molybdenum in the protein source was unavailable to the chick, the basal diet would contain only 0.0228 ppm of "available" molybdenum supplied by the mineral mixture. However, this remains to be proven. The turkey mineral mixture contained 0.3 ppm of molybdenum and supplied 0.0287 ppm to the basal ration. It would appear that both the chick and poult have a nutritional requirement for molybdenum under the conditions of these studies.

Summary. 1) The results of 2 studies with chicks and one study using Broad Breasted Bronze turkey poults have indicated that addition of molybdenum to purified basal diets resulted in a growth advantage of 13.5-19.1% over the unsupplemented group. 2) The feeding of a reconstituted ash mixture which simulated the ash of distillers dried solubles resulted in significant increases in growth rate of both chicks and poults. 3) From the data presented it appears that under conditions of these studies a nutritional requirement for molybdenum by chick and poult may be demonstrated.

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4,4'-Bisacetamidocarbanilide, a Metabolite of Nicarbazin. (22721)

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Nicarbazin, a chemical complex of 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4, 6-dimethyl pyrimidine (HDP), is an effective and widely used anticoccidial agent for chickens(1,2). After nicarbazin is absorbed by chickens, the complex separates into its components, which are rapidly eliminated from the tissues(3). Clark, *et al.*(4) used nicarbazin labelled with C^{14} in the benzene ring of the DNC to determine if metabolites, which were undetected by chemical assay, remained in the tissues. These studies showed that most radioactivity found in tissues of chickens fed or dosed with C^{14} labelled nicarbazin was accounted for as colorimetrically assayable DNC. Although traces of metabolites of DNC were present in tissues of chickens, they were rapidly destroyed or excreted.

During the performance of tolerance studies with large doses of nicarbazin(5),

crystalline deposits were noted in the renal tubules of calves. Therefore, studies were conducted to isolate this material, which was identified as 4,4′-bisacetamidocarbanilide, a metabolite of nicarbazin.

Methods. Nicarbazin was fed to 8-10-week-old calves for 4 to 6 weeks. The nicarbazin, in daily dosages of 0.04, 0.2 or 1.0 g/kg, was suspended in about one-half of the calves' morning milk ration. When this was consumed, the balance of the milk was fed. Water, hay and a commercial calf-starter were fed *ad lib.*

Results. The calf on the lowest dosage of nicarbazin (0.04 g/kg) consumed 93 g of the drug during 6 weeks of administration. This calf gained in weight from 108 to 167 lb, appeared normal and maintained a good appetite. When this calf was sacrificed at end of dosage period, all organs were grossly and histologically normal. Another calf was fed a total of 565 g of nicarbazin in a daily dosage of 0.2 g/kg. During the first week of administration, this calf lost 10 lb in weight but afterwards gained slowly, increasing in weight from 153 to 183 lb. Although this calf continued to have a good appetite and appeared normal clinically, at autopsy the kidneys were enlarged and showed evidence of damage. The calf fed nicarbazin at the rate of 1 g/kg received 1524 g of the compound. Failure to gain in weight was evident during the first week of administration. During the next 3 weeks this calf lost 34 lb in weight, refused to eat normally, weakened and died. Necropsy examination showed the kidneys were enlarged. The cortex was pale and firm. The medulla revealed gray streaks next to the cortex and in its inner portion there were groups of minute tan-colored foci suggestive of crystalline deposits.

Histopathological examinations were made on the myocardium, aorta, bone marrow, lymph nodes, thymus, thyroid, lungs, pancreas, stomach, intestines, urinary bladder, adrenals, striated muscle, liver and kidneys. The only significant pathologic alterations were in the liver and kidney. The liver lesion was found only in the calf fed the highest dosage of nicarbazin (1 g/kg). The lesion

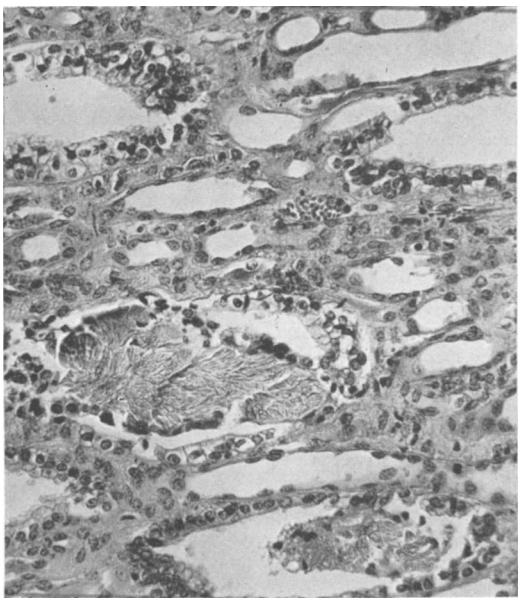

FIG. 1. Crystals of 4,4′-bisacetamidocarbanilide in collecting tubules of calf kidney.

was confined to the portal area and consisted of a slight to moderate bile duct proliferation and fibrosis. The kidney changes included precipitation of crystals (Fig. 1) mainly in the distal portions of the collecting tubules and a few in the convoluted tubules, probably the distal ones. The tubular epithelium directly adjacent to the crystals showed hyperplastic changes consistent with a foreign body reaction. The cortex was characterized by a focal, granulomatous, interstitial reaction. Within these areas of the cortex, hyperplastic changes were present in the tubules and small blood vessels, even in the apparent absence of crystals. Some tubules were filled with neutrophilic leucocytes and eosinophilic casts. Degenerative changes were noted in other convoluted tubules.

To isolate and identify the crystalline material, portions of the kidneys were ground with a meat chopper and digested overnight at 37°C in artificial gastric juice. After straining and sedimenting, the material was washed with distilled water and centrifuged several times until the supernatant was clear. Microscopic examination of the sediment showed that considerable fine tissue debris was present. Therefore, the crystalline ma-

terial was suspended briefly in normal NaOH solution, centrifuged and repeatedly washed with several changes of distilled water. The crystals were assayed for DNC but a negative test was obtained.

The crystalline material was gray in color, insoluble in water, dilute acid and the common organic solvents. Likewise in dilute alkali the solubility was essentially nil. It burned somewhat sluggishly and left a slight residue which exhibited a typical brick-red color characteristic of calcium salts. The crude crystals darkened at temperatures above 300°; a slight residue was left unmelted at 350°. The melting point and solubility data suggested that the dinitrocarbanilide rather than the hydroxy dimethylpyrimidine portion of nicarbazin was the precursor of the isolated material. To obtain purified crystals of the metabolite, a 20 mg sample was dissolved in 2 ml of hot dimethylformamide, centrifuged to separate from foreign material, and then carefully diluted with 1 ml of water. After chilling and collecting the solid, the recrystallization was repeated once more. A third crystallization was then made from about 1 ml of dimethylformamide (without subsequent dilution with water). At this point the material was colorless, darkened above 335°C, and melted with decomposition >345°C. After drying at 100°C for 1 min, the material was subjected to elemental analysis and infra-red study. Qualitative tests for S and halogen were negative while a positive test for N was obtained. Found: C, 62.51, H, 5.45; N, 17.20. The infra-red spectrum (Nujol) contained bands, at 3.0-3.03, 6.0, 6.05, 6.32, 6.48, and 6.57 u. These bands are consistent with NH, amide carbonyl and aromatic residues. A check of the literature indicated that the properties of the isolated compound were in agreement with the previously described 4, 4'-bisacetamidocarbanilide(6). Analysis Calc. $C_{17}H_{18}O_3N_4$ C_1 62.5; H_1 5.56, N_1 17.17.

An authentic sample of the acetamidocarbanilide was prepared by fusion of p-acetamidoaniline and urea(6). No depression of melting point was observed for the mixture of the authentic sample and the isolated metabolite. Also the infra-red spectra of the 2 compounds were identical.

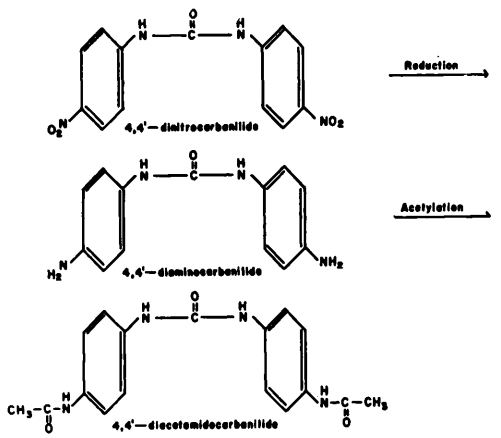

FIG. 2. Metabolic degradation of 4,4'-dinitrocarbanilide in the bovine.

To duplicate the proposed metabolic pathway (Fig. 2), a sample of 4,4'-dinitrocarbanilide was reduced catalytically to the diamine and subsequently acetylated via the Schotten-Baumann procedure. This preparation likewise yielded a compound identical with the metabolite from the kidneys of the calf.

Discussion. In calves, excessively large doses of nicarbazin produced kidney damage and crystalline deposits in the renal tubules. The material has been identified as 4,4'-bisacetamidocarbanilide and it is postulated that the compound was formed by reduction of the aromatic nitro groups of the dinitrocarbanilide component of nicarbazin. The newly formed amino groups were then acetylated into an insoluble metabolite. The pathological lesion and metabolic acetylation of this compound is similar to that resulting from administration of certain sulfonamides (7).

Other studies* have shown that large doses of nicarbazin produced kidney damage associated with crystal deposits in rats but not in dogs. It is well-known that rats possess a marked acetylating capacity whereas dogs are poor in this respect. Therefore, these *in vivo* observations support the suggestion that the metabolic pathway in the formation of 4, 4'-

* Unpublished data.

bisacetamidocarbanilide involves a reduction of DNC to the soluble diaminocarbanilide, which is readily excreted by dogs but is converted to an insoluble acetylated derivative in rats and calves. The *in vitro* demonstration of the reduction and acetylation of DNC to a compound identical with that found in the kidney tubules further supports the probability of this method for the metabolic degradation of nicarbazin in the bovine.

Summary. The feeding of large doses of nicarbazin to calves produced moderate nephrotoxicity associated with precipitation of crystalline deposits in the renal tubules. These crystals were identified as 4, 4′-bisacetamidocarbanilide. Additional evidence is presented that, after absorption, nicarbazin splits into its components, dinitrocarbanilide and hydroxydimethylpyrimidine, which are metabolized separately. The dinitrocarbanilide is first reduced to soluble diaminocarbanilide and then acetylated to insoluble 4,4′-bisacetamidocarbanilide. The renal tissue reaction and the metabolic degradation of 4,4′-dinitrocarbanilide resembles that known for the sulfonamides.

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Demonstration of Central Nervous Mediation of Acute Pulmonary Edema Produced by Intravenously Administered Epinephrine. (22722)

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Experiments have been previously reported (1) on the phenomena associated with production of acute pulmonary edema in mice and rats. The most straightforward interpretation of the observations was obtained on the assumption that the genesis of the acute pulmonary edema either produced by air blast or by massive doses of epinephrine was mediated via the central nervous system. As other interpretations were not entirely ruled out, it was considered important to obtain a more rigorous proof of the central nervous mediation, especially when produced by epinephrine. Usually the physiological effects of intravenously administered epinephrine are interpreted in terms of its well-known peripheral effects. However, it can be easily demonstrated that massive and even lethal doses of epinephrine can be administered intravenously to cats without any evidence of acute pulmonary edema production, while the typical acute edema can often be produced by injection of epinephrine into the hypothalamic regions of the brain of the cat. A natural interpretation of these observations is that the cat has a blood-brain barrier more impervious to epinephrine than that of rodents.

To prove conclusively the central nervous mediation of the acute edema in rats, the experiment was performed of severing the spinal cords of a group of rats at a position between the second and third vertebrae. The respiration was maintained with a mechanical respir-

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