

tion and vasodilatation. The larger doses also caused bronchomotor activity. However, the lack of intratracheal pressure changes with pulmonary vasodilating doses of acetylcholine or vasoconstricting doses of norepinephrine, and the absence of pulmonary hypertension with nebulization of acetylcholine indicated that pulmonary vascular resistance alterations were not dependent upon changes in bronchomotor tone.

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1. Hamilton, W. F., Woodbury, R. A., and Vogt, E., *Am. J. Physiol.*, 1939, v125, 130.
2. Friedberg, L., Katz, L. N., and Steinitz, F., *J. Pharmacol. and Exp. Therap.*, 1943, v77, 80.
3. Rodbard, S., *Am. J. Med.*, 1953, v15, 356.
4. Daly, I. de Burgh, Elsdon, S. R., Hebb, C. O., von Ludany, G., and Petrovskaja, B., *Quart. J. Exp. Physiol.*, 1942, v31, 227.
5. Borst, H. G., McGregor, M., Whittenberger,

J. L., and Berglund, E., *Circulation Research*, 1956, v4, 393.

6. Rose, J. C., Freis, E. D., Hufnagel, C. A., and Massullo, E. A., *Am. J. Physiol.*, 1955, v182, 197.

7. Franklin, K. J., *J. Physiol.*, 1932, v75, 471.

8. Gaddum, J. H., and Holtz, P., *ibid.*, 1933, v77, 139.

9. Rose, J. C., Broida, H. P., Hufnagel, C. A., Rabil, P. J., Gillespie, J. F., and Freis, E. D., *J. Applied Physiol.*, 1955, v7, 580.

10. Dawes, G. C., and Comroe, J. H., Jr., *Physiol. Rev.*, 1954, v34, 167.

11. Aviado, D. M., Jr., and Schmidt, C. F., *ibid.*, 1955, v35, 247.

12. Rose, J. C., and Lazaro, E. J., *J. Pharmacol. and Exp. Therap.*, in press.

13. Marchand, P., Gilroy, J. C., and Wilson, V. H., *Thorax*, 1950, v5, 207.

14. Daly, I. de Burgh, Duke, H. N., Unzell, J. L., and Weatherall, J., *Quart. J. Exp. Physiol.*, 1952, v37, 149.

15. Harris, P., Fritts, H. W., Jr., Clauss, R. H., Odell, J. E., *Proc. Soc. Exp. Biol. and Med.*, 1956, v93, 77.

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### Molybdenum in Poults Nutrition.\* (23070)

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Molybdenum has been reported to be associated with xanthine oxidase in the rat(1,

2). The maximum level of molybdenum required for optimum xanthine oxidase activity was estimated by De Renzo(3) to be 0.2-0.3  $\mu\text{g}/\text{day}$ ; however, rate of growth of weanling rats was unaffected by molybdenum supplementation. It was reported by Reid *et al.* (4) that addition of molybdenum to purified diets for chicks or poults resulted in stimulation of growth rate similar to that obtained with distillers dried solubles ash. Remy and Westerfeld(5) were unable to demonstrate an effect of liver residue on xanthine dehydrogenase in the chick as had been obtained in the rat. Higgins *et al.* obtained molybdenum deficiency through addition of 4.5-9.4 mg% tungsten in the ration of chicks. Feeding of molybdenum restored xanthine dehydro-

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genase to normal and corrected other deficiency symptoms. The ash of distillers dried solubles, dried whey and fish solubles has been shown to stimulate growth of both chicks and poults fed purified type diets(6,7) and chicks under practical conditions(8). The present study was initiated to determine effect of feeding distillers dried solubles ash and molybdenum on xanthine dehydrogenase activity in the turkey poult.

**Methods.** Broad Breasted Bronze turkey poults obtained from dams fed a breeder ration containing alfalfa meal, fish solubles and dried whey were used. The poults were divided into groups of 10 birds at 1 day of age and 2 replicates were fed on each dietary treatment. The basal diet was the same as reported by Reid *et al.*(4) and consisted of soybean protein (Drackett C-1) and starch supplemented to meet requirements of the turkey poult. Poults were maintained in batteries throughout the 4 week experimental period. Feed and water were supplied *ad libitum*. Ten birds from each treatment (5 birds replicate) were sacrificed for xanthine dehydrogenase determinations on liver and intestine according to the procedure of Remy *et al.*(9). In addition, the left tibia was removed for bone ash determinations. Molybdenum determinations on livers, bones and diets were carried out according to the procedure of Bertrand(10) and Marmoy(11).

**Results.** Addition of 6% distillers dried solubles to the basal diet produced a 14.9% ( $P < 0.05$ ) increase in growth rate of turkeys at 4 weeks of age (Table I). A similar response (14.4%) was obtained with 0.0254 ppm added molybdenum. This level of molybdenum was found to correspond to one-fourth of that supplied by distillers dried solubles. Distillers dried solubles used in this study was assayed and contained 1.8 ppm molybdenum. The ash of distillers dried solubles was approximately twice as active as the untreated product in stimulating growth of poults (Table I). These data indicate that a portion of molybdenum or other minerals present in distillers dried solubles was unavailable to poults for growth. A mixture of distillers dried solubles, dried whey, fish solubles, an antibiotic fermenta-

TABLE I. Effect of Molybdenum, Distillers Dried Solubles and Ash of Distillers Dried Solubles on Growth, Bone Ash and Xanthine Dehydrogenase in Turkey Poults at 4 Weeks of Age.

| Supplements to basal diet               | Molybdenum content of diets, ppm | Avg wt at 4 wk, g | Response over basal, % | Bone ash, % | Molybdenum content of bone ash, ppm | Xanthine dehydrogenase activity, $\text{mm}^3 \text{O}_2/20 \text{ min.}/283 \text{ mg tissue (wet wt)}$ | Liver molybdenum content (dry wt basis), ppm | Ratio of liver enzyme activity to molybdenum content |
|-----------------------------------------|----------------------------------|-------------------|------------------------|-------------|-------------------------------------|----------------------------------------------------------------------------------------------------------|----------------------------------------------|------------------------------------------------------|
| None                                    | 1.58                             | 459.7             |                        | 54.7        | .240                                | 40.4                                                                                                     | 2.54                                         | 27.9                                                 |
| 6% distillers dried solubles            | 1.68                             | 528.0             | 14.9†                  | 56.4        | .216                                | 47.3†                                                                                                    | 2.96                                         | 25.4                                                 |
| .0254 ppm molybdenum (as molybdic acid) | 1.61                             | 526.0             | 14.4†                  | 56.6        | .444                                | 56.2†                                                                                                    | 3.15                                         | 26.7                                                 |
| Distillers dried solubles ash           | 1.68                             | 588.5             | 28.0†                  | 57.0        | .312                                | 52.3†                                                                                                    | 3.20                                         | 25.4                                                 |
| U. G. F. mixture*                       | 1.98                             | 666.0             | 44.9†                  | 56.7        | .374                                | 64.5†                                                                                                    | 5.95                                         | 23.6                                                 |
| L. S. D. .05                            | —                                | 65.5              | 14.2                   |             |                                     | 4.7                                                                                                      |                                              |                                                      |
| .01                                     | —                                | 86.7              | 18.9                   |             |                                     | 6.4                                                                                                      |                                              |                                                      |

\* 3% distillers dried solubles, 6% fish solubles, 6% dried whey, 3% antibiotic fermentation residue, and 3% forage juice.

† Significant at .05 level of probability over unsupplemented group.

‡ " " .01

tation residue, and forage juice produced a 44.9% increase in growth ( $P < 0.01$ ). It is suggested from these data that a portion of the unidentified factor response may be explained on the basis of molybdenum in the factor sources.

Slight increases in bone ash values were obtained with supplemented diets. A 1.7% increase in bone ash was obtained with 6% distillers dried solubles, 1.9% increase with molybdenum, 2.3% with distillers dried solubles ash, and 2% with the unidentified factor mixture. Morrison *et al.* (12) have reported significantly higher ( $P < 0.02$ ) bone ash values in chicks upon feeding of ash of a mixture of distillers dried solubles, dried whey, fish solubles, forage juice and penicillin mycelia meal; bone ash was increased from 45.07% for the basal group to 47.30% for the supplemented group in this report.

Molybdenum determinations on bone ash of turkey poulters demonstrated slightly more of the element present in ash from birds fed molybdic acid, distillers dried solubles ash or the U.G.F. mixture as compared to the basal group. There was slightly less molybdenum present in the bone ash of 6% distillers dried solubles fed poulters (Table I) than in control birds.

Intestinal xanthine dehydrogenase activities, determined using hypoxanthine as substrate with methylene blue as electron acceptor, were increased significantly ( $P < 0.01$ ) over that of the control group by all supplements tested. Enzyme activity exhibited by the group fed 0.0254 ppm added molybdenum as molybdic acid was significantly greater ( $P < 0.01$ ) than that obtained with the distillers dried solubles group (Table I). The ash of distillers dried solubles was more active ( $P < 0.05$ ) in stimulating intestinal xanthine dehydrogenase activity than the untreated product, but was not significantly different from molybdenum in this regard (Table I).

The mixture of supplements was more effective than any of the supplements tested in eliciting a response in intestinal xanthine dehydrogenase. These data again suggest that a portion of molybdenum in distillers dried

solubles was unavailable to the poults, since one-fourth the level of molybdenum in distillers dried solubles added as molybdic acid was significantly more effective than was distillers dried solubles. Ash of distillers dried solubles, although more active in this respect than the unashed product, was no more active than was molybdenum fed at a level of one-fourth that supplied in the ash.

Liver xanthine dehydrogenase values showed significant increases ( $P < 0.05$ ) due to addition of either distillers dried solubles, molybdenum (0.0254 ppm) or distillers dried solubles ash to the basal diet (Table I). However, no significant differences in liver enzyme activities between these 2 supplements were obtained. A highly significant response in enzyme activity was obtained with the mixture. The liver molybdenum contents corresponded quite well with level of enzyme activity exhibited. A correlation coefficient of 0.955 was obtained which proved significant at the 0.05 level of probability. The ratio of enzyme activity to molybdenum content of liver was also fairly constant over levels of the element determined, with the average for all groups at 25.8.

*Summary.* (1) Weights of turkey poulters fed a purified type diet were increased significantly upon addition of distillers dried solubles, distillers dried solubles ash, molybdenum (0.0254 ppm) or a mixture of distillers dried solubles, dried whey, fish solubles, an antibiotic fermentation residue, and forage juice. Although differences of as much as 2% in bone ash were observed, statistical significance was not obtained. (2) Both liver and intestinal xanthine dehydrogenase activities were significantly higher in supplemented groups as compared to control birds. A significant ( $P < 0.05$ ) correlation was determined between liver molybdenum content and level of enzyme activity. (3) Under the conditions of this study a portion of growth promoting activity of distillers dried solubles ash would appear to be explained on the basis of the molybdenum content.

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1. De Renzo, E. C., Kaleita, E., Heytler, P. G., Oleson, J. J., Hutchings, B. L., and Williams, J. H., *J. Am. Chem. Soc.*, 1953, v75, 753.

2. Richert, D. A., and Westerfeld, W. W., *J. Biol. Chem.*, 1953, v203, 915.
3. De Renzo, E. C., Kaleita, E., Heytler, P. G., Oleson, J. J., Hutchings, B. L., and Williams, J. H., *Arch. Biochem. Biophysics*, 1953, v45, 247.
4. Reid, B. L., Kurnick, A. A., Svacha, R. L., and Couch, J. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1956, v93, 245.
5. Remy, C., and Westerfeld, W. W., *J. Biol. Chem.*, 1951, v193, 659.
6. Dannenburg, W. N., Reid, B. L., Rozacky, E. E., and Couch, J. R., *Poultry Sci.*, 1955, v34, 1023.
7. Morrison, A. B., Scott, M. L., and Norris, L. C., *ibid.*, 1955, v34, 738.
8. Camp, A. A., Reid, B. L., and Couch, J. R., *ibid.*, 1956, v35, 621.
9. Remy, C., Richert, D. A., and Westerfeld, W. W., *J. Biol. Chem.*, 1951, v193, 649.
10. Bertrand, D., *Compt. Rend.*, 1939, v208, 2024.
11. Marmoy, F. B., *J. Soc. Chem. Ind.*, 1939, v58, 275.
12. Morrison, A. B., Dam, R., Norris, L. C., and Scott, M. L., *J. Nutrition*, 1956, v60, 283.

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### Isolation and Identification of Infectious Bovine Rhinotracheitis Virus in Tissue Culture. (23071)

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A disease of cattle characterized by severe inflammation of upper respiratory passages and trachea, accompanied by excessive nasal discharge, salivation, dyspnea and fever has been reported since 1950 in about 15 western states(1,2,3). This condition, observed in both dairy and beef cattle areas, has been described under various names such as acute upper respiratory infection, infectious necrotic rhinotracheitis, or more commonly, red nose disease. At a meeting of the U. S. Livestock Sanitary Assn. in Nov. 1955, it was generally agreed to call this infectious bovine rhinotracheitis (IBR). The disease could be reproduced regularly by intranasal inoculations of cattle with nasal washings from naturally infected cases, and protection tests in cattle, using infectious material from different parts of the country, indicated that the illness was caused by the same etiological agent(4,5). However, attempts to isolate this agent in chick embryos, weanling and suckling mice, guinea pigs and rabbits were unsuccessful(5). In a preliminary report, it was shown that with the use of bovine embryonic tissues in tissue culture, a virus producing a cytopathogenic effect on cells was isolated from infectious material of cattle(6). This paper presents additional information concerning this

virus, and shows that it is the etiological agent of infectious bovine rhinotracheitis.

*Material and methods. Tissue culture procedures.* The cortex of bovine kidneys obtained from 8-9 months old feti was minced and trypsinized by a method similar to that described by Youngner(6). The prepared cells were suspended in nutrient medium consisting of 0.5% lactalbumin hydrolysate, and 5% to 10% horse serum made up in Earles basic salt solution. To this was added 200 units of penicillin and 200  $\mu$ g of streptomycin/ml. This mixture was then dispensed in 2 ml amounts in tubes and allowed to stand in a stationary position for 5 days, or until good cellular growth was observed. At this time, the medium was changed and tubes placed in roller drums for use. Similar preparations were made with bovine testicular tissue. Tubes made with lung tissue, however, were prepared by a plasma-clot technic employing small pieces of minced tissue(7). *Sources of infectious material.* Various tissues, as well as nasal washings, were obtained during the early acute phase of illness from cattle naturally infected with IBR disease in Colorado, California, and Ohio. These specimens were placed in screw-cap vials and frozen immediately in an alcohol dry-ice bath, and stored at