reasons why reserpine does not exert sedative activity when room temperature is 37° or 0°C. We believe that the stress reaction with release of catecholamines takes place at 0° and at 37°C. In this regard recent experiments may be recalled in which DOPA, a precursor of noradrenaline, antagonizes the sedative activity of reserpine (*13*).

The increase of brain serotonin at 37°C may be considered as a situation analogous to that occurring after iproniazid administration, when reserpine does not show sedation (*14*).

Note added in proof. Since this communication was submitted for publication we checked our results with a more specific method for evaluation of brain serotonin [D. F. Bogdanski *et al., J. Pharmacol. Exptl. Therap.* **117**, 82 (1956)]. Thus, using a spectrofluorimetrical technique, we confirmed that reserpinized rats kept at a room temperature of 37°C have a higher brain serotonin (0.36 ± 0.009 μg) than the animals treated with reserpine at a room temperature of 22°C (0.16 ± 0.008 μg).

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Demethylation of Chlorpromazine-(N-Methyl)-C^{14}

Abstract. Chlorpromazine methyl-C^{14} was administered to three groups of rats —orally to one group following chronic oral administration of the unlabeled drug; orally to an unprimed group; and intravenously to another unprimed group. Appearance of $C^{14}O_2$ in the air expired by all animals indicated that the rat metabolized the side chain of the labeled drug.

Previous studies on the metabolism of the psychopharmacologic (*1*) agents containing the phenothiazine nucleus have resulted in little information about the fate of the N-aliphatic side chain of this class of compounds. Berti and Cima (*2*) suggested that this side chain was unchanged in all of the urinary compounds examined in their study on the fate of chlorpromazine administered to rabbits. Salzman and Brodie (*3*) failed to detect any demethylation of chlorpromazine upon incubation of the drug with rabbit-liver homogenate, even though La Du *et al.* (*4*) showed an active demethylation system for drugs to be present in liver. As a part of our program concerned with the metabolism of phenothiazine compounds, we have studied the fate of the side chain of chlorpromazine by using chlorpromazine-(N-methyl)-C^{14}. This report describes the extensive in vivo demethylation of the drug manifested by the appearance of $C^{14}O_2$ in the air expired by the rat.

Chlorpromazine was labeled with carbon-14 by reacting 2-chloro-10-(methylaminopropyl)-phenothiazine with formaldehyde-C^{14} (*5*). The product was identified as 2-chloro-10-(dimethylaminopropyl)-phenothiazine hydrochloride by ultraviolet spectrum analysis (maximum, 255 mμ) and by paper chromatography in isoamyl alcohol, water, formic acid, and ethanol (100:100:10:15) and in isoamyl alcohol, *n*-butanol, ammonia, and water (8:8:1:3). Its specific activity, 2.42×10^6 count/min mg (3.05 μc/mg), was determined by oxidation to carbonate, according to the wet-persulfate procedure (*6*). The radioactivity was counted as $BaCO_3$ in a thin-window gas-flow counter to a statistical accuracy of 1 percent. All counts were corrected to samples of infinite thinness by reference to a standard self-absorption curve for $BaCO_3$.

Expiration of radioactive CO_2 was followed in groups of adult male rats of the Long Evans strain. One group of five animals was fasted overnight prior to the oral administration of 15 mg of chlorpromazine per kilogram (approximately 13.9×10^6 count/min kg) in 2 ml of distilled water, by stomach tube. In order to study the effect of chronic administration of the drug upon the metabolism of the rat, a second group of five rats was pretreated with 15 mg of chlorpromazine per kilogram *per os* daily for 10 days prior to fasting and the oral administration of the same amount of labeled chlorpromazine with an average activity of 11.9×10^6 count/min kg. The third group of three animals received 2 mg of chlorpromazine per kilogram (approximately 4.86×10^6 count/min kg) intravenously in 0.5 ml of physiological saline solution by injection into the tail vein. After administration of the labeled drug, the animals were maintained in an all-glass metabolism chamber, and the expired air was collected in sodium hydroxide solution. All animals were studied for 6 hours, and two rats from each that had received the drug orally were followed for 12 hours. Radioactivity was recovered from the absorptive solutions at hourly intervals as $BaCO_3$ and determined according to the procedure used for the radioactive drug.

Figure 1 shows the release of radioactivity by the three groups of animals. Each point represents the average of the amount of radioactive CO_2 released during the collection interval, expressed as a percentage of the radioactivity administered to the animals. In all instances the label was detected during the first hour after administration of the chloropromazine-C^{14}. The radioactivity expired by the animals that had received a single administration of chlorpromazine-C^{14} was significantly greater ($p = 0.01$) than that released by the primed animals. Somewhat less $C^{14}O_2$ was expired during this period by the animals given the drug intravenously. Subsequently the $C^{14}O_2$ released declined and was not significantly different for any group beyond the first hour after administration.

Release of the label was extensive, since during the 6-hour collection period the unprimed oral group expired 16.7 percent of the administered radioactivity as $C^{14}O_2$. This was significantly greater ($p = 0.05$) than the amount expired by the primed animals, which re-

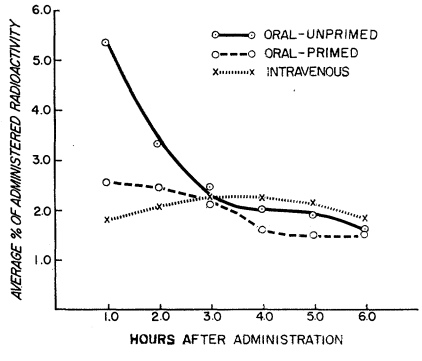

Fig. 1. Comparison of average percentages of administered radioactivity expired by rats following oral intravenous administration of chlorpromazine-(N-methyl)-C^{14} (see text).

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.

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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*).

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