leased 12.1 percent of the administered dose, or than that expired by the animals that received the drug intravenously; these released 12.3 percent of the administered dose as $C^{14}O_2$. A sustained release of radioactivity occurred, since the four animals maintained for 12 hours continued to expire from 0.7 to 2.0 percent of the dose each hour until the termination of the experiments.

These results show that the rat is capable of demethylating chlorpromazine-C^{14} and oxidizing the methyl group so that it appears as expired $C^{14}O_2$ following oral or intravenous administration of the drug. The tissues responsible for this transformation are the subject of current investigations.

JOHN J. ROSS, JR.
RICHARD L. YOUNG
ALFRED R. MAASS
Smith, Kline, and French Laboratories, Philadelphia, Pennsylvania

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Zinc Requirement for Growing Swine

Abstract. Swine fed rations containing 16, 21, 26, 31, 36, 41, or 46 parts per million of zinc gained 0.06, 0.13, 0.25, 0.52, 0.61, 0.69, and 0.76 lb, respectively, per day. Feed required per unit of gain decreased with each increment of zinc. Parakeratosis occurred in pigs fed 16, 21, 26, 31, or 36 ppm of zinc.

It has been shown by Bertrand and Bhattacherjee (*1*) and by Elvehjem and his associates (*2*) that zinc is a necessary constituent of the diet for the normal growth of mice and rats. O'dell *et al.* (*3*) and Roberson *et al.* (*4*) demonstrated that the growth of chicks was stimulated if zinc was added to a semipurified diet. A dermatitis of swine which Kernkamp and Ferrin (*5*) described in detail and designated parakeratosis has been shown to be greatly affected by inorganic and organic compounds present. Tucker and Salmon (*6*) reported that the symptoms of parakeratosis could be prevented or cured by the addition of zinc to the diet. Their work also showed that an excess of calcium or phosphorus, or both, markedly increased the incidence and severity of the disease. Recently these results were confirmed by Lewis *et al.* (*7*), Leucke *et al.* (*8*), and Conrad and Beeson (*9*) for both natural and purified diets, different calcium and phosphorus levels, and both $ZnCO_3$ and ZnO as sources of supplemental zinc. Prevention or cure was effected by these workers with levels of supplemental zinc from 50 to 100 ppm of the ration.

Table 1. Performance of pigs fed different levels of zinc.

Item	Eight-week summary						
	1	2	3	4	5	6	7
Added zinc*	0	5	10	15	20	25	30
Total zinc (ppm)	16	21	26	31	36	41	46
Av. final wt. (lb)	15.0	18.0	24.9	40.2	44.9	49.9	53.5
Av. daily gain (lb)	0.06	0.13	0.25	0.52	0.61	0.69	0.76
Av. daily feed (lb)	0.82	0.82	1.04	1.28	1.40	1.54	1.59
Feed per pound gain (lb)	13.9	6.5	4.1	2.4	2.3	2.2	2.1
Parakeratosis (pigs)	6	6	6	4	3	0	0
Deaths	2	0	0	0	0	0	0

* Added zinc was U.S.P. grade zinc oxide.

In view of the previous work our experiment was designed to determine whether parakeratosis of swine was a true zinc deficiency and, if so, what quantitative level of zinc is required.

Forty-two Duroc pigs weaned at the age of 3 weeks were allotted into seven groups containing six animals each. The average initial weight was 11.0 lb, and the animals were allocated on the basis of live weight, litter number, and sex. One female and one barrow were placed in a 3.5- by 7-ft concrete-floored pen which had a wooden platform covering the front half of the floor. No bedding was used, and all feeders and waterers were lined with glass. The pigs had free access to the feed. Chlorinated tap water containing 0.2 ppm of zinc was treated with a Zeolite water softener before it was put in the glass-lined waterers. The basal ration consisted of Drackett C-1 protein, 25 percent; cerelose, 30.8 percent; starch, 30.8 percent; corn oil, 5 percent; celluflour, 3 percent, and all minerals and vitamins known to be required by swine. The basal ration contained 0.66 percent calcium, 0.47 percent phosphorus, and 16 ppm of zinc. U.S.P. grade zinc oxide was added to the basal ration to provide the following levels of total zinc for treatments 1 through 7, respectively: 16, 21, 26, 31, 36, 41, and 46 ppm of the ration.

The summary of results at 8 weeks, as given in Table 1, shows that the growth rate was stimulated by each increment of added zinc. Statistical treatment of these data showed that the increase was linear. The pigs on treatments of 36, 41, and 46 ppm of zinc were continued on the trial for two additional weeks; and the average daily growth rates for the 10 weeks were 0.70, 0.84, and 0.95 lb, respectively. The first noticeable difference in growth rate occurred in the pigs on the basal ration during the third week of experiment. On the 23rd day of the trial, lesions of the epidermis were noticed on the underline of one pig in treatment 1 (no added zinc). Within a few days several pigs in lots 1 and 2 showed similar lesions of the skin; the dermatitis became more severe and spread to all parts of the body surface as the experiment progressed. Lesions appeared in lots 3 and 4 during the sixth week and in two pigs in lot 5 during the eighth week. The severity of the parakeratosis was in inverse proportion to the zinc content of the ration. The lesions were very similar to those described by Kernkamp and Ferrin (*5*) and by Tucker and Salmon (*6*). In addition, loss of appetite, severe scours, and greasy skin accompanied the dermatitis. Two pigs on the basal ration died. Careful weekly examinations of all pigs failed to show any symptoms of parakeratosis in the pigs fed 41 or 46 ppm of zinc.

Feed required per pound of gain decreased with each increment of zinc. However, the greatest differences were between the four lowest levels (16, 21, 26, and 31 ppm).

Four pigs with parakeratosis showed marked improvement in appetite, growth rate, and appearance of skin when they were placed on the basal ration fortified with 50 ppm of zinc. Appetite was noticeably improved within 2 days. During the 4-week recovery period, the pigs grew from an average weight of 29.6 lb to an average of 60.4 lb; the average rate was 1.18 lb/day.

Under the conditions of this experiment, symptoms of parakeratosis did not appear in growing pigs fed 41 ppm of zinc. A further increase in zinc to 46 ppm improved the growth rate 14 percent, indicating that the optimum requirement for this element is at least 46 ppm of the total ration. In subsequent studies the availability of zinc in the ration should be considered (*10, 11*).

W. H. SMITH
M. P. PLUMLEE
W. M. BEESON
Department of Animal Science, Purdue University, Lafayette, Indiana

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10. We express our appreciation to Mr. Vernon R. Heaton of the Calcium Carbonate Company, Quincy, Ill., for the zinc and calcium analyses of the rations.
11. This report is journal paper No. 1302, Purdue University Agricultural Experiment Station.

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Inactivations of Oxytocin Suggesting Peptide Denaturation

Denaturation, a phenomenon associated with proteins (*1*), is frequently described by occurrences such as loss of biological activity, loss of crystallizability, change in solubility, increase in levorotation, and changes in parameters such as viscosity which indicate a change in the shape of the molecule. The term implies a subtle change in molecular structure, and the phenomenon, which is frequently base-catalyzed, is usually attributed to change in the configuration of the molecule due largely to a loss of hydrogen bonding, as is thought to occur with many proteins in concentrated solutions of urea. The role in the denaturation process in urea of sulfhydryl-disulfide interactions with resultant changes in cross-linking and molecular weight has also been recognized and has been studied for certain proteins (*2*).

Study of the inactivation of oxytocin, the cystine-containing octapeptide which is the chief oxytocic principle of the posterior pituitary, and of the material resulting from inactivation, suggests that denaturation, at least as defined by many of the criteria used with proteins, may occur with polypeptides of relatively small size. Two separate inactivations of oxytocin (molecular weight 1007) are described in this report (*3, 4*); both result in a large increase in levorotation.

Inactivation of oxytocin has been found to occur rather readily under the mildly alkaline conditions frequently used to form dinitrophenyl (-DNP) derivatives of peptides and proteins. A less soluble, higher molecular weight form of the hormone is obtained. This inactivation has been characterized as an intermolecular disulfide interchange. By treatment of the inactivation product with certain sulfhydryl reagents it has been found possible to regenerate the biological activity.

Mono-DNP-cystine (*5*), allowed to stand in 10.5 percent solution in the presence of five equivalents of $NaHCO_3$ at room temperature for 22 hours, was found to result in a mixture of approximately equimolar amounts of cystine, mono-DNP-cystine, and di-DNP-cystine (*6*), as indicated by paper chromatography. Oxytocin, represented by the structure (*7*)

Cys-Tyr-Ileu-Glu(NH_2)-Asp(NH_2)-Cys-Pro-Leu-Gly(NH_2)

is also, as are many proteins, an unsymmetrically substituted cystine derivative, or mixed disulfide. Under similar conditions, *p*H 8.3, a sample of the hormone with a potency of 350 unit/mg, as determined by the chicken depressor assay of Coon (*8*), gradually lost biological activity and deposited a white solid in 40 percent yield; $(\alpha)^{22.5}D$, $-104°$ (*c*, 0.5; 1*N* acetic acid); compare $(\alpha)^{22.5}D$, $-23.1°$ for oxytocin acetate. Loss of activity was not inhibited under these conditions by the addition of N-ethyl maleimide and proceeded much more rapidly than in the absence of $NaHCO_3$. The rate of inactivation was markedly decreased at low hormone concentrations.

Analysis (*9*) of a hydrolyzate (6*N* HCl, 14 hours, 120°C) of the solid showed approximately the same composition as oxytocin. Probably due to its low solubility, the material remained at the origin on paper electrophoresis in sodium acetate buffer, *p*H 5.5, or sodium glycinate buffer, *p*H 9.5, or on paper chromatography with *n*-BuOH–H_2O–HAc (5:5:1), while oxytocin traveled 6.8 cm and 3.5 cm, $R_f = 0.3$, respectively. On electrophoresis in 5*N* acetic acid it moved toward the cathode somewhat faster than oxytocin. It rapidly deposited an amorphous flavianate in contrast to the slowly formed, more soluble crystalline flavianate characteristic of oxytocin (*10*).

Treatment of the inactivated material (7 unit/mg) (*11*) at a concentration of 0.15 mg/ml at *p*H 5.7 with 8 μmole/ml of the following reagents led after 3 days to the following biological activities in units per milligram: cysteine, 235 (*12*); glutathione, 150; H_2S (saturated solution), 200; homocysteine, 140; thioglycollic acid, < 9; thiomalic acid, < 6; ergothioneine, < 5; ovalbumin, < 3; ascorbic acid, 5; oxidized glutathione, 4. Sodium in NH_3 led to 22 unit/mg; $Na(NH_3)$ followed by cysteine, to 42. It was also found that similar treatment of oxytocin (480 unit/mg) with cysteine, H_2S, or thioglycollic acid (*13*) led to approximately the same potencies as were regenerated from the inactivated material (7 unit/mg) with each of these reagents, suggesting that the same equilibrium mixture is attained starting from either oxytocin or the inactivated oxy-

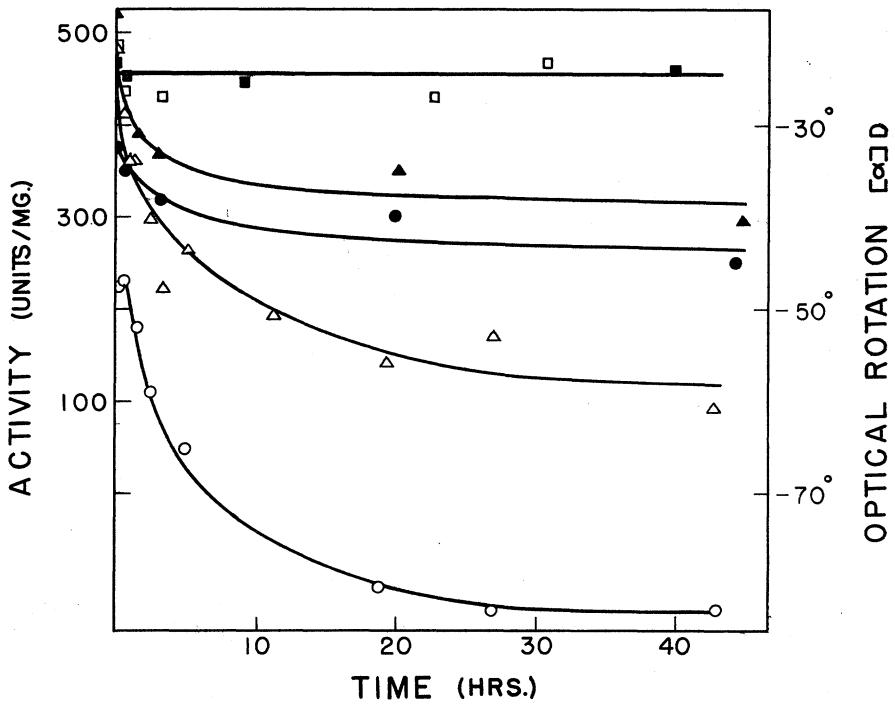

Fig. 1. Effect of urea on the biological activity and optical rotation $(\alpha)D$ of oxytocin, concentration 0.5 percent, at 22°C. Open circles, $(\alpha)D$; open triangles, activity, 7*M* urea, *p*H 8. Solid circles, $(\alpha)D$; solid triangles, activity, 7*M* urea, *p*H adjusted to 4.3 with acetic acid. Open squares, activity, 0.02*M* sodium barbital buffer, *p*H 8. Solid squares, activity, 0.02*M* sodium acetate buffer, *p*H 4.3.