not a structural analog of serotonin. This, and the lack of competitive antagonism, would argue against its being an antimetabolite of the hormone. Present data indicate that the action is indirect. Thus, nicotinamide was found to inhibit the muscle-contracting action of Ca^{++}, whereas a specific antimetabolite of serotonin, BAS (*8*), did not (see Table 2). If the action of serotonin on muscle and nerve cells is to transport Ca^{++} into them (*6, 9*), then a specific antiserotonin should not interfere with the serotonin-like action of Ca^{++}. Conversely, an agent which did not antagonize directly the basic action of serotonin, but which interfered with some other steps of the contractile process of muscle (for example, the energy-yielding ones), should also interfere with the action of Ca^{++}. This is what nicotinamide was found to do. Such indirect inhibitions might be of DPNase or the transmethylation processes which are known to be sensitive to nicotinamide. Elucidation of the precise mechanism must await further study.

The main purposes of this note (*10*) are to point out that nicotinamide affects the behavior of normal animals and that its effects are not incompatible with its ability to act as an antagonist to serotonin and to acetylcholine.

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10. I wish to acknowledge the technical assistance of N. K. Campbell and M. Gallagher.

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Researches on the Mechanism of Reserpine Sedative Action

In recent years experimental evidence has accumulated on the possibility that reserpine acts as a sedative through release of serotonin (*1*). Such a hypothesis has been suggested and supported by Brodie *et al.*, who have shown (i) that release of serotonin takes place only with *Rauwolfia* alkaloids which have sedative action (*2*); (ii) that central antagonists of serotonin, such as lysergic acid diethylamide (LSD) (*3*), are also antagonists of reserpine (*4*); and (iii) that substances which increase brain serotonin, such as iproniazid, can reverse the sedative activity of reserpine (*4, 5*).

Recently Lessin and Parkes (*6*) stressed the importance of hypothermia occurring after administration of reserpine as an explanation of its sedative activity. But serotonin too can decrease body temperature (*7*). This observation may explain the potentiation of barbiturates by serotonin, since the hypnotic effects of barbiturates are increased when hypothermia is induced (*8, 9*). Finally, in Pletscher's experiments, iproniazid antagonized both serotonin metabolism and hypothermia after reserpine (*10*).

These data prompted us to determine whether a relationship between the sedative action of reserpine, serotonin release, and hypothermia is evident under different experimental conditions.

The sedative action of reserpine was evaluated by the potentiation of sleeping-time after administration of pentobarbital (20 mg/kg, intraperitoneally). Brain serotonin was extracted according to the method of Bogdanski *et al.* (*11*) and measured spectrophotometrically at 275 mμ by the method of Udenfriend *et al.* (*12*). Rectal temperature was determined with a resistance thermometer. The experiments were carried out with 200-g female albino rats kept in constant-temperature rooms at 0°, 22°, 29°, or 37°C and injected intraperitoneally with 2.5 mg of reserpine per kilogram 4 hours before the reported determinations. The results are summarized in Table 1.

Table 1. Results. The figures in parentheses show the number of animals in each series. Each brain serotonin value represents an analysis of the pooled brains of two to three animals.

Treatment	Room temperature (°C)	Time (hr)	Rectal temperature (°C ± S.E.)	Brain serotonin (μg/g ± S.E.)	Sleeping-time after pentobarbital ± S.E.*	Animals that went to sleep/ treated animals
Control	0	21	36.6 ± 0.15 (10)	0.93 ± 0.11 (6)		
Reserpine	0	21	34.3 ± 0.26† (10)	0.99 ± 0.024 (10)		
Control	22	21	37.3 ± 0.09 (25)	0.91 ± 0.05 (30)	25′ ± 1′37″	11/22
Reserpine	22	21	32.6 ± 0.3† (15)	0.26 ± 0.02† (8)	53′ ± 2′13″†	20/22
Control	29	4	37.3 ± 0.4 (5)	0.96 ± 0.03 (4)	24′ ± 1′41″	6/8
Reserpine	29	4	34.4 ± 0.3† (5)	0.29 ± 0.037† (4)	63′ ± 1′45″†	7/8
Control	29	21	37.2 ± 0.18 (5)	1.02 ± 0.034 (4)	24′ ± 1′52″	5/8
Reserpine	29	21	34.5 ± 0.19† (5)	0.31 ± 0.035† (4)	54′ ± 3′35″†	7/8
Control	37	4	37.5 ± 0.14 (5)	1.15 ± 0.04 (4)	23′ ± 2′20″	6/8
Reserpine	37	4	37.2 ± 0.13 (5)	0.85 ± 0.35 (4)	28′ ± 1′14″	5/8
Control	37	21	37.1 ± 0.1 (15)	1.25 ± 0.074 (6)	24′ ± 1′04″	8/16
Reserpine	37	21	37.0 ± 0.12 (15)	1.72 ± 0.24† (10)	24′ ± 1′24″	7/16

* The average includes only the number of animals that went to sleep after receiving pentobarbital.
† Significant beyond the 0.01-level of probability.

Our results show that increasing room temperature from 22° to 37°C does not change body temperature and sleeping-time after pentobarbital but does induce a small increase in the content of brain serotonin. After reserpine is injected, sedation is present only if serotonin is released and body temperature decreases (22° and 29°C). At 37°C, body temperature is unchanged and brain serotonin increases after administration of reserpine; under these conditions there is no evidence of sedative activity. At 0°C reserpine significantly decreases body temperature, with no change in brain serotonin. The measurement of sleeping-time after pentobarbital was very difficult to evaluate because the cold provokes large variations between animals. However, when the rats which had been at 0°C were brought to normal room temperature, it was evident that animals treated with reserpine did not show typical sedation and ptosis. But after 1 to 2 hours at normal temperature sedation appears. An experiment, carried out by keeping the animals after reserpine treatment for 4 hours at 0°C and for 4 hours at 22°C, showed in fact a decrease of brain serotonin (0.63 ± 0.08 μg/g).

Thus, at 22°, 29° and 37°C, there is a parallelism between the onset of the sedative activity of reserpine, as indicated by an increase of barbiturate narcosis, serotonin release, and hypothermia. In contrast, at 0°C we obtained hypothermia after reserpine injection, without sedative activity or serotonin release. It is not simply a delayed action, for 8 hours after reserpine treatment at 0°C we observed no decrease in the content of brain serotonin (1.17 ± 0.06 μg/g).

Our results agree with the hypothesis that the sedative action of reserpine takes place only when there is serotonin release. The onset of hypothermia is not always associated with sedation, and it is not correlated with serotonin release. This is true also in experiments in which we kept the animals in ice bath until their rectal temperatures reached 30°C. Under these conditions no decrease of brain serotonin occurs (1.07 ± 0.1 μg/g).

It is interesting to speculate on the

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-

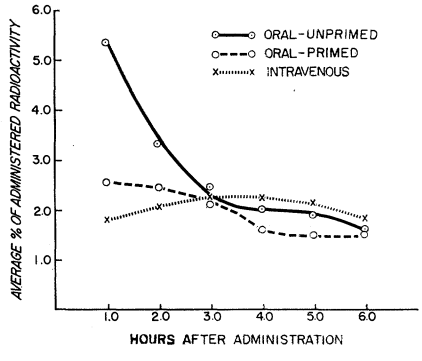

Fig. 1. Comparison of average percentages of administered radioactivity expired by rats following oral intravenous administration of chlorpromazine-(N-methyl)-C^{14} (see text).