitamin E and have not been confirmed.

Infectious abortion in cattle had been given some attention by the National Research Council beginning with 1920. In 1925 the Council appointed a joint committee of the Divisions of Biology and Agriculture and Medical Sciences under the chairmanship of Theobald Smith. The personnel consisted of workers in both the nutritional and bacteriological fields, with one geneticist. The committee held its first meeting in Chicago June 11 and 12, 1926, and outlined procedures for the further study of nutrition and *Brucella abortus* separately or in combination as related to reproduction in cattle.

At this time (1927), from South Africa, Theiler, Green, and Du Toit[43] reported a significant investigation on minimum mineral requirements in cattle. These authors state:

> The paper presents a summary of a preliminary series of orientation experiments concerning minimum requirements of growing cattle for calcium, phosphorus, sodium, potassium, and chlorine. Main attention was concentrated upon phosphorus deficiency in the hope of producing clear clinical cases of "aphosphorosis" for subsequent pathological study and comparison with the naturally occurring disease Styfsiekte; but since the question of ratio of minerals in dietaries has attracted so much attention of recent years, this factor is also considered. The vitamin factors come in for incidental review.

The vitamin factors caused us to review these studies comprising eight duplicate experiments on sixteen heifers. The basal ration consisted of 3½ pounds of hay, poor in minerals but otherwise of fair quality; 2 ounces of blood meal; and 5 pounds or more of Fanko. This last, locally produced, consisted of the rolled endosperm of maize, high in calorific value, fair in protein content, but very low in mineral constituents. All the animals used, except those in experiment 8, were on diets low in vitamin A, which was limited to the amount in the 3½ pounds of hay. Fanko, it was stated, contained even smaller quantities of vitamins than polished rice, and blood meal also very little. In experiment 8, a check on the vitamin factor, part of the hay was replaced by 4 pounds daily of young, fresh, green forage.

The experiment, therefore, from the standpoint of this vitamin arranged itself into 2 control animals (experiment 8) and 14 animals (experiments 1 to 7) on diets low in vitamin A. The 2 control animals in experiment 8 gave birth to normal calves. Of the 14 heifers low in vitamin A along with other deficiencies, 8 produced weak or dead calves, 2 died without having calves, and 4 produced normal calves in June or July, 1926, after having been on the diets from September, 1924.

The summary leaves open the explanation of the observed abnormalities in calving except in the case of phosphorus deficiency, in which definitely abnormal calves may be born. According to present knowledge vitamin A was probably a factor in the failure of reproduction, the 3½ pounds of poor-quality ripe hay not furnishing enough for normal reproduction in all cases. Similar reproductive failure in sheep fed this ration was reported in two subsequent experiments by Du Toit, Malan, and Groenwald.[44, 45]

In 1929 Hadley and Hawn[46] and in 1932 Hart, Hadley, and Humphrey[47] reported experiments of the Wisconsin group following procedures outlined by the committee on abortion of the National Research Council to answer the question: "Is it possible to lower the resistance of cattle by feeding a ration low in both lime and protein so that they become more susceptible to contagious-abortion infection?"

Forty-four Holstein heifers were divided into two lots. Lot 1 received the good ration consisting of alfalfa hay, corn silage, and a grain mixture consisting of corn, oats, oil meal, wheat bran, bone meal, and cod-liver oil. Iodized salt was used, and in summer the animals had access to both alfalfa and sweet-clover pasture. Lot 2 received a relatively poor ration consisting of corn silage, timothy hay from acid soils, and a grain mixture of corn, oats, and gluten meal. Common salt was used and in the summer they were pastured on timothy and blue-grass. Both groups did well, and eventually all were bred and calved at term.

They were milked for 3 months, bred again for second calves, and when proved by rectal examination to be pregnant were divided into five groups, each containing approximately the same number of animals from lots 1 and 2. Four of these groups were exposed to infection with the abortion organism while the fifth was left as controls.

This experiment showed that the good ration did not increase resistance to infectious abortion, nor did the poor one increase susceptibility. There were eleven abortions on the good ration; eight on the poor. The experiment also showed that cows could adjust themselves to a low lime intake and that the efficiency of lime utilization increased as the amount in the ration decreased. There was also evidence that cod-liver oil in the good ration depressed milk-fat production. E. B. Hart, who had charge of the nutrition of the animals in this experiment, commented in the discussion of the 1929 report as follows:

> We chose this poor ration because the roughage used was grown on acid soil, which is a condition that is common in the northern part of the United States. For milking cows it is no doubt too low in its lime content. The phosphorus content, as Dr. Hadley said, is at a rather low level. If you analyze that and compare it with the alfalfa ration, you are sure to have one distinct deficiency with two others on the border line. The one distinct deficiency in dairy-cow nutrition in the northern states is the use of these poor roughages with their low lime content. You may not know, but it is a fact that with rations still poorer than this poor one that we have, those made from straws and grains, you can disturb reproduction. The deficiency may be lime or it may be one of the vitamins, particularly vitamin A. It was that set-up that led us to this experiment. But we put the poor ration as one that is not uncommon on farms. You may say that is not a very poor ration. Indications are that these animals have gotten along very well on it. There has not been much difference in the results from the two rations up to date, it is true. Maybe we will have to revise our idea that the ration we call the poor ration was not so poor after all.(46)

These experiments demonstrated clearly that the specific disease, bovine infectious abortion, was not materially influenced by nutrition. Because of the controversial nature of the subject, the results left in the minds of many the idea that restricted nutritional regimens could not interfere with normal reproduction in the bovine. The cited experiments of Hart and his associates, however, had already proved that restricted nutritional regimens involving low lime and vitamin A could prevent normal reproduction. In the experiments on the effect of nutrition on susceptibility to infectious abortion, an abundance of vitamin A was supplied in the poor ration for lot II, while attention was being focused on low calcium. The result showed increased efficiency of calcium utilization at low levels and indicated that vitamin-A deficiency was the more important cause of the failure of reproduction in the Wisconsin nutrition experiments between 1907 and 1924.

In 1926 Jones, Eckles, and Palmer[30] showed vitamin A to be an indispensable factor in the diet of calves. They concluded that wheat straw is a good source of this vitamin for ruminants. In a calf from a stall-fed cow consuming less than 40 per cent wheat straw in its ration, however, symptoms of vitamin-A deficiency developed. The vitamin-A storage in the liver of calves at birth, which depends on the diet of their dams, was considered to be an important factor. We believe that the amount of the vitamin in the milk, the time of cutting the grain after it has ripened, and the method of handling the straw are also important factors.

In 1928 Bechdel, Honeywell, and Dutcher[48] studied the effect of feeding five heifers, one to two and a half years of age, a ration deficient in vitamin A. They produced edema in the front legs with declining appetite and increased respiratory rate. One animal went completely blind. In two cases of pregnancy the calves were born dead, one being more than two months premature. No statement is made regarding the possibility of abortion infection in these cases.

Halverson and Sherwood[49] in 1930 reported investigations on the feeding of cottonseed meal to cattle. They showed very conclusively that the so-called poisoning of livestock from cottonseed feeding was due to deficiencies rather than to the gossypol content, which had generally been held to be the cause since Withers and Carruth[50] had isolated this phenol-like substance from the seed and showed it to be toxic for animals. The deficiency primarily responsible for the abnormalities was vitamin A, although vitamin B and calcium were low in the diets and in some cases when yeast and calcium were supplied some improvement was observed. Though these experimental animals showed reproductive difficulties similar to those in the Wisconsin experiments, the data are confusing because infectious abortion existed in some of the cows. Nevertheless, the lowered body weight at birth, the weakness and high mortality in the offspring, and the presence of eye lesions in the calves from cows on the rations low in vitamin A as compared with the controls are significant.

In 1932 Meigs and Converse[51] reported the physiological effects of rations containing low-grade roughage on cows free from infectious abortion. After fairly long periods on grain and U. S. No. 3 timothy hay their animals uniformly gave birth to premature dead or weak and blind calves. The hay was graded No. 3 because it had been cut in the seed stage without other damage. When a better grade of timothy was fed to other cows in the same herd the proportion of normal calves was larger; and when the roughage consisted of U. S. No. 1 alfalfa hay, reproduction was quite satisfactory.

Deficiency of vitamin A in the No. 3 hay was considered an important factor in producing the results obtained. Experiments on rats showed that U. S. No. 1 alfalfa contains thirty times as much vitamin A as U. S. No. 3 timothy hay. Even the best timothy hay was found to contain only about one-tenth as much vitamin A as the best alfalfa.

All critical students of the subject, therefore, must realize that even in cattle vitamin A is a necessary food essential for normal physiological processes. Reproduction is affected when vitamin A is too low in the diet, and the result is the birth of premature, dead, or weak calves with or without eye lesions, accompanied by retention of the placental membranes. These may be the only symptoms noticed. They are so similar to those of infectious abortion of cattle, a widespread infectious disease which is far more likely to be the cause, that possibility of the latter must always be eliminated by bacteriological and serological procedure before the existence of vitamin-A deficiency can be established. All previous evidence of the existence of this condition has been based on the work with experimental groups of cattle on more or less highly restricted nutritional regimens.

We now desire to present evidence that conditions to which range cattle are more or less regularly subjected may be such that they will not be getting sufficient of this vitamin in their forage and supplements and that severe losses may result.

EVIDENCE OF VITAMIN-A DEFICIENCY UNDER RANGE CONDITIONS

According to the evidence previously cited, range feed loses its vitamin-A content upon drying and bleaching. In the bovine, as in other species, the concentration of vitamin A in the liver varies with feed conditions. The reserve supply that has accumulated in the body during the green-feed season, together with the limited additional supply obtained in different areas from browse, moss, grass from moist places around streams and waterholes, etc., is sufficient to carry animals through the dry season under average conditions.

The amount of this substance required by an animal varies with age, rapidity of growth, pregnancy, and lactation. With unusual prolongation of the dry-feed period, therefore, manifestations of the existence of deficiency may occur. During the five-year period in which our range-feed investigations have been carried on, more or less severe losses of calves were reported over wide areas on the range in California. In the fall and winter of 1929–30 and again in 1932–33 these losses were particularly heavy. In these years the feed dried early in the spring, and the

first rains came in late autumn followed by cold weather, so that green feed was not available until January or February of the following year. Under such conditions, the calves are born dead or are weak and die soon after birth. Retention of the placenta by the cows and diarrhea in the weak calves are also common. In general the calves born dead are late in gestation; but evidence exists that they may be expelled at any time during the second half of pregnancy. Reports have not been infrequent that as many as one hundred head of pregnant animals have aborted in this way. At the same time stockmen have generally agreed that the losses cease and the remaining pregnant cows calve normally after green forage has been available for a short time. Blood samples, collected by us and by official veterinarians from a sufficient number of cases in widely scattered herds, have been negative to the abortion agglutination test, so that bovine infectious abortion can be eliminated as the cause of the trouble. Even though some of these herds contained an occasional reactor to the blood test for abortion, the number of abortions, weak calves, and retained placentae occurring in the nonreactors shows that some other cause besides infectious abortion is operating.

These manifestations are identical with those described in the controlled experiments cited. Further to substantiate the lack of vitamin A in the diet as the cause of this condition, livers of calves were studied chemically by the colorimetric method of Carr and Price[21] during the winter of 1932–33.

The livers of new-born animals tend to be comparatively low in vitamin A irrespective of the diet of the dam during gestation. Under favorable conditions of intake, storage accumulates with age and reaches a maximum in mature animals. We have demonstrated that in normal new-born calves solutions representing from 25 to 100 milligrams of their liver tissue are necessary to produce a color reaction with antimony trichloride, while as little as 1 milligram of adult cow liver produces the same reaction. Pieces of liver tissue were examined in four aborted calves whose dams were under dry-range conditions in widely separated herds, where heavy losses had been reported. Solutions representing as much as 10 grams of liver tissue from these fetuses failed to give any color reaction, and this quantity justifies the assumption that no vitamin A was present. In a band of ewes on dry range feed and bean straw about 40 head of lambs were born dead. The liver of one brought to the laboratory was negative for vitamin A. When the ewes were changed to alfalfa hay, the loss of lambs ceased. Negative results were also obtained with liver samples from swine and poultry on vitamin-A-deficient rations. In chickens and turkeys definite vitamin-A deficiency and high mortality have existed while the color reaction could still be demonstrated in the

livers of the dead fowls. We recognize the nonspecific nature of the color reaction with antimony trichloride. Negative reactions are valuable because they demonstrate that no vitamin A, carotenoids, or any other substances that may produce the reaction are present. From the quantitative standpoint positive reactions must be interpreted with caution. In our experience this test has checked sufficiently well with clinical observations to be of value quantitatively even in the absence of spectrographic confirmation.

Despite the apparently large accumulation possible in the livers of adult cattle, if they subsequently go on rations deficient in vitamin A there will be a utilization of reserves and a reduction in the quantity secreted with the milk that can be definitely demonstrated after a period of a few weeks on the deficient ration. Also Halverson and Sherwood[49] showed that cows on sufficiently low intake of the vitamin may manifest symptoms and be at the point of death in about 200 days. Steers fed heavily in dry lot on cottonseed cake and hulls will break with vitamin-A deficiency as soon as 100 to 125 days. Evidently, then, under range conditions, reserves could be depleted to the point where reproductive failure would occur without other definite clinical symptoms in the dam. This condition probably results because the rapidly growing fetus in utero is utilizing relatively large amounts of the vitamin. Its greater susceptibility to eye lesions as compared with adult animals is further substantiation.

NATURALLY OCCURRING VITAMIN-A DEFICIENCY IN EXTREME FORM

During the past winter we have been able definitely to diagnose vitamin-A deficiency in cattle under natural conditions and to study its various manifestations in animals of different ages. This was possible because weather conditions and management on one ranch in southern Tulare County caused its development in such a severe form that 100 animals died and many more showed symptoms in a herd of about 250 head. The ranch comprises 3,000 acres, practically all farmed to wheat and barley. The owner maintains 75 head of dairy cows. Calves from these cows, together with others purchased when a few days old from dairy farmers, nurse these animals. The calves are finally sold for veal.

In the spring of 1932 rains ceased early, so that the feed was dry and the grain fields were yellow by May 15. In the fall the first rains came about the middle of December, followed by such cold weather that green feed did not become available until the middle of February, 1933.

During this unusually long dry-feed period, covering nine months, part of the cattle grazed the unharvested grain fields through the sum-

mer. Thirty-five of the cows were left on a leased adjoining natural range until August 10, when they were placed on stubble from which wheat and barley had been harvested. Early in the fall and through the winter they were fed roughage from a stack adjoining the corral. This consisted of straw mixed with wheat and barley hay showing some traces of green color. The hay came from the borders of the fields that were harvested for grain. Barley was the main concentrate feed. This was later supplemented with whole cottonseed. A group of about fifty steers purchased in October was brought in from native grass range and fed in dry lot. These steers, in addition to the straw, grain hay, and barley, received 2 pounds per head daily of a proprietary concentrate feed containing 12 per cent protein.

Symptoms.—The adult cows manifested less marked deficiency than the young stock. Their general condition was poor, although 6 to 7 pounds of barley was fed per head daily in addition to the straw and grain hay. When two parts of cottonseed and one part of barley were substituted for the barley alone there was evidence of improvement, but deficiency symptoms were not relieved. The most interesting symptom was night blindness noticed in the twilight and after darkness when an electric light was turned on in the corral. If attempts were made to handle the cattle at this time they ran into each other or into objects, becoming nervous and excitable. A few showed ophthalmia with ulceration of the cornea. Their milk and cream entirely lacked color, as did butter made from the latter.

These cows started to calve the latter part of August. The early calves were normal and although they became unthrifty, showed no definite symptoms until about 12 weeks of age. Birth of normal calves continued till late in the fall. During December and January, however, when 25 to 30 cows calved, all the calves were born weak, developed severe diarrhea, and died when one to five days old. At this same time, calves purchased from dairymen on alfalfa ranches remained normal for six to eight weeks after being brought on the ranch and nursing the same cows. This fact indicated the noninfectious nature of the diarrhea.

One bull was kept on the ranch. He was in poor condition during the winter, but no data regarding his fertility are available. Ability to serve cows was not completely lost. Many of the cows did not come in estrum for five or six months after calving, when green feed became available.

The feed-lot steers varied in age from yearlings to two-year-olds. The younger cattle were most affected. Eye lesions were the principal symptom, beginning with profuse lacrymation followed by clouding and ulceration of the cornea.

The young animals, varying in age from a few weeks to one year, showed the most marked and varied symptoms and the mortality was high. The eye lesions varied from profuse lacrymation and slight clouding of the cornea to extensive ulceration, loss of aqueous humor, lense opacity, and shrinking of the eyeball, with complete and permanent blindness.

The animals exhibited capricious appetites, intermittent diarrhea, general unthrifty appearance and pulmonary complications. With periods of inclement weather and exposure, pneumonia was the terminal condition causing death in nearly all cases.

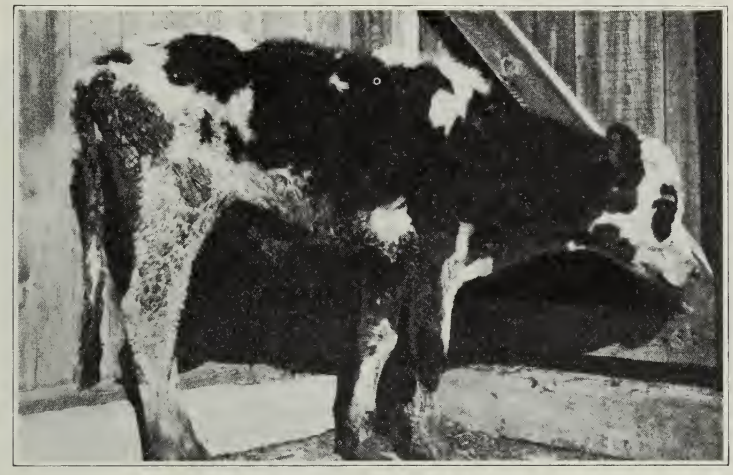

Fig. 1.—One-year-old steer in advanced stage of vitamin-A deficiency. Feeding of cod-liver oil in addition to the feeds it had been receiving resulted in a gradual improvement and a gain in weight of 125 pounds in 2 months.

Liver tissue from four of the dead animals was brought to the laboratory and was tested colorimetrically for vitamin A. In all cases solutions representing as much as 10-gram samples were negative.

On February 28, 1933, three animals were taken to the University Farm. No. 1 was a steer born on the ranch in March, 1932, and weaned September 1. After weaning it was fed straw, grain hay, barley, and salt. It first showed symptoms early in January. On February 28 it was extremely gaunt, totally blind, discharging profusely from the nostrils, very unsteady on its legs, and without appetite (fig. 1). Drenching with reconstituted dried skim milk was resorted to, and in addition 30 mils of good-quality cod-liver oil was given daily. After four days the appetite began to improve, the discharge from the nostrils ceased, and the muzzle became moist. The steer was continued on straw, barley, and cotton-

seed meal, plus the cod-liver oil, and made a complete recovery except for the loss of sight.

No. 2, a steer born December 1, 1932, came from an alfalfa ranch and grew normally during the first six weeks. While still nursing it developed severe diarrhea and eye lesions and showed evidence of lung involvement on January 19. On February 28, it was extremely emaciated; the left cornea was badly ulcerated, the right cornea clouded, and respiration rapid (figs. 2, 3, and 4.) It was selected for pathological study without any thought of its surviving. It died on March 3, and showed extreme lung involvement which had gradually progressed over a period of six or seven weeks to be the final cause of death.

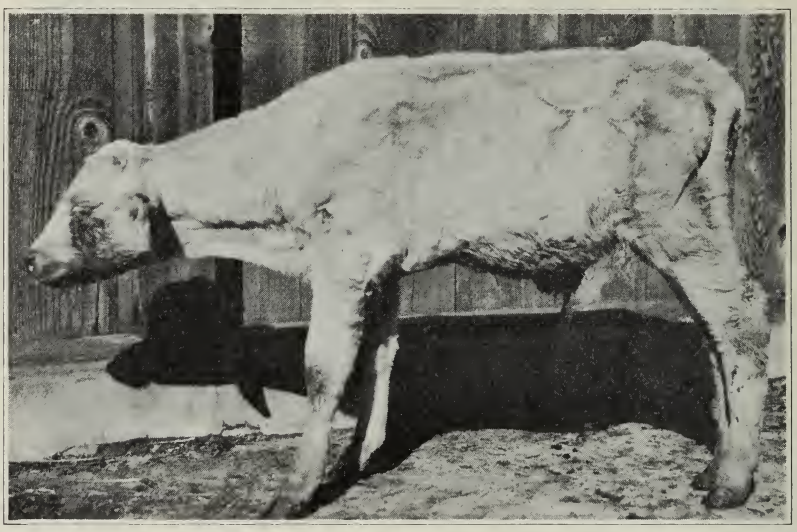

Fig. 2.—Three-month-old steer in moribund condition from vitamin-A deficiency. It died of pneumonia 2 days after this picture was taken.

No. 3 was a heifer born on the ranch November 15, 1932, and still nursing its dam. Although it was in nearly normal condition of flesh, the right eye was badly affected with severe ulceration of the cornea (fig. 5). On February 28, its appetite was good, and it was kept on the same diet as at the ranch except that reconstituted dried skim milk was substituted for the milk of its dam. No cod-liver oil was given. It gradually became worse, showing emaciation, intermittent diarrhea, irregular appetite, and some evidence of lung involvement by rapid breathing under slight exertion. Its regression was very gradual, considering the low vitamin-A intake and the condition on arrival. It was killed March 10 in order to have fresh tissues for pathological study.

Fig. 3.—Lung of steer shown in figure 2. Notice the nodules in the pneumonic area. These contained air and pus and are evidence of the subacute nature of the lung involvement.

Fig. 4.—Left eye of steer No. 2 removed after death for photographing. It shows the severe ulceration of the cornea.

All the animals on the ranch have recovered since the advent of green feed except for blindness in two cases, which will be permanent. This heavy loss could have been obviated entirely had the owner purchased alfalfa hay instead of cottonseed in the fall.

The feeds on this ranch were inadequate in vitamin A, rather than completely devoid of it. The diet was also relatively low in calcium. The liberal feeding of grain, coupled with adequate protein when cotton-

Fig. 5.—Animal No. 3, a heifer, three and one-half months old, showing the typical appearance of the advanced eye lesions in cases of vitamin-A deficiency.

seed was added, stimulated growth and milk production and probably explains the severe incidence of deficiency manifestations. Under natural range conditions with deficient protein and phosphorus and with low energy intake, the manifestations have been limited largely to reproductive difficulties, although the dry range forage is probably equally low in vitamin A.

It is evident that vitamin-A deficiency in cattle is manifested in a variety of ways, and some of its most outstanding symptoms are such that one must carefully differentiate it from other well-known and widely prevalent diseases.

DIFFERENTIAL DIAGNOSIS

Vitamin-A deficiency in some of its manifestations, may be symptomatically similar to one of two and possibly three other troubles. Thus the ophthalmia is readily confused with infectious keratitis (pink eye), the abnormalities in reproduction with bovine infectious abortion, and possibly the diarrhea with white scours in calves. Careful observations and, in some cases, laboratory tests, coupled with history and knowledge of conditions under which the animals are being kept will serve to distinguish which trouble is present in most cases.

Infectious keratitis is a rapidly spreading condition that affects all ages of animals irrespective of condition and feed supply. The infection is commonly transmitted by flies, the conjunctival sac is first involved, and clouding of the surface of the cornea quickly ensues. Both eyes are usually affected, and there is general systemic disturbance with fever followed by recovery. Ulcerations of the cornea occur in some cases.

In ophthalmia from lack of vitamin A, young animals will be affected more than mature cows. One eye only is more frequently affected; the animals are usually emaciated and are on feed lacking the vitamin. Clouding of the cornea may start as small opaque spots in the deeper layers and not cover the entire surface so quickly as in infectious keratitis. It progresses much more slowly, and ulceration of the cornea is common. There may be complete blindness with no abnormalities of the visible structures of the eye (amarosis) because of involvement of the optic nerve, and night blindness is common. After ulceration has occurred it is impossible to differentiate the eye lesion alone from the ulceration that sometimes follows infectious keratitis, but the general condition of the animal and knowledge of its surroundings will greatly assist in diagnosis.

The abnormalities of reproduction resemble those of infectious abortion, and from field observations alone, when the fetus is aborted, are quite indistinguishable. In range cattle terminating their gestation periods before green feed becomes available, the manifestations are similar to those obtained in the controlled experiments of other investigators as outlined at the beginning of this article. Under these range conditions, however, the cows are subjected to other deficiencies besides vitamin A, of which low phosphorus and low protein are the most outstanding in addition to possible low total energy intake. The actual expulsion of the fetus before it is viable or while it is too weak to live, long attributed to infectious abortion or to some other local cause such as eating acorns or mistletoe, we now believe to result from vitamin-A deficiency. The dif-

ferential diagnosis from infectious abortion requires laboratory examination. Blood samples must be taken from the aborting cows and the serum tested in the bacteriological laboratory for the presence of agglutinins with *Brucella abortus* antigen. If any positive reactors are found, infectious abortion is present and must be eliminated before the identity of the vitamin-A deficiency can be established. In testing for vitamin-A deficiency, liver samples from the aborted fetuses or from dead calves are forwarded to the nutrition laboratory to be examined chemically by the colorimetric test. For this purpose samples consisting of about ½ pound of tissue placed in pint fruit jars must reach the laboratory before decomposition has occurred. Decomposition can best be prevented by chilling or freezing before shipment. A negative chemical test, if the agglutination tests of the cows are negative, indicates that vitamin-A deficiency is causing the trouble; if the agglutination tests are positive, vitamin-A deficiency may be present along with abortion infection. The diagnosis of vitamin-A deficiency is further strengthened by the finding of ophthalmia in the new-born calves. Though the presence of this condition has been definitely reported on the ranges, we have so far not found it in any of the limited number of cases we have examined.

Animals in advanced stages of vitamin-A deficiency develop a severe diarrhea. If these are young calves born deficient and getting practically no vitamin A in their mothers' milk, the diarrhea may develop early enough to be confused with white scours. History of the cases and analysis of liver tissues may be necessary for positive diagnosis.

DISCUSSION

The progress of the authors' studies on range forage as related to nutrition in animals shows more and more clearly that single deficiencies, uncomplicated by other factors, do not usually occur under natural conditions. We have demonstrated that range forage becomes deficient in phosphorus, protein, and vitamin A. The energy intake may be a limiting factor when feed is scarce or when the appetites are diminished by specific deficiencies in the forage.

Since the rigorous conditions to which animals on unsupplemented range forage may be subjected vary from mild deficiencies to actual starvation, the symptoms vary widely. Some knowledge of these symptoms, together with information on the feed essentials in supplements, is necessary in order to meet most efficiently and economically the nutritive requirements of the animals.

The ability of cattle to store vitamin A in times of abundance against periods of privation is extremely important under California range con-

ditions. Thus cottonseed cake, rich in protein and phosphorus, will maintain cattle on dry forage in thrifty condition as long as sufficient reserves of vitamin A remain. If, however, the period on forage deficient in vitamin A is extended until the reserves are depleted, this essential then becomes the limiting factor; and alfalfa hay, green forage, carrots, or other substances containing it must be fed if failure in reproduction or other manifestations of its deficiency are to be avoided.

According to evidence from varied sources, manifestations of single deficiencies are produced more rapidly by the stimulating effects of an otherwise complete ration. The feeding of cottonseed cake, for example, will result in vitamin-A deficiency after a shorter period than when growth and production are more severely limited by the multiple deficiencies of range forage alone.

Experiments are in progress to determine the period required to deplete animals of vitamin A after the most ideal conditions for storage and to ascertain the minimum amount of good alfalfa hay necessary to prevent the abnormal termination of gestation. Until more information is available, the range livestock man can do much to prevent losses by observing his animals closely and by so supplementing the range as to keep them in thrifty condition.

Experiments have clearly shown that when cod-liver oil is incorporated into a finely ground feed, vitamin A is rapidly oxidized. This occurs because the thin oil film surrounding the particles presents a relatively enormous surface exposed to the air. Obviously, therefore, cod-liver oil or a similar source of vitamin A, mixed in so-called vitamized minerals for animal feeding, would not long retain its potency. Even though it did, the amount ingested with the small amount of mineral consumed would be insignificant in relation to the requirements of the animals.

When animals are not doing well or when definite losses occur, some reason exists. Bacterial diseases, parasitism, nutrition, and hereditary conditions are all to be considered. Stockmen confronted with these conditions can do their part in adding to the knowledge of the range by reporting their losses to those who are investigating the subject. Failure to do so is often prompted by a feeling that such losses are a personal reflection on the owner, and thus the industry is deprived of the increased knowledge that might be obtained through investigation of such reports.

CONCLUSIONS

The existence of vitamin-A deficiency in cattle under natural conditions has been demonstrated. Conditions are present during the dry-feed season which if unusually prolonged will cause the manifestation of various degrees of this deficiency. The symptoms most commonly seen are as follows:

1. Birth of dead or weak calves with or without eye lesions and associated with retention of the placenta. This condition simulates infectious abortion.

2. Severe diarrhea in weak newborn calves which simulates white scours.

3. Eye lesions, particularly in immature animals, which simulate infectious keratitis.

The manifestation in animals is more severe when the diet is otherwise complete and supplied in amounts above maintenance. Under the multiple deficiencies of the range the manifestations are commonly limited to reproductive failure toward the end of the dry-feed season.

Data on the nature and occurrence of vitamin A and the experimental evidence on the relation of vitamin A to reproduction have been reviewed.

The best practical source of vitamin A now available for livestock feeding is green feed or hay. The vitamin-A potency of hay is associated with the degree of green color.

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