drolysis from thoracic duct studies in the rat (7,8).

The technical assistance by J. Gürtler is greatly acknowledged.

This work is part of investigations supported by Swedish Medical Research Council.

1. Linder, E., Blomstrand, R., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 653.

2. Blomstrand, R., Dahlbäck, O., Linder, E., *ibid.*, 1958, v100, 802.

3. Borgström, B., *Acta Physiol. Scand.*, 1952, v25, 101, 111; 1954, v30, 231.

4. Reeves, R. E., *J. Am. Chem. Soc.*, 1941, v63, 1476.

5. Borgström, B., Tryding, N., Westöö, G., *Acta Physiol. Scand.*, 1957, v40, 241.

6. Blomstrand, R., Tryding, N., Westöö, G., *ibid.*, 1957, v37, 91.

7. Bernhard, K., Wagner, H., Ritzel, G., *Helv. Chim. Acta*, 1952, v35, 1404.

8. Reiser, R., Bryson, M. J., Carr, M. J., Kuiken, K. A., *J. Biol. Chem.*, 1952, v194, 131.

Received July 16, 1959. P.S.E.B.M., 1959, v102.

Comparative Toxicity of 3-Nitro 4 Hydroxyphenylarsonic Acid and Its Formaldehyde Dimer to Chicks. (25193)

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The arsenical, 3-nitro 4 hydroxyphenylarsonic acid (3-nitro), is widely used in poultry and swine rations. In the feed or drinking water of poultry, 3-nitro has a growth stimulating and coccidiostatic effect(1). In swine feeds, 3-nitro is used for growth promotion and as an aid in controlling infectious enteritis(2). Toxicosis has been reported in both species when 3-nitro was fed at recommended levels for growth stimulation or disease control(3,4). Polybenzarsol, a mixture of polymers formed from the reaction of formaldehyde and 4-hydroxy benzenearsonic acid, has been reported to have a low order of toxicity in rats, mice, dogs and man, following oral administration(5). This report suggests the possibility that the reaction product of formaldehyde and 3-nitro 4 hydroxyphenylarsonic acid (hereafter called the dimer) might have reduced toxicity to poultry.

Methods. The dimer of formaldehyde and 3-nitro was synthesized using the method of Faith(6). This was fed to chicks in 2 experiments. The basal diet was a practical broiler feed deemed adequate in all known nutrients. The birds were housed in electrically heated batteries equipped with raised wire floors and allowed access to feed and water *ad lib.* In the first experiment, duplicate lots of 10 male broiler strain chicks were given the following treatments: control; 3-nitro at levels of 49.5 and 198.0 mg/kg of feed; the dimer of 3-nitro at levels of 51.15 and 204.6 mg/kg; and procaine penicillin at a level of 4.4 mg/kg. All treatments in the second experiment were triplicated. Each lot contained 5 male and 5 female chicks. Treatments were as follows: control; 3-nitro at levels of 49.5, 74.25, and 198.0 mg/kg of diet and the dimer of 3-nitro at levels of 51.15, 76.72, and 204.6 mg/kg. At end of the 4-week test, triplicate liver arsenic determinations were made for each level of arsonic fed.* In both experiments all chicks were wing banded and individual weights taken at initiation and at termination of test. Feed consumption was determined by group at weekly intervals. Acute toxicity was studied using 4-week-old male chicks given a single oral dose of 3-nitro at levels of 100 and 200 mg/kg body weight and the dimer at levels of 103.2 and 206.4 mg/kg. The technic has been described(7).

Results. Data obtained in Exp. 1 and 2 are shown in Table I. In the first experiment, 4.4 mg penicillin, 49.50 mg 3-nitro and 51.15 mg of the dimer/kg diet respectively significantly improved growth over that obtained on the basal diet ($P = 5\%$ or less). 3-nitro appeared to be more effective in stimulating growth than either penicillin or the dimer, the latter 2 being essentially identical in their

* Arsenic determinations were made by J. B. Thompson of Trace Metal Research Labs., Chicago Heights, Ill.

TABLE I. Effect of 3-Nitro and Its Dimer on Chick Growth, Feed Efficiency and Liver Storage of Arsenic.

Arsenic addition (mg/kg)		Avg wt gain (g) 0-4 wk		Feed efficiency*		PPM As_2O_3
3-nitro	Dimer	Exp. 1†	Exp. 2‡	Exp. 1	Exp. 2	in liver
		317	305	1.87	1.78	.17‖
49.50		390	346	1.67	1.81	.86
	51.15	376	328	1.77	1.72	1.60
74.25			352		§	1.14
	76.72		375		§	2.0
198.00		284	293	1.78	1.79	2.0
	204.60	307	289	1.79	1.67	2.9
(4.4 procaine penicillin)		361		1.89		

* g feed/g gain. † Avg of replicated lots. ‡ Avg male and avg female/2; avg of triplicated lots. § 4th wk feed consumption data misplaced. ‖ Avg of 3 assays—each sample consisting of composited whole liver from 1 ♂ and 1 ♀ chick.

effect; however, the differences were not statistically significant. At the level of 198.0 mg/kg, 3-nitro significantly depressed growth ($P = 5\%$) whereas 204.6 mg/kg of its dimer, compared with the controls, was without significant effect.

In Exp. 2, growth was significantly improved ($P < .01 > .001$) by all additions except the highest level of 3-nitro and its dimer and the 51.15 mg level of the dimer. The difference between growth on the 76.72 mg/kg level of the dimer and the 49.5 mg/kg level of 3-nitro approached significance ($P > .05$). Growth was not significantly depressed by the highest level of arsonics. At each level of comparison, the dimer resulted in greater liver stores of arsenic than did 3-nitro (Table I).

As shown in Table II, there was essentially no difference in toxicity, expressed as number and weight change of survivors, between a single dose of 100 or 200 mg 3-nitro/kg body weight and an equivalent dose of the dimer. All treated birds lost weight. And, although the birds given the dimer appeared to be less affected, there were no statistically significant differences.

Discussion. The data obtained in both feeding trials suggest that at a level comparable to 49.5 mg of 3-nitro/kg of diet, the dimer of 3-nitro is slightly less growth stimulatory. This level corresponds to the commercial usage of 3-nitro, i.e., 45 g/ton. At an intermediate level, the dimer produced greater (but non-significant) weight gains. In one experiment in which growth was depressed by highest levels, the dimer was less detrimental.

TABLE II. Toxic Effect of a Single Oral Dose of 3-Nitro and Its Dimer.

Treatment	Avg change in body wt (g), 3 days
Control	+64.7 (10)*
100 mg 3-nitro/kg	−40.7 (7)
103.2 " dimer/kg	−36.6 (8)
200 " 2-nitro/kg	−38.0 (1)
206.4 " dimer/kg	(0)

* No. surviving of 10 chicks started.

Wharton *et al.* reported the LD_{35} dose of 3-nitro, administered/*os* to chicks 4 weeks of age, was 100 mg/kg of body weight(7). Weight loss of survivors averaged 48 g. This is in good agreement with LD_{30} and 40 g weight loss obtained in this study with the 100 mg/kg level of 3-nitro. The dimer, with an apparent LD_{20} and 36 g weight loss at a comparable dose, is little, if any, less toxic. 200 mg of 3-nitro/kg body weight is essentially the LD_{100} dose for chicks(7). Our data show that LD_{100} of the dimer is no greater than 200 mg/kg.

The consistently higher liver arsenic stores in chicks receiving the dimer suggest that it is excreted at a slower rate than 3-nitro.

Toxicity to chicks of 3-nitro was not greatly different from that of its formaldehyde dimer. This is in contrast to the report that the formaldehyde polymer of 4 hydroxy benzenearsonic acid has greatly reduced toxicity to rats, mice and dogs. Rats tolerated the polymer at levels of 400 mg/kg/day for 8 weeks and there was no mortality in mice given 4 g/kg intragastrically as a single dose(5).

Summary. The formaldehyde dimer of 3-nitro 4 hydroxyphenylarsonic acid was slightly less growth stimulatory than 3-nitro when fed to chicks at a level equivalent to 49.5 mg of 3-nitro/kg diet. Both arsenicals

stimulated growth when fed at a level equivalent to 74.25 mg of 3-nitro/kg diet, with the dimer somewhat more effective. There was little difference between the 2 when fed at a level equivalent to 198 mg 3-nitro/kg diet. Liver arsenic stores were greater in chicks fed the dimer at each of the 3 levels of comparison. Administered as a single oral dose at levels approximating 100 and 200 mg/kg body weight, survival out of 10 chicks treated was 7 and 1 respectively, for 3-nitro and 8 and 0 for the dimer.

1. Morehouse, N. F., Mayfield, O. J., *J. Parasitol.*, 1946, v32, 20.
2. Carpenter, L. E., Larson, N. L., *J. Animal Sci.* 1952, v11, 283.
3. Milligan, J. L., Wilcke, H. L., Marr, J. E., Bethke, R. M., *Poultry Sci.*, 1955, v34, 794.
4. Becker, D. E., Terrill, S. W., Meade, R. J., Edwards, R. M., *Antib. and Chemo.*, 1952, v2, 421.
5. Jones, W. R., Newberne, J. W., Forney, R. B., Begley, R. W., Alexander, W. M., Abreu, B. E., Weaver, L. C., *ibid.*, 1958, v8, 400.
6. Faith, H. E., *J. Am. Chem. Soc.*, 1950, v72, 837.
7. Wharton, F. D., Jr., Fritz, J. C., Schoene, R. B., Smidt, M. J., *Am. J. Vet. Research*, 1959, v20, 655.

Received July 17, 1959. P.S.E.B.M., 1959, v102.

Hypoglycemic Activity of Tris Buffer in Man and Dog. (25194)

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Tris(hydroxymethyl)aminomethane [$(CH_2OH)_3 \cdot C\text{-}NH_2$; tris buffer, THAM] has been used widely in biochemical work as a buffer. Nahas(1) discovered that it can be safely infused into dogs and corrects profound respiratory acidosis. Stimulated by this observation, our laboratory participated in a study of the alkalinizing effects of intravenous tris in healthy, fasting adult man. Since little was known of *in vivo* properties of tris, we set out to characterize its effects in terms of chemical measurements available in our laboratory. We were surprised to find a hypoglycemic effect.

Studies in man. After an oral water load, 4 subjects breathed room air for about half an hour and then 2.3 or 3.4% CO_2 for a similar period. Intravenous tris (0.3M in 0.03M NaCl and 0.005M KCl) was given immediately thereafter, during CO_2 breathing, over 30 to 64 minutes. A recovery period followed. Arterial blood was sampled from an indwelling femoral cannula. Serum glucose(2) fell distinctly (Table I) in 3 subjects during and at the end of tris infusion. Hypoglycemia occurred in J.F., who received the largest dose (8.8 mM/kg or 0.49 ml/kg/min over 60 minutes). This was accompanied by hunger and profuse sweating superseded by other toxic manifestations which persisted for 24 hours. Glucose returned toward pre-infusion values within 57 minutes after infusion in J.F. Glucose fell substantially in S.B., 2.8 mM tris/kg (0.19 ml/kg/min over 49 minutes) and in J.D., 4.1 mM/kg (0.21 ml/kg/min over 64 minutes). The fall in J.L. to 65 mg/100 ml occurred 26 minutes after tris infusion was over (3.0 mM/kg or 0.33 ml/kg/min for 30 minutes). Serum inorganic phosphate fell progressively with glucose in J.F. from 3.6 to 1.9 mg/100 ml, from 3.5 to 2.9 mg/100 ml in J.D., from 3.8 to 3.3 mg/100 ml in J.L., but was unchanged in S.B. Serum potassium did not fall in any of the subjects. Other chemical and physiologic observations are reported elsewhere(3,4).

Studies in dogs. Tris was then given intravenously to 3 fasting, anesthetized (pentobarbital), heparinized, female dogs during room air breathing. 0.3M saline was infused at 0.5 ml/kg/minute over 1 hour; then 0.3M tris (in 0.03M NaCl and 0.005M KCl) at the same rate (slow infusion, Table II) for 2½ to 3 hours followed by 1 hour at 0.7 to 1.0 ml/kg/minute (fast infusion, Table II). Femoral arterial serum glucose concentrations rose during anesthesia and manipulation of 2 of the animals and then tended to remain unchanged or return to pre-anesthetic levels during saline infusion. Glucose declined during slow infusion of tris in all dogs. Values fell to below 50 mg/100 ml in 2 dogs. Rapid infusion produced a precipitous further reduc-