Effect of Zinc Deficiency and Restricted Feeding on Wound Healing in the Bovine.* (29865)

W. J. Miller, J. D. Morton, W. J. Pitts and C. M. Clifton
(Introduced by William L. Williams)
Department of Dairy Science and School of Veterinary Medicine, University of Georgia, Athens

There has been considerable research related to the effects of protein or amino acids and vitamin C on wound healing in a few species of animals(1,2). The relationship of many other nutrients to healing is not clear. Nevertheless, it seemed reasonable to speculate that other deficiencies might have important influences. A relationship between wound healing and zinc deficiency is certainly suggested from the nature of this syndrome(3,4). In zinc-deficient animals, the skin on certain parts of the body develops parakeratosis which is characterized by thickened, dry, keratinized skin; loss of hair; as well as other changes(3,4,5). The portion of the skin affected has generally been the head, neck, legs, abdomen and scrotum(3,4,5). However, other workers have reported that the back region was most generally involved (6). Previously there has been no satisfactory explanation of why these differences should occur.

The purpose of these experiments was to evaluate the effects of a zinc deficiency on wound healing in the bovine. It has been shown that a zinc deficiency results in a lower feed consumption(3). Thus the studies were designed to separate the effects of zinc deficiency *per se* from the indirect effect on level of feed intake. Also, the research was conducted to determine whether the effects of the zinc deficiency were reversible.

Materials and methods. Uncastrated male Holstein calves were fed one of the following dietary regimens: a) zinc-deficient basal, fed *ad libitum*; b) control, fed *ad libitum*; and c) restricted control, allowed the same level of total feed intake as was consumed by the zinc-deficient group. The zinc-deficient diet was similar to the purified diets which have been used previously to produce experimentally a zinc deficiency in the bovine and the goat(3,7). It contained the following: dried egg albumin (autoclaved), 8 kg; glucose, 28.4 kg; stabilized lard, 3.0 kg; cellulose (Solka-Floc), 10 kg; $KHCO_3$, 1.5 kg; $NaHCO_3$, 3.0 kg; dried whole whey, 20 kg; dicalcium phosphate, 2.0 kg; $CaCO_3$, 1.0 kg; urea (feed grade), 1.0 kg; corn starch, 20.0 kg; Na_2SO_4, 200 g; KCl, 550 g; NaCl, 484 g; MgO (56% Mg), 165 g; $Fe_2(SO_4)_3 \cdot XH_2O$ (72% Fe by assay), 22 g; $MnSO_4 \cdot H_2O$, 4.4 g; $CoCO_3$ (45-50% Co by assay), 0.02 g; KI, 0.018 g; $CuSO_4$, 3.1 g; menadione sodium bisulfite (63%), 0.33 g; d-biotin, 0.026 g; dl-alpha tocopherol acetate, 1.8 g; vit D_3 (200,000 I.U./g), 2.2 g; vit A palmitate (250,000 I.U./g), 22 g; choline Cl (70%), 264 g; and oxytetracycline (25%), 88 g.

The control diet differed from the zinc deficient in that 40 ppm of zinc as ZnO was added. It was formulated to be nutritionally complete by generally accepted standards(8). However, the level of digestible protein did not materially exceed requirements. Calves fed the zinc deficient and restricted control diets only ate about 60% as much as the controls. Those fed the restricted control diet ate all their feed rather quickly after being fed.

Five calves were used in the zinc-deficient group and 4 for each of the other treatments. At the time the wounds were imposed they ranged in age from 11 to 17 weeks and had received their dietary treatment for several weeks. Analyses of the data indicated that age did not affect the results.

The wounds were surgically imposed on the gluteal (rump) and the para lumbar fossa (triangle in front of hips) regions on the right side of the calves (Fig. 1). These areas were chosen because parakeratosis is not normally exhibited here and they are well

* Journal Paper No. 380 College Exp. Station, Univ. of Georgia, Athens. Supported in part by PHS research grant AM 07367-01 NTN from Nat. Inst. of Arthritis and Metab. Dis., USPHS.

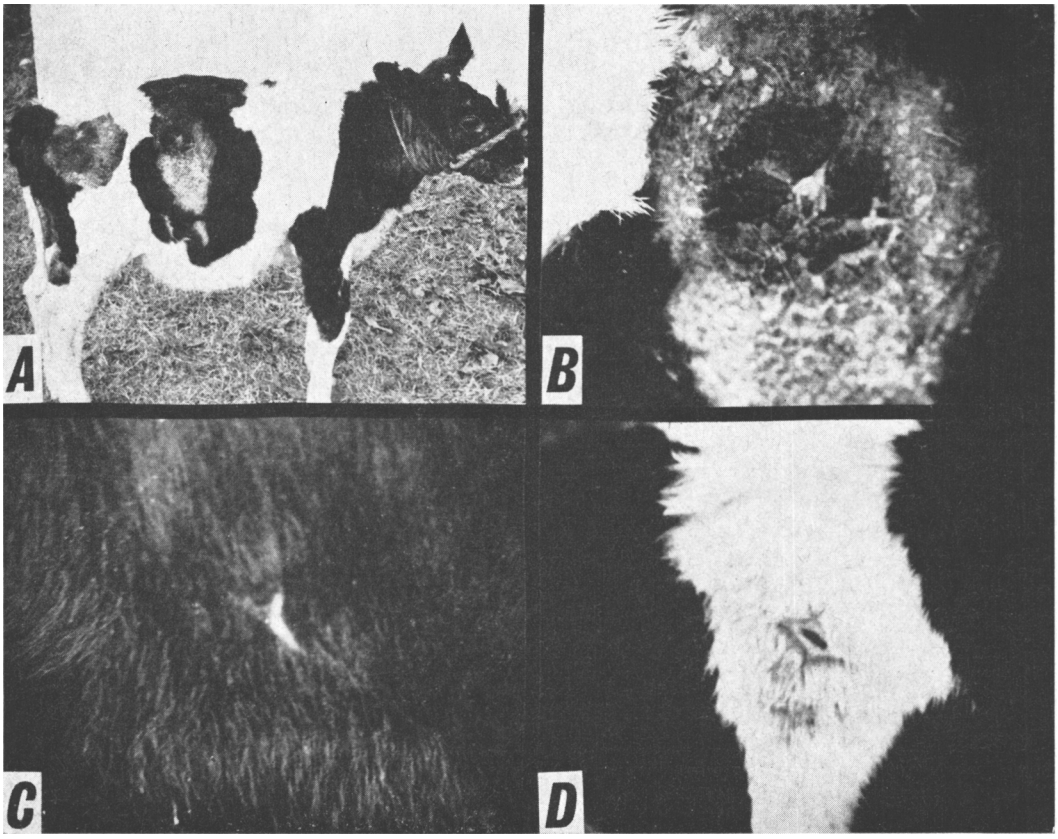


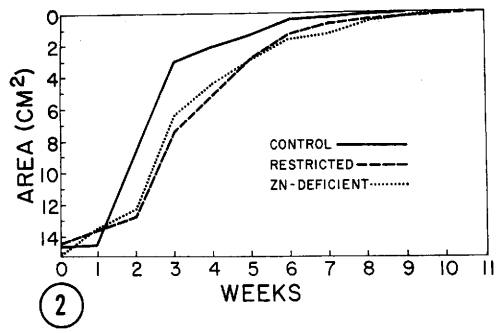
FIG. 1. Photographs of calves made 6 weeks after wounds were surgically imposed on gluteal and para lumbar fossa regions of the skin (A). Calves fed zinc-deficient diet (A, B) developed parakeratosis over large areas around wounds. Wounds on calves fed control diet *ad libitum* (C) healed normally and at a rapid rate. When control diet was fed in restricted amounts (D) the type of healing was normal but rate of new skin growth was retarded.

protected against accidental injury. The wounds were made by surgically removing diamond shaped areas of skin which had diagonals of 6.0 cm and 4.5 cm in length. These were permitted to heal without sutures or medication, except for scarlet oil which was applied daily. The scarlet oil contains: menthol, carbolic acid, oil camphor, eucalyptus oil, tar oil, thyme oil, pera balsom, biebrick scarlet red and a vegetable oil base.

Following surgery, the lengths of the diagonals across the area over which skin was absent were measured immediately and at weekly intervals until new skin had completely covered the areas.

After the first experiment was completed all the calves were fed a nutritionally complete, practical-type diet, *ad libitum* for several weeks. This diet was composed of corn; barley; soybean meal; ground coastal bermudagrass hay; and mineral, vitamin, and antibiotic supplements. While they were continued on this diet the wound experiment was repeated using areas near those used initially.

Results and discussion. It was quite apparent that each of the 3 treatment groups of calves responded in a characteristic manner (Fig. 1 A, B, C, D). The control individuals all healed normally and at a rapid rate. Healing in those fed the control diet in restricted amounts was normal in appearance but the generation of new skin was at a much slower rate (Fig. 2). The growth rate of new skin was also retarded in those fed the zinc-deficient diet. In addition, large areas around the wounds became parakeratotic (Fig 1 A, B). The calves were confined to stanchions, thus having a minimum of



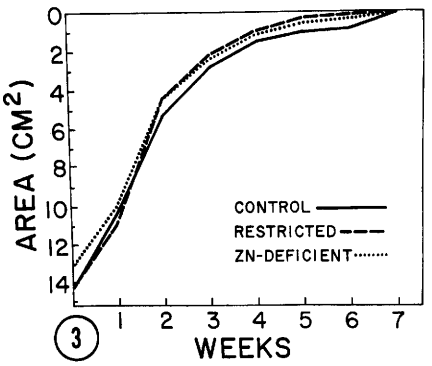


FIG. 2. Area over which skin was absent for calves fed the 3 dietary regimes. About 2½ weeks longer were required for skin to be generated in calves that were fed the zinc-deficient and the control diets in restricted amounts in relation to that of the controls.

FIG. 3. Experiment shown in Fig. 2 was repeated after the calves had been fed a common control diet for several weeks. Rate and type of healing were normal for all calves indicating that adverse effects of a zinc deficiency and limited dietary intake were completely reversible.

opportunity for licking or rubbing the affected areas. When the calves were removed from the stanchions for photographing and other purposes, the zinc-deficient animals expressed a strong tendency to bite or scratch the wound areas. This was not noted in calves fed the other rations.

New skin covered the entire wound area 2½ weeks sooner in the control than in the other calves (Fig. 2). The performance of the calves within each group was quite uniform.

Development of parakeratosis on the para lumbar fossa and the gluteal regions of zinc-deficient calves, following surgery, suggests that the basic physiological picture is not different in the skin on these areas than on those which normally develop parakeratosis. It appears, however, that external or secondary factors such as trauma influence the development of parakeratosis. Thus the differences observed by various workers in relation to the location of the parakeratosis(3,5, 6) may be due to the way the animals are managed, housed, etc.

The mechanism, whereby a zinc deficiency causes parakeratosis of the skin has not been determined. However, the knowledge that it does not appear to be due to any basic physiological or biochemical difference in skin from various sections of body should be quite useful in research attempting to determine the mechanism.

The results of these studies suggest that methods might be developed for evaluating zinc deficiency from the response to standardized mild degrees of trauma. This could conceivably reduce the variability in studies in which parakeratosis is one of the important criteria of zinc deficiency. It also might be a more sensitive measure of zinc deficiency than those currently available and it could also be very simple.

The pronounced effect of restricting the level of total feed intake on rate of regeneration of new skin is a very interesting phenomena. Whether the effect would apply to other species, to animals which are not in such a rapid portion of the growth cycle and to other types of tissue healing is not known. However, the implications are interesting.

Effects reversible. The results of the healing experiment conducted after the calves had all been fed a common practical-type control diet are presented in Fig. 3. All of the calves healed normally and at a rapid rate. The calves on the zinc deficient treatment had been very severely deficient for an average of 3 months. Thus, insofar as ability to heal and regenerate new skin was concerned, the calves had completely recovered from the effects of a zinc deficiency and the restricted dietary intake.

Summary and conclusions. Large areas around surgically imposed wounds developed parakeratosis in zinc-deficient calves. The regions involved normally do not develop parakeratosis. Thus secondary factors such as mild trauma appear to determine the loca-

tion of parakeratosis. This also indicates that the basic condition of the skin in a zinc deficiency is the same throughout the body. Reducing the total amount of feed consumed, either by zinc deficiency or by limited feeding, substantially retarded the rate of generation of new skin following surgical removal of the skin. The effects of zinc deficiency and dietary restriction were reversible.

The authors thank Dr. P. W. Warga and Dr. J. T. Steele for pathological evaluation of skin biopsies; Kraft Foods Co. for dried whey; Chas. Pfizer Co. for antibiotics and vitamins; American Cyanamid Co. for chlortetracycline; Hoffmann-LaRoche Co. for biotin; Commercial Solvents Co. for choline; Basic, Inc. for magnesium oxide; and Abbott Labs. for menadione.

1. Edward, L. C., Dunphy, J. E., New Engl. J. Med., 1958, v259, 275.
2. Sisson, R., Lang, S., Serkes, K., Pareira, M. D., Sicher, N., Surgery, 1958, v44, 613.
3. Miller, J. K., Miller, W. J., J. Nutr., 1962, v76, 467.
4. Miller, W. J., Miller, J. K., J. Dairy Sci., 1963, v46, 1285.
5. Legg, S. P., Sears, L., Nature, 1960, 186, 1061.
6. Haaranen, S., Nord. Vet. Med., 1962, v14, 265.
7. Miller, W. J., Pitts, W. J., Clifton, C. M., Schmittle, S. C., J. Dairy Sci., 1964, v47, 556.
8. Committee on Animal Nutrition, Nat. Acad. of Sci., Nat. Research Council, 1958, Publ. 464.

Received October 7, 1964. P.S.E.B.M., 1965, v118.

Effect of 3-Aminopropanol on Choline-Deficiency in Rats.* (29866)

JOSEPH W. HUME, DWIGHT J. MULFORD AND PERCY J. RUSSELL
Department of Biochemistry, University of Kansas Medical Center, Kansas City, Kan.

We reported(1) that addition of ethanolamine to low-choline diets containing either 18 or 42% casein increased the incidence of both hemorrhagic kidneys and hemorrhagic eyes in weanling male rats consuming these diets for 7 days. In the same animals the liver fat accumulation was reduced by addition of ethanolamine. Addition of choline to the diet containing added ethanolamine markedly decreased the incidence of both hemorrhagic kidneys and hemorrhagic eyes.

Recently we have studied the effect of 3-aminopropanol (3AP), the homologue of ethanolamine, in choline-deficient rats. The present communication shows that 3-aminopropanol increased the severity of kidney hemorrhagic degeneration, and unlike ethanolamine, markedly elevated the liver fat. The results reported here suggest that 3AP behaves very much like 2-amino-2-methylpropanol (2A2MP) which has been shown to increase not only the severity of kidney hemorrhagic degeneration(2,3) but also the liver fat(4) in rats consuming a choline-deficient diet.

Experimental and results. Weanling male rats (18 to 22 days of age; 35 to 55 g) derived from the Sprague-Dawley strain and born to mothers fed Laboratory Chow (Ralston-Purina Co., St. Louis, Mo.) were placed in raised cages and fed experimental diets *ad libitum* for 7 days. Water was available to the animals at all times. Food consumption and body weights were recorded each day. At the end of the 7-day period the rats were sacrificed by decapitation and the kidneys and eyes were examined for hemorrhage.

The composition of the diet (diet A) used in these experiments and the method for measuring the liver fat as shown in the following Tables have been described earlier (4).

Table I shows that when either 3AP or 2A2MP was incorporated into diets and fed to rats, the incidence of hemorrhagic kidneys

* The material presented in this paper was taken from the thesis of Joseph W. Hume, which was submitted in partial fulfillment of the requirements for the degree of Master of Arts, Univ. of Kansas, 1964. This investigation was supported by a research grant from Nat. Inst. of Arthritis & Metab. Dis., Nat. Inst. Health, USPHS (AM 07054-02).