gested that the rate of intestinal absorption, for any series of similar, water-soluble compounds, is inversely related to polarity.

1. Barrett, H. W., and Elliott, G. L., *Science,* 1955, v121, 62.

2. Barrett, H. W., and West, R. A., *J. Am. Chem. Soc.,* 1956, v78, 1612.

3. Best, C. H., and Taylor, N. B., *The Physiological Basis of Medical Practice,* 5th ed., p587, The Williams and Wilkins Co., Baltimore, Md.

4. Olson, J. K., Ely, R. E., and Reneke, E. P., *J. Biol. Chem.,* 1947, v168, 681.

5. Soodak, M., Pircio, A., and Cerecedo, L. R., *ibid.,* 1949, v181, 713.

6. Christensen, H. N., *ibid.,* 1945, v160, 425.

7. Astwood, E. B., *J. Pharmacol. Exp. Therap.,* 1943, v78, 79.

8. Barrett, H. W., Gassner, F. X., and Dittmer, K., *Endocrinology,* 1951, v48, 197.

9. Gibson, Q. H., and Wiseman, G., *Biochem. J.,* 1951, v48, 426.

10. Agar, W. T., Hird, F. J. R., and Sidhu, G. S., *Biochim. Biophys. Acta,* 1956, v22, 21.

11. Höber, R., *Physical Chemistry of Cells and Tissues,* The Blakiston Co., Philadelphia, Pa., 1945.

Received April 9, 1957. P.S.E.B.M., 1957, v95.

Effect of Distillers Dried Solubles and Molybdenum on the Growing Chick.* (23219)

A. A. KURNICK,† B. L. REID, R. N. BURROUGHS, H. D. STELZNER AND J. R. COUCH

Departments of Poultry Science and Biochemistry and Nutrition, Texas A. and M. College System, College Station.

The role of molybdenum in animal nutrition has been ably reviewed by Dick(1) and the association of this element with xanthine oxidase in the rat by Westerfeld and Richert (2). The first indication of a possible requirement for molybdenum by the chick and poult fed a purified type diet was reported by Reid *et al.*(3). The addition of 0.0254 ppm of molybdenum to a purified diet has been subsequently shown by the same group of workers to stimulate the growth of poults and the activity of both intestinal and liver xanthine dehydrogenase(4). Furthermore, a portion of the growth promoting activity of the ash of distillers dried solubles(5,6) was reported by Reid *et al.*(4) to be due to the molybdenum content of this unidentified nutrient source.

The present study was initiated to determine the effect of feeding distillers dried solubles ash, after the molybdenum has been removed from this substance, on the growth, bone ash, molybdenum distribution in tissues and on xanthine dehydrogenase activity of chicks.

Method. New Hampshire chicks, from dams fed an all vegetable protein diet containing no added unidentified growth factors (5), were distributed at random into groups of 12 birds each. Each treatment was replicated once. The basal diet (Extracted soybean protein and Cerelose supplemented to meet the requirements of the chick) and the care and management of the birds was the same as previously reported by Reid *et al.*(3). At the close of the experiment, the chicks were sacrificed; the liver and a portion of the

* This work was supported in part by Robert A. Welch Fn., Houston, Texas. The following products were supplied through the courtesies as indicated: Inositol and Cerelose (monohydrate), Corn Products Refining Co., Argo, Ill.,; folic acid and aureomycin, Lederle Laboratories Division, Am. Cyanamid Co., Pearl River, N. Y.; biotin, Hoffmann-LaRoche, Inc., Nutley, N. J.; soybean oil, the Buckeye Cellulose Corp., Cincinnati, O.; methionine and glycine, Dow Chemical Co., Freeport, Texas; stabilized vit. A, Stabilized Vitamins, Inc., New York; vit. D_3, Bowman Feed Products Co., Holland, Mich.; diphenylparaphenylenediamine, B. F. Goodrich Chemical Co., Cleveland, O.; alpha tocopheryl acetate, menadione, riboflavin, thiamin HCl, D-calcium pantothenate, para-aminobenzoic acid, pyridoxine HCl, niacin, choline chloride and vit. B_{12}, Merck & Co., Rahway, N. J.

† Predoctoral Research Fellow of National Cancer Institute, N.I.H., P.H.S., Bethesda, Md.

TABLE I. Effect of Ash of Distillers Dried Solubles, after Removal of Molybdenum, on Growth, Bone Ash and on Molybdenum Distribution in Tissues of Chicks Fed a Purified Diet.

Supplements to basal diet	Mo content of diets (ppm)	Avg wt at 3 wk (g)	Response over basal (%)	Bone ash (%)	Mo content of bone ash (ppm)	Mo content of whole blood (γ/ml)	Mo content of liver, dry wt (ppm)
None	1.16§	228.3 (24)¶		51.2 (15)¶	.756**	.032**	1.84
3% distillers dried solubles	1.21	271.1 (24)	18.8††	50.9 (15)	.665	.026	2.08
Distillers dried solubles ash*	1.21	249.0 (22)	9.1	52.3 (15)	.658	.031	2.81††
Idem,* (molybdenum removed)	1.16‖	225.9 (24)	−1.1	52.7 (15)	.775	.028	1.32
Sodium molybdate, .03 ppm Mo†	1.19	258.5 (24)	13.2††	51.8 (15)	.570	.038	2.17
" ,.3 ppm Mo†	1.46	258.6 (23)	17.8††	50.3 (15)	3.210	.062	6.24††
U. G. F. mixture‡	1.51	268.9 (22)	13.2††	51.1 (15)	.706	.042	3.11††
L. S. D., .05		27.1	11.9				.64
" .01		35.8	15.7				.87

* Supplemented in amt equivalent to 3% distillers dried solubles. † Supplied as $Na_2MoO_4 \cdot 2H_2O$. ‡ 6% distillers dried solubles, 2% antibiotic fermentation residue, 3% dried whey, 3% fish solubles and 3% forage juice. § Molybdenum content of basal diet based on 6 replicate analyses. ‖ Contained 0.004 ppm added molybdenum (not extracted). ¶ Figures in parentheses indicate No. of chicks or samples on which avg figures were based. ** Determination carried out on pooled samples within groups. †† Significant to the 0.05 level of probability over unsupplemented group. ‡‡ Significant to the 0.01 level of probability over unsupplemented group.

jejunum were removed for xanthine dehydrogenase determinations according to the procedure of Remy *et al.*(7). Homogenate nitrogen was determined by microKjeldahl according to the method recommended by A.O.A.C. (8). Blood was collected by heart puncture prior to sacrificing the birds. The left tibia from each sacrificed bird was severed and retained for bone ash and molybdenum determinations. All samples for molybdenum determination were digested with conc. HNO_3, H_2SO_4 and 70% $HClO_4$ according to the method described by Piper(9) and molybdenum content of the digest determined according to the method of Purvis and Peterson(10) using ethyl ether as extraction solvent. Molybdenum was removed from the ash of distillers dried solubles by digestion with HNO_3 and $HClO_4$, followed by ether extraction of the NH_4SCN-molybdenum complex produced in the digest with NH_4SCN and $SnCl_2$. Extraction of the complex was continued until after the concentration of molybdenum in the ether extract was determined colorimetrically and found to correspond to the molybdenum concentration previously found in 5 replicate samples (18.74 ppm). The residue was evaporated to dryness, taken up in redistilled water and neutralized with NH_4OH. All supplements were added at the expense of glucose.‡ The duration of the experiment was three weeks.

Results. Addition of either distillers dried solubles, sodium molybdate (0.03 or 0.3 ppm Mo) or a mixture of 5 unidentified nutrient sources to the basal diet resulted in a significant growth increase compared to that observed in the control group (Table I). The growth response due to the ash of distillers dried solubles (9.1%) was one-half that observed with distillers dried solubles (18.8%). These data are in agreement with previous reports(5,6). However, the response of the group fed this ash sample was not sufficiently great to be statistically significant. Removal of molybdenum from the ash resulted in a 1.1% growth depression as compared to the basal group.

With the exception of the ash of distillers

‡ Cerelose.®

TABLE II. Effect of Ash of Distillers Dried Solubles, after Removal of Molybdenum, on Intestine and Liver Xanthine Dehydrogenase Activity of Chicks as Compared to Other Sources of Dietary Molybdenum.

Supplement to basal diet	Mo content of diet (ppm)	Xanthine dehydrogenase activity,¶ $cmmo_2$/20 min. Intestine, per 283 mg (wet wt)	Liver, per 283 mg (wet wt)	Liver, per mg of N
None	1.16§	22.5	100.4	7.95
3% distillers dried solubles	1.21	34.4‡‡	120.2‡‡	8.92††
Distillers dried solubles ash*	1.21	30.7††	101.4	8.11
Idem,* (molybdenum removed)	1.16‖	25.0	96.9	7.53
Sodium molybdate, 0.03 ppm Mo†	1.19	31.8††	128.4‡‡	10.02‡‡
" " , 0.3 ppm Mo†	1.46	37.0‡‡	137.4‡‡	10.53‡‡
U. G. F. mixture‡	1.51	32.3††	137.5‡‡	10.01‡‡
L. S. D., 0.05		8.0	11.6	.89
" , 0.01		10.6	15.4	1.18

*–‖ See Table I.

¶ A 1:6 dilution of tissue homogenate was used in these studies.

dried solubles, all supplements which supplied at least 0.03 ppm of molybdenum above that found in the basal diet exerted a significant growth response. This was not unexpected in view of the previously reported growth increases when only 0.0126 ppm of molybdenum as molybdic acid was added to the basal diet(3). These data would indicate that the chick can utilize higher levels of molybdenum for optimum growth. However, addition of higher molybdenum levels (0.3 and 0.35 ppm) from either organic or inorganic sources, did not result in increased growth above that observed with the lower supplemented levels (0.03-0.05 ppm).

Slight differences in bone ash values found in the groups fed the supplements were not statistically significant (Table I). However, further investigations are warranted in view of the work of Morrison *et al.*(11) in which elevated bone ash values resulted in birds fed a purified diet supplemented with the ash of a mixture of unidentified factor sources.

Molybdenum contents of the tissues studied indicate that low levels of molybdenum supplied either by distillers dried solubles, the intact ash of the supplement or by sodium molybdate did not significantly affect the molybdenum concentration of bone ash or whole blood; but did affect the molybdenum concentration of liver (Table I). The addition of 0.3 ppm of molybdenum as sodium molybdate to the basal diet increased molybdenum deposition in the tissues studied. Since bone ash and blood samples were pooled for the molybdenum determinations, the differences found are not considered to be significant.

The livers of chicks fed the molybdenum free ash exhibited the lowest liver molybdenum concentration; whereas, the group fed the untreated ash showed a significant ($P<0.01$) increase in molybdenum deposition over the basal group (Table I). The ash of distillers dried solubles supplied 0.05 ppm of molybdenum to the diet which was equal to that supplied by the unashed material; however, untreated distillers dried solubles failed to affect the level of molybdenum in liver. Livers of chicks fed 0.3 ppm of molybdenum as sodium molybdate showed the highest concentration of the element ($P<0.01$). The liver molybdenum deposition in this group was significantly higher than that observed in the group fed the mixture of unidentified growth factors which supplied 0.35 ppm of molybdenum.

Liver and intestine xanthine dehydrogenase activity were significantly increased by the added molybdenum sources (Table II). The treated ash (molybdenum removed) failed to increase the enzyme activity either in intestine or liver above that observed in the basal group. The enzyme activity of the group fed the intact ash exhibited a significant response ($P<0.05$, Table II), in intestinal xanthine dehydrogenase, but failed to increase the liver enzyme activity. Rat intestine was

found by Westerfeld and Richert(2) to be a better test object than liver tissue for the unidentified liver residue factor. This may help to explain the difference in response observed with the 2 tissues on the same treatment.

No significant differences between intestinal enzyme activity of the dietary treatments other than the control group were noted. Since the supplements supplied varying levels of molybdenum (0.05-0.35 ppm), it was assumed that a saturation level has been reached similar to that observed in the rat(12).

The response of liver xanthine dehydrogenase activity to the added supplements was less consistent than that observed in intestinal homogenates. The liver enzyme activities of the groups fed the higher level of sodium molybdate (0.30 ppm) and the U.G.F. mixture (added Mo 0.35 ppm) were significantly higher ($P<0.01$) than the activity noted in the livers of birds fed distillers dried solubles supplying 0.05 ppm of molybdenum. When xanthine dehydrogenase activity was expressed on a nitrogen basis the differences observed above were quantitatively less, but still statistically significant ($P<0.05$).

The ratio of enzyme activity to liver molybdenum content was more erratic than that reported in turkey poults(4). However, a correlation of 0.617 ($P<0.05$) was found to exist between the results of the two determinations.

Summary. 1) Growth of chicks was significantly improved by the addition of distillers dried solubles, sodium molybdate or a mixture of unidentified factor sources. Removal of the molybdenum from the ash of distillers dried solubles resulted in the same growth rate exhibited by the control group. The ash of this product produced a 9.1% increase in growth; which was not quite significant. 2) Bone ash values were not affected by the dietary treatments. Intestinal xanthine dehydrogenase activities were significantly increased in all cases except the "molybdenum-free" distillers dried solubles ash-fed group.

1. Dick, A. T., *Soil Sci.*, 1956, v81, 229.
2. Westerfeld, W. W., and Richert, D. A., *Ann. N. Y. Acad. Sci.*, 1954, v57, 896.
3. Reid, B. L., Kurnick, A. A., Svacha, R. L., and Couch, J. R., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 245.
4. Reid, B. L., Kurnick, A. A., Burroughs, R. N., Svacha, R. L., and Couch, J. R., *ibid.*, 1957, v94, 737.
5. Dannenburg, W. N., Reid, B. L., Rozacky, E. E., and Couch, J. R., *Poultry Sci.*, 1955, v34, 1023.
6. Morrison, A. B., Scott, M. L., and Norris, L. C., *ibid.*, 1955, v34, 738.
7. Remy, C., Richert, D. A., and Westerfeld, W. W., *J. Biol. Chem.*, 1951, v193, 649.
8. Official Methods of Analysis, A. O. A. C., 7th Ed., 1950, Washington 4, D.C.
9. Piper, C. S., *Soil and Plant Analysis,* Interscience Publishers, 1950, New York.
10. Purvis, E. R., and Peterson, N. K., *Soil Sci.*, 1956, v81, 223.
11. Morrison, A. B., Dam, R., Norris, L. C., and Scott, M. L., *J. Nutrition,* 1956, v60, 283.
12. Westerfeld, W. W., and Richert, D. A., *ibid.*, 1953, v51, 85.

Received April 10, 1957. P.S.E.B.M., 1957, v95.

Cold-Pressor Responses in Families of Rabbits with Spontaneous Hypertension.* (23220)

NATALIE ALEXANDER, WILLIAM J. TIBBS† AND DOUGLAS R. DRURY
Department of Physiology, School of Medicine, University of Southern California, Los Angeles.

For the past 5 years we have bred rabbits with spontaneous hypertension(1). A high percentage of their progeny inherit the hypertension(2). We recently began raising families of control rabbits under the same conditions as those of the spontaneously hy-

* This investigation was supported by research grants from Am. Heart Assn. and Life Insurance Medical Research Fund.

† In part derived from thesis to Univ. of S. California Graduate School in partial fulfillment of requirements for the M.S. in Physiology.