patterns seem to be related more to the state of the organism at the moment the after-discharge is produced than to the precise anatomic location of the electrodes or to the parameters of stimulation. Although the activity emanating from symmetrical electrode placements may show differences in pattern, there are systematic relationships between the right and the left which would indicate some bilaterally expressed control on the after-discharge. Extremely high voltage electrical after-discharge may be present in the hippocampal formation with only minimal disturbance in the dorsolateral cortex, at least by transverse recording, and without the precipitation of a major convulsion.

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## The Effect of Molybdenum on Chick and Poult Growth. (22720)

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The ash of unidentified factor sources has been reported to stimulate the growth of both chicks and poults when supplemented to a purified type diet(1,2). The ash of dried whey, fish solubles or distillers dried solubles has also been found to stimulate the growth of chicks fed a practical type diet under field conditions(3).

Single additions of 40 elements to a purified diet for chicks were ineffective in duplicating the growth response obtained with the ash of a mixture of 6 unidentified factor sources(8). A reconstituted ash mixture, which was made up of chemically pure, reagent grade chemicals and supplied the elements found in the ash of distillers dried solubles upon spectrographic analysis, stimulated the growth rate of both chicks and poults (4). A similar reconstituted ash mixture produced a growth response under practical conditions (3).

Molybdenum has been found to be a co-factor of xanthine oxidase in the rat(5,6). The maximum molybdenum required by the rat for xanthine oxidase activity has been estimated as 0.2-0.3 $\mu$g per day(7). However, the growth rate of rats receiving the diets low in molybdenum was not affected. Molybdenum deficiency in the chick was associated with the feeding of 4.5-9.4 mg % tungsten in the ration(9). Such a deficiency could be overcome through addition of molybdate to the diet. The deficiency was characterized by growth depression, mortality, lowered tissue molybdenum concentrations, reduced xanthine dehydrogenase activity in the liver, intestine, kidney and pancreas, and decreased uric acid excretion.

*Methods.* Chicks were obtained from dams which had been reared from one day of age on an all-vegetable protein diet deficient in unidentified growth factors. The maternal diet used was the same as previously reported by Dannenburg *et al.*(1). Two types of chicks were used, New Hampshire and a New Hampshire X Barred Plymouth Rock Cross.

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TABLE I. Basal Diets.

| Ingredients | Chicks | Poults |
|---|---|---|
| | g/kg | |
| Cerelose | 625.7 | 528.8 |
| Soybean protein (Drackett C-1) | 250 | 325 |
| Soybean oil | 30 | 35 |
| $CaCO_3$ | 14.5 | 16.03 |
| $CaHPO_4 \cdot 2H_2O$ | 42.1 | 60.10 |
| NaCl | 9.5 | 5 |
| $MnSO_4 \cdot 1H_2O$ | .78 | .81 |
| $FeC_6H_6O_7 \cdot 5H_2O$ | 1.6 | 1.60 |
| $CuSO_4 \cdot 5H_2O$ | .016 | .02 |
| $ZnCl_2$ | .014 | .014 |
| KI | .040 | .040 |
| $CoCl_2 \cdot 6H_2O$ | .0005 | .0005 |
| KCl | 5.72 | 6 |
| $MgSO_4 \cdot 7H_2O$ | 5.7 | 5.76 |
| | Amt/kg | |
| Thiamin HCl | 4 mg | 5 mg |
| Riboflavin | 6 | 8 |
| *D*-Calcium pantothenate | 15 | 16 |
| Pyridoxine HCl | 4 | 4 |
| Para-Aminobenzoic acid | 20 | 20 |
| Alpha-tocopheryl acetate | 24 | 24 |
| Inositol | 1,000 | 1,000 |
| Menadione | .5 | .5 |
| Biotin | .2 | .2 |
| Niacin | 100 | 120 |
| Choline chloride | 2,000 | 2,000 |
| Folic acid | 2 | 2 |
| Aureomycin HCl | 10 | 50 |
| Vitamin A | 10,000 I.U. | 10,000 I.U. |
| " $D_3$ | 2,000 I.C.U. | 2,000 I.C.U. |
| " $B_{12}$ | 30 μg | 50 μg |
| DPPD | 125 mg | 125 mg |
| *DL*-methionine | 7.5 g | 7.5 g |
| Glycine | 4 g | 4 g |

The Broadbreasted Bronze poults were obtained from dams which had not been depleted of unidentified factors. The birds in each experiment were distributed at random into groups of 10 to 16 birds each, and in the case of the chicks each treatment was replicated once. Four replicates were used on the basal ration in the chick studies and two in the poult studies conducted. The birds were maintained in batteries with raised wire floors throughout the four-week experimental periods. Feed and water were supplied *ad lib.*

The basal diets used are shown in Table I. The diets for both the chicks and poults have been checked for adequacy by feeding higher protein levels, a combination of the 11 essential amino acids at levels of 0.1 of the N.R.C. recommended levels, additional vitamins as well as double and four times the trace minerals without producing additional growth. The soybean protein (Drackett C-1) was extracted with either 70% isopropanol or hot water (pH 4.6) in chick diets in order to remove any unknown growth factors associated with this dietary component. The ash of distillers dried solubles was prepared by ignition over an open flame followed by ashing in a muffle furnace at 600°C until a constant weight was obtained (8 to 10 hours). The composition of the reconstituted ash mixture used which consisted of the elements present in the ash upon spectrographic analysis is shown in Table II.

*Results.* Feeding molybdenum (0.0126 ppm) in a purified diet for chicks resulted in an 18.7% increase in growth (Exp. 1, Table III). In the same experiment, distillers dried solubles produced an increase in growth of chicks to 4 weeks of 97 g over the unsupplemented group. Approximately one-half of the growth response obtained with distillers dried solubles was produced by feeding the ash of this product at a level equivalent to 3%. The reconstituted ash mixture was equivalent to distillers dried solubles ash in growth-promoting properties (Table III).

In the study using crossbred chicks, a 13.5% growth increase was obtained through addition of 0.0126 ppm of molybdenum to the basal diet. This response was found to be significant at the 0.05 level of probability. The reconstituted ash mixture in this experiment produced an 11.4% increase in growth while the higher level of molybdenum (3.3 ppm) produced a growth response of 9.7%. Both of these growth rates were significantly greater than that of the unsupplemented control group (Table III).

Using Broadbreasted Bronze turkey poults, a 29.1% growth response was obtained with reconstituted ash mixture fed at a level equivalent to 6% distillers dried solubles. Inclusion of 0.0126 ppm of molybdenum in the basal diet produced a 19.1% increase in growth rate.

It is interesting to note that growth responses obtained with molybdenum in these

TABLE II. Composition of Reconstituted Ash Mixture.

| Ingredient | Formula | Amt equivalent to 100 g ash* mg | g |
|---|---|---|---|
| Aluminum sulfate | $Al_2(SO_4)_3 \cdot 18\ H_2O$ | 370.8 | |
| Barium hydroxide | $Ba(OH)_2 \cdot 8H_2O$ | 13.7 | |
| Sodium borate | $Na_2B_4O_7 \cdot 10H_2O$ | 264.0 | |
| Chromic oxide | $Cr_2O_3$ | 1.46 | |
| Cobalt chloride | $CoCl_2 \cdot 6H_2O$ | 4.04 | |
| Copper sulfate | $CuSO_4$ (anhydrous) | | 3.93 |
| Ferric phosphate | $FePO_4 \cdot 4H_2O$ | | 12.0 |
| Magnesium sulfate | $MgSO_4$ (anhydrous) | | 3.04 |
| Manganese chloride | $MnCl_2 \cdot 4H_2O$ | | 1.08 |
| Molybdic acid | $H_2MoO_4 \cdot H_2O$ | 56.26 | |
| Nickelous carbonate | $NiCO_3 \cdot 2Ni(OH)_2 \cdot 4H_2O$ | 64.08 | |
| Ca. phosphate (dibasic) | $CaHPO_4 \cdot 2H_2O$ | | 7.33 |
| Potassium chloride | KCl | | 5.73 |
| Sodium meta-silicate | $Na_2SiO_3 \cdot 9H_2O$ | | 3.04 |
| Sodium chloride | NaCl | | 7.63 |
| Strontium chloride | $SrCl_2 \cdot 6H_2O$ | 304 | |
| Titanium oxide | $TiO_2$ | 5 | |
| Zinc chloride | $ZnCl_2$ | 62.53 | |
| Lead nitrate | $Pb(NO_3)_2$ | 159 | |
| Silver nitrate | $AgNO_3$ | 47.2 | |

* 451.3 mg of reconstituted ash mixture were equivalent to 1 g of distillers dried solubles ash.

studies were equivalent to those obtained with either distillers dried solubles ash or reconstituted ash mixture in the chick experiments, while the results of the poult study indicate an added growth advantage through the feeding of reconstituted ash at a higher level. These data would indicate a higher requirement for molybdenum in the turkey poult under the conditions of these studies. The molybdenum contents were determined to be 0.972 ppm and 1.58 ppm, respectively, for the chick and poult diets. It is difficult to understand how inclusion of only 0.0126 ppm of molybdenum in these diets would produce an increase in growth, unless a large portion of the molybdenum present in the basal rations was unavailable. Richert and Westerfeld(7) have indicated that the molybdenum in soybean oil meal was largely unavailable to the rat. The molybdenum content of soybean protein (Drackett C-1), before alcohol or water extraction, was found to be 4.77 ppm; while the extracted protein contained 3.84 ppm of molybdenum. If it were assumed that

TABLE III. Effect of Minerals on Chick Growth.

| Supplements to basal diet | Exp. 1 (New Hampshire chicks) 4-wk avg wt (g) | Response over basal (%) | Exp. 2 (New Hampshire × Barred Rock) 4-wk avg wt (g) | Response over basal (%) | Exp. 3* (Broadbreasted Bronze poults) 4-wk avg wt (g) | Response over basal (%) |
|---|---|---|---|---|---|---|
| None | 289.7 | — | 346.1 | — | 403 | — |
| 3% distillers dried solubles | 376.7‡ | 26.2 | — | — | — | — |
| Distillers dried solubles ash equivalent to 3% | 330.3† | 14.0 | — | — | — | — |
| Reconstituted ash equivalent to 3% distillers dried solubles | 335.2† | 15.7 | 385.6† | 11.4 | 520‡ | 29.1 |
| Molybdic acid, 0.0126 ppm Mo | 344.0† | 18.7 | 399.9† | 13.5 | 480† | 19.1 |
| " " 3.3 " " | — | — | 379.8† | 9.7 | — | — |

* The reconstituted ash was fed at level equivalent to 6% Distillers Dried Solubles.
† Significant at 0.05 level of probability.
‡ " " 0.01 " " " .

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),