TABLE II.
Effect of Once- vs. 5-Times Feeding per Day on Fasting Katabolism of Rats.

	Mean age days	Mean body wt g	Mean heat production per day per kg% kcal
6 rats fed 8* g per day once	91	161	78.3 ± 2.3
Same rats fed 1.6 g per day 5 times	91	162	78.2 ± 2.3

*N.B. These rats fed *ad lib.* ate 24, 14, 15, and 14 g of the same food on the first to the 4th day respectively after the respiration trial.

probably our method of using each rat for each of the two conditions, thus eliminating the influence of individual variation between rats on the difference between the means. Our trial shows that this individual variation in the metabolic rate between different rats is much greater than a difference resulting from a change in the number of daily feedings. For the measurement of the fasting metabolism it is thus sufficient to equalize the previous daily rations between experimental and control rats without regard to their feeding habit.

11776

Studies of Phenothiazine. X. Further Observations on Oxidation of Phenothiazine.*

FLOYD DEEDS.

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Several years ago an interesting observation was made on the oxidation of phenothiazine in the dry state. At that time no practical importance was attached to the observation, and it was not reported. As will be shown later, recent developments on the use of phenothiazine as a fungicide have pointed to the importance of this observation, which has now been repeatedly confirmed, and deemed worthy of a report.

A porcelain mortar and pestle had been used for intimately mixing a small quantity of phenothiazine with the basic rat diet routinely used in this laboratory. Subsequently the same mortar was wiped out, but not washed, and used for mixing bentonite with rat diet. It was observed that after a few minutes of mixing the whole con-

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tents of the mortar had assumed a pink tint. Realizing that traces of phenothiazine might have been left in the mortar, and believing that an oxidation to thionol could account for the color developed, the following experiments were performed.

The mortar and pestle were thoroughly washed, and a sample of bentonite alone was thoroughly ground. No coloration developed. After washing the mortar and pestle, phenothiazine alone was ground and again no color developed. The experiment was then repeated with a mixture of phenothiazine and bentonite. After a few moments of grinding the mixture showed a definite pink color which rapidly developed into a deep brick-red as the grinding proceeded. Repetition of the experiment by other members of the staff yielded the same result. Substitution of talc for bentonite gave the same results, but the color development was somewhat slower, indicating that talc was less effective than bentonite in facilitating the oxidation of phenothiazine.

To determine whether or not the grinding was an essential part of the oxidation mechanism, a sample of bentonite and phenothiazine was placed in a test tube and the contents were mixed by repeated but gentle inversions of the tube. In the course of several hours a definite degree of color was observed. The tube was stoppered and set aside. The color continued to deepen and after several weeks was grey-black. Apparently grinding is desirable only insofar as it facilitates the process.

Twenty-five grams of phenothiazine and 100 g of bentonite were placed in a ball bill and ground for 48 hours. Within a few minutes after starting the ball mill, a pronounced brick-red color developed. The mill was stopped and a sample of the mixture removed. Attempts to remove a colored product from the mixture by extraction with water, or by extraction with ether and chloroform, gave negative results. After 48 hours of grinding the mixture was nearly black. From this mixture a red dye could easily be extracted with alcohol, ether or chloroform. When a chloroform extract was filtered to remove bentonite, and evaporated to dryness, the residue consisted of a mixture of a reddish-black powder and a light-colored powder which was probably unchanged phenothiazine. This residue was completely soluble in alcohol, but upon the addition of water the solution yielded a fine silky precipitate characteristic of phenothiazine. Removal of this precipitate left a clear, light-red aqueous solution. Chloroform extraction of this solution permitted recovery of a phenothiazine-free dye. An aqueous solution of this dye exhibited reactions characteristic of thionol. It was easily reduced by stannous chloride and sodium hydrosulfite and reoxidized by hydrogen peroxide, or simply by shaking during exposure to air.

Further identification of the dye as thionol has not been attempted, nor have tests been made for the presence of the intermediate oxidation product, namely, phenothiazone.

The results obtained suggest that the oxidation of phenothiazine which normally takes place slowly in the presence of moisture, light, and air is greatly facilitated by the distribution of the phenothiazine over the extensive surface present in finely divided bentonite. That this is a fairly general phenomenon is shown by preliminary experiments in which dimethylacridan and diphenylamine have been oxidized to colored compounds by grinding with bentonite.

If compounds of low-water solubility are of insecticidal and fungicidal value by virtue of slow oxidation to a more potent compound, it may well be that a mixture of these compounds with bentonite will improve their efficiency.

Recently, M. C. Goldsworthy and E. L. Green of the Bureau of Plant Industry, U. S. Department of Agriculture, claimed to have discovered that the fungicidal action of phenothiazine is due to its partial oxidation to phenothiazone, according to the following statement quoted from U. S. Department of Agriculture press release dated February 11, 1940:

"An attempt has been made to find a way to spray the chemical so that some of it would change to phenothiazone and kill fungi and the remainder keep its original form as an insect killer. Some promising results were obtained by mixing phenothiazine with bentonite—a fine clay—and hydrated lime. This caused the spray to stick to the fruit and build up a residue. The under part of the spray film apparently retains its insecticidal value while that exposed to light on the surface of the film changed to a fungicide."

The experiments reported here suggest that the distribution of phenothiazine over the extensive surface presented by bentonite permits intimate contact with atmospheric oxygen, thereby facilitating oxidation, an action which may be further aided by the alkalinity of the hydrated lime.

11777 P

Electrophoretic Analysis of Digested Antitoxic Sera.

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Recent work has shown that certain antitoxins can be partially digested[1] with pepsin without losing their abilities to neutralize