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INHIBITION OF THE METHYLATION OF NICOTINAMIDE BY CHLORPROMAZINE*

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Abstract—The administration of chlorpromazine to mice inhibits *in vitro* the activity of a liver enzyme that N-methylates nicotinamide. Inhibition of nicotinamide methylation was also observed after the addition *in vitro* of chlorpromazine to a 25-fold purified enzyme isolated from rat liver. Kinetic studies indicated that this inhibition was due to a marked reduction in the affinity constant of the enzyme for nicotinamide. In the presence of saturating amounts of nicotinamide, chlorpromazine increased rather than decreased the methylation of nicotinamide.

A LARGE increase in the diphosphopyridine nucleotide (NAD) concentration occurs in mouse liver, brain, and spleen after the injection of nicotinamide.[1] Chlorpromazine, reserpine, and other chemically related tranquilizing drugs prolong the period that the NAD concentration remains elevated in mouse liver if these drugs are administered prior to nicotinamide.[2–4] It is possible that nicotinamide is degraded at a slower rate in the presence of tranquilizing drugs, whereas the synthesis of NAD is unaffected. Under these conditions, the synthesis of NAD would be expected to continue at a high rate for an extended period, causing a prolonged elevation of tissue NAD levels.[4, 5] In this study, catalysis of the methylation of nicotinamide to form N^1-methylnicotinamide was examined *in vitro*, with rat and mouse liver as the enzyme source. The enzyme of rat liver was purified partially and used to determine in greater detail the effect of chlorpromazine. This enzyme resembles the nicotinamide-methylating enzyme described previously,[6, 7] but the pH optimum is different.

METHODS

Enzyme assay. The nicotinamide-methylating activity of mouse and rat liver preparations was determined *in vitro* by measuring the formation of N^1-methylnicotinamide from nicotinamide and S-adenosylmethionine.‡ Reaction mixtures contained nicotinamide, S-adenosylmethionine, enzyme, and buffer in a final volume of 50 to 75 μliters. Reaction mixtures were prepared either in duplicate or triplicate and incubated 2 hr at 38°. The reaction was stopped by adding trichloroacetic acid

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‡ S-adenosylmethionine iodide was obtained from California Biochemicals, Los Angeles, Calif; nicotinamide-7-^{14}C, 0·6 mc/mmole, from New England Nuclear Corp., Boston Mass. Male Sprague-Dawley rats were obtained from Holtzman Farm and DBA mice from Rockland Farm.

to a final concentration of 5%. Precipitated protein was removed by centrifugation, and aliquots (10 to 30 μl) of the supernatants were assayed in duplicate for N^1-methylnicotinamide by the methyl ethyl ketone method.[8] Fluorescence was measured in the Farrand fluorometer (primary filter 5860; secondary filters 3387, 4308). Protein was determined by the method of Warburg and Christian[9] or Lowry *et al.*[10] N^1-methylnicotinamide iodide standards were routinely incubated with all components of the system except enzyme which was added after trichloroacetic acid addition.

Experiments in vivo. Mice were injected i.p. with chlorpromazine (2·5, 10, 25, or 100 mg/kg) or an equal volume of saline, and decapitated after 1 hr. Livers were excised and chilled in 0·05 M Tris. HCl buffer, pH 8·1, at 0°, weighed separately, and homogenized in the same buffer in the Potter-Elvehjem tissue grinder (3:1, v/w). Supernatants, free from nuclear and mitochondrial material, were obtained by centrifugation of the whole homogenate for 20 min at 25,000 *g* in the refrigerated Servall at 4°. Both the supernatant preparations and whole homogenates were assayed for their ability to catalyze the methylation of nicotinamide.

Intracellular localization of the nicotinamide-methylating enzyme of rat liver. Rat liver rather than mouse liver was chosen for the intracellular localization of the nicotinamide-methylating enzyme because of the higher methylating activity of the rat liver whole homogenate and the 25,000 *g* supernatant fluid. Cell fractionation was accomplished by differential centrifugation at 4°.[11] The major portion of nicotinamide-methylating activity was found in the 100,000 *g* supernatant fluid (Table 1).

TABLE 1. INTRACELLULAR LOCALIZATION OF THE NICOTINAMIDE-METHYLATING ENZYME OF RAT LIVER

Cell fraction	Protein	Formation of N^1-methylnicotinamide	
		Enzyme activity	Total enzyme activity of fraction
	(g)	(mmole/kg protein/hr)	(mmole/hr)
I. Whole homogenate	1·59	4·4	7·0
II. Mitochondria	0·25	7·8	1·9
III. Supernatant fluid (25,000 *g*)	0·94	11·5	10·8
Microsomes	0·16	6·7	1·0
Supernatant fluid (100,000 *g*)	0·78	12·6	9·7

Reaction mixtures contained $1{\cdot}5 \times 10^{-1}$ M nicotinamide, $6{\cdot}7 \times 10^{-3}$ M S-adenosylmethionine, enzyme (0·5 mg protein), 5×10^{-3} M Tris·HCl buffer, pH 8·1.

Partial purification of the nicotinamide-methylating enzyme of rat liver. The procedure is described below and the data are summarized in Table 2.

Protamine sulfate. Three adult male rats were decapitated and livers were excised and cooled at 0° in Tris, 0·05 M, pH 8·1. Excess moisture was removed by blotting on filter paper, and livers were weighed and homogenized in buffer (660 ml Tris to 220 g liver). The homogenate was spun 1 hr at 100,000 *g*. The supernatant fluid from this centrifugation was decanted and adjusted to pH 5·7 with acetic acid (8·5 N). The precipitate which formed was removed by centrifuging for 30 min at 25,000 *g*. Protamine sulfate (1%) was adjusted to pH 6·5 with NaOH and added by drops to the supernatant fluid (3 mg protamine/mg protein) while stirring continuously. The

precipitate that formed was discarded. The enzyme activity of the supernatant liquid increased twofold (B, Table 2).

TABLE 2. PARTIAL PURIFICATION OF THE NICOTINAMIDE-METHYLATING ENZYME OF RAT LIVER

Fraction	Enzyme activity (N^1-methylnicotinamide formed) (mmole/kg protein/hr)	Purification	Recovery (%)
A. Supernatant fluid (100,000 *g*)	3·1		100
B. Protamine sulfate	6·9	2·2	73
C. Ammonium sulfate	47·0	15·2	54
D. Calcium phosphate gel supernatant fluid	77·5	25·0	25
E. Extraction of gel ppt with 0·05 M KH_2Po_4 pH 6·6	44·5	14·2	19

Ammonium sulfate fractionation. A saturated solution of ammonium sulfate was adjusted to pH 7·4 with NH_4OH. The supernatant fluid (B) from the protamine sulfate step was brought to 60% of saturation with respect to ammonium sulfate (0°) and stirred for 30 min. The precipitate that formed was removed by centrifugation and dissolved in 0·1 M sodium acetate at pH 5 (C, Table 2).

Calcium phosphate gel. Gel[12] was added to the sodium acetate fraction (0·5 mg gel dry weight/mg protein) and stirred for 45 min. The gel was removed by centrifugation, discarded, and a second gel addition was made to the supernatant fluid (3 mg gel/mg protein). After centrifugation, the supernatant liquid (D, Table 2) contained the major portion of methylating activity. Additional enzyme could be recovered by extracting the gel precipitate with 0·05 M KH_2PO_4 buffer, pH 6·6 (E, Table 2). Overall purification by these procedures was about 25-fold for fraction D and 14-fold for E. These preparations lost little enzyme activity when stored at −20° for 3 months, despite repeated freezing and thawing.

Identification of N^1-methylnicotinamide as the product of the reaction. Reaction mixtures contained $1·4 \times 10^{-2}$ M nicotinamide-7-C^{14} (0·44 μc), $7·1 \times 10^{-3}$ M S-adenosylmethionine, rat enzyme (118 μg D protein, Table 2), 0·2 M Tris·HCl, pH 7·6. In addition, chlorpromazine (1×10^{-3} M) was added to some reaction mixtures. At the end of the incubation period, the reaction was stopped by heating at 80° for 15 min, and the protein was removed by centrifugation. Aliquots of the supernatants were assayed chemically for N^1-methylnicotinamide and also chromatographed on Whatman no. 1 paper with 80% acetone as the solvent in an ascending system. Vertical strips 1 inch wide and the length of the chromatography paper were monitored for radioactivity.[5] Chlorpromazine (R_f 0·96) and S-adenosylmethionine (R_f 0, at origin) were detected by their absorption of ultraviolet light. The N^1-methylnicotinamide (R_f 0·37) area fluoresced bluish-white when treated with 1 M NaCN and subsequently exposed to methyl ethyl ketone:NH_4OH vapors.[13] Nicotinamide (R_f 0·78) and S-adenosylmethionine did not react with cyanide under these conditions, whereas chlorpromazine fluoresced slightly. Only the nicotinamide area formed a yellow spot when exposed to cyanogen bromide; it turned black when sprayed with benzidine and orange with *p*-aminobenzoic acid.[14]

RESULTS

I. Inhibition of enzyme activity

The catalysis in vitro *of the methylation of nicotinamide by the livers of mice given chlorpromazine.* Four groups of eight mice each were injected i.p. with chlorpromazine (10, 25, 100 mg/kg) or saline, deprived of food and water for 1 hr, and decapitated. The liver supernatant fluids obtained from centrifugation of each of the whole homogenates of mouse liver at 25,000 *g* were assayed *in vitro* for their ability to catalyze the methylation of nicotinamide as described in Table 3 and Methods. The methylation of nicotinamide was inhibited 32% to 48% when liver supernatant preparations from chlorpromazine-treated mice (Table 3) were used as the enzyme source. In other experiments not reported, the administration of chlorpromazine *in vivo* also inhibited the nicotinamide-methylating activity of whole liver homogenate.

TABLE 3. FORMATION OF N^1-METHYLNICOTINAMIDE *IN VITRO* BY LIVER PREPARATIONS OF MICE GIVEN CHLORPROMAZINE

Treatment	Enzyme activity (mmole/kg protein/hr)†	Enzyme inhibition %	P value*
Saline	2·5 ± 0·41		
Chlorpromazine			
10 mg/kg	1·7 ± 0·30	32	<0·01
25 mg/kg	1·6 ± 0·42	36	<0·05
100 mg/kg	1·4 ± 0·29	48	<0·01

The liver of each mouse (8 mice per group) was assayed *in vitro* 1 hr after the i.p. injection of chlorpromazine or saline. The reaction mixture contained 1×10^{-2} M nicotinamide, 4×10^{-3} M S-adenosylmethionine, mouse liver enzyme (3 to 4 mg protein of Fraction III, Table 1), 1×10^{-2} M Tris·HCl buffer, pH 8·1.
* The mean of each chlorpromazine-treated group is compared with the mean of the saline-treated group by the Student's *t* test.
† Mean ± standard deviation.

Effect of chlorpromazine in vitro. Inhibition of nicotinamide methylation was observed *in vitro* by the addition of chlorpromazine to incubation mixtures containing the partially purified enzyme from rat liver (D, Table 2). The inhibition of nicotinamide methylation by chlorpromazine at pH 6 and pH 8 is illustrated by the Michaelis constants for nicotinamide and S-adenosylmethionine. These data are plotted according to Lineweaver and Burk (Figs. 1–4). The apparent competitive nature of the inhibition is shown by the change of the slopes in the presence of chlorpromazine and by the values for the K_m estimated from these plots (Table 4).

TABLE 4. MICHAELIS CONSTANTS FOR NICOTINAMIDE AND S-ADENOSYLMETHIONINE

Substrate	K_m at pH 6 (M)	K_m at pH 8 (M)
Nicotinamide	1×10^{-5}	1×10^{-4}
Nicotinamide with chlorpromazine (1×10^{-3} M)	50×10^{-5}	30×10^{-4}
S-Adenosylmethionine	6×10^{-6}	4×10^{-5}
S-Adenosylmethionine with chlorpromazine (1×10^{-3} M)	25×10^{-8}	8×10^{-5}

Details of incubation procedure are presented in Figs. 1–3.

Requirements for the methylation of nicotinamide in vitro. The reagents required for the nicotinamide-methylating system include nicotinamide, S-adenosylmethionine, and enzyme.

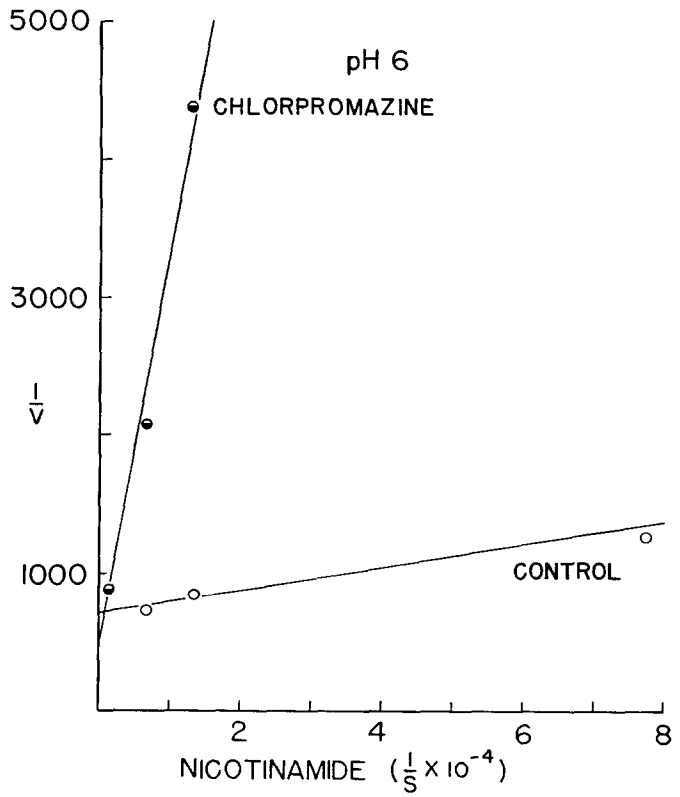

FIG. 1. Effect of nicotinamide concentration on reaction velocity at pH 6 in the presence and absence of chlorpromazine. Incubations contained 6×10^{-5} M to $1{\cdot}2 \times 10^{-2}$ M nicotinamide, $1{\cdot}2 \times 10^{-3}$ M S-adenosylmethionine, rat liver enzyme (23 μg D, Table 2), $1{\cdot}2 \times 10^{-2}$ M sodium acetate buffer (pH 6), and $1{\cdot}2 \times 10^{-3}$ M chlorpromazine where indicated. The reaction velocity in the absence of chlorpromazine is also shown (control).

Identification of N^1-methylnicotinamide as product of the reaction. Incubations containing nicotinamide-7-^{14}C as substrate were assayed chemically for N^1-methylnicotinamide and also chromatographically. Chemical assay of incubation supernatants according to the method described indicated the formation of N^1-methylnicotinamide. Aliquots of these supernatants were also chromatographed, and sharp radioactive peaks appeared at positions corresponding to the R_f of nicotinamide and N^1-methylnicotinamide. No other radioactive peaks resulted from addition of chlorpromazine to the incubation. Radioactivity appeared only at the R_f of nicotinamide in the absence of incubation or omission of either S-adenosylmethionine or enzyme from the reaction mixture. These results provide evidence for the enzymatic formation of

N^1-methylnicotinamide *in vitro*. It also indicates that no unknown complex or product is formed in the presence of chlorpromazine which could react in the chemical assay for N^1-methylnicotinamide.

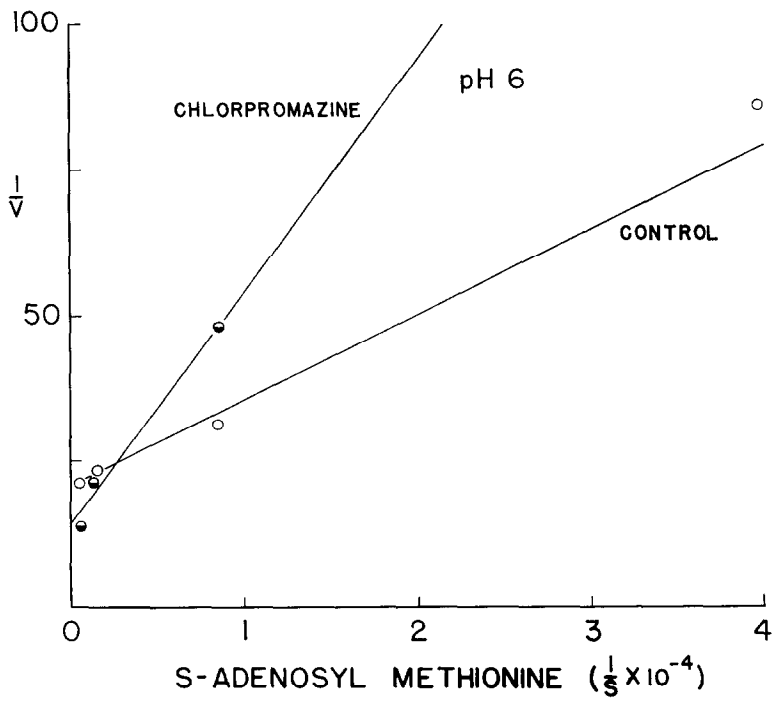

FIG. 2. Effect of S-adenosylmethionine concentration on reaction velocity at pH 6 in the presence and absence of chlorpromazine. Incubations contained 6×10^{-6} M to $1{\cdot}2 \times 10^{-3}$ M S-adenosylmethionine, 6×10^{-3} M nicotinamide, rat liver enzyme (50 μg D, Table 2), $1{\cdot}2 \times 10^{-2}$ M sodium acetate buffer (pH 6), and $1{\cdot}2 \times 10^{-3}$ M chlorpromazine where indicated.

pH optimum. The maximal rate of nicotinamide methylation occurred at about pH 8·5 (Fig. 5, curve B) with either 25,000 *g* mouse or rat liver supernatant fluid, or the partially pure rat liver enzyme (D, Table 2). The pH optimum was altered only slightly by the addition of chlorpromazine to the reaction mixture (Fig. 5, curve A). This figure also shows that under conditions of high nicotinamide concentration ($7{\cdot}6 \times 10^{-2}$ M), chlorpromazine increases enzyme activity at all pH values. A description of the stimulatory effect of chlorpromazine on nicotinamide methylation in the presence of high substrate concentration is presented below. The pH measurement s were made at room temperature immediately before and after incubation.

II. Increase of enzyme activity

Effect of chlorpromazine on the methylation of nicotinamide in vitro. As described above, the addition *in vitro* of chlorpromazine inhibited nicotinamide methylation over a wide range of substrate concentrations at pH 6 and pH 8. However, under

conditions of high substrate concentration, chlorpromazine *increased* rather than decreased the rate of formation of N^1-methylnicotinamide. This can be seen by careful examination of the Lineweaver-Burk plots in the region of high substrate concentrations (Figs. 1–4) and the pH activity curve (Fig. 5, curve A). For example, addition of $1{\cdot}3 \times 10^{-2}$ M chlorpromazine to a reaction mixture containing $1{\cdot}3 \times 10^{-2}$ M nicotinamide and 4×10^{-3} M S-adenosylmethionine increased the rate of formation of N^1-methylnicotinamide from 10 to 25 mmoles/kg protein per hr. With 1×10^{-2} M nicotinamide, a stimulation of enzyme activity was observed when the concentration of chlorpromazine ranged from $1{\cdot}25 \times 10^{-3}$ M to $1{\cdot}25 \times 10^{-2}$ M. However, this effect did not occur with $1{\cdot}25 \times 10^{-5}$ M chlorpromazine (Table 5). As shown in Fig. 5 (curve A), an increase in enzyme activity caused by chlorpromazine occurred throughout the pH range tested.

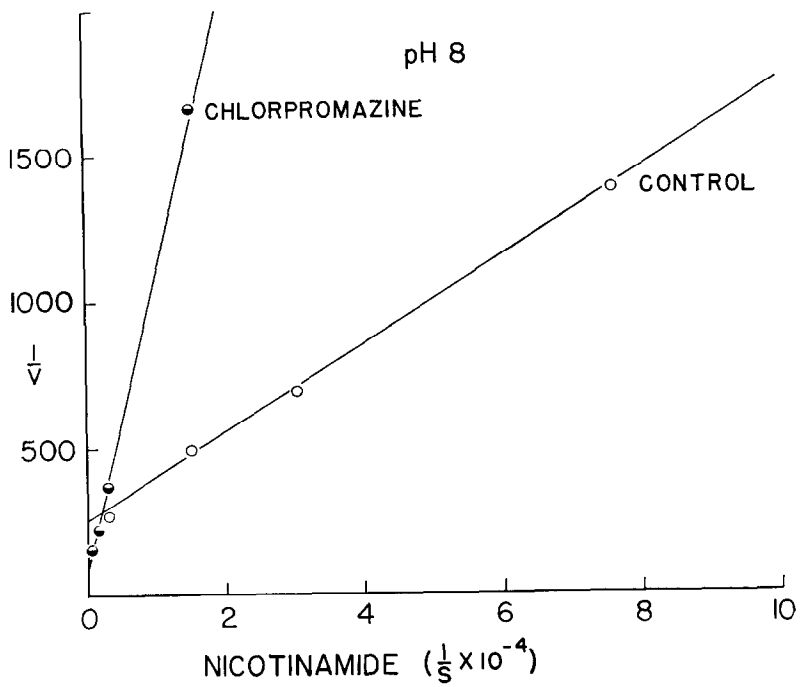

FIG. 3. Effect of nicotinamide concentration on reaction velocity at pH 8 in the presence and absence of chlorpromazine. Incubations contained $1{\cdot}3 \times 10^{-5}$ M to $1{\cdot}3 \times 10^{-2}$ M nicotinamide, $1{\cdot}3 \times 10^{-3}$ M S-adenosylmethionine, rat liver enzyme (50 μg D, Table 2), $7{\cdot}5 \times 10^{-2}$ M Tris·HCl buffer (pH 8), and $1{\cdot}2 \times 10^{-3}$ M chlorpromazine where indicated.

Protection of the enzyme by chlorpromazine during incubation. A loss of methylating activity occurred if enzyme and buffer were preincubated 30 min at 38° prior to the addition of the remaining components of the reaction mixture (Table 5). This loss of activity was prevented by addition to the preincubation mixture of S-adenosylmethionine ($6{\cdot}7 \times 10^{-3}$ M) or chlorpromazine ($1{\cdot}25 \times 10^{-5}$ M). Under these conditions, the enzyme was equally protected by both reagents. Preincubation with both S-adenosylmethionine and chlorpromazine present gave only slightly higher activity.

TABLE 5. EFFECT OF PREINCUBATION WITH S-ADENOSYLMETHIONINE AND CHLORPROMAZINE ON THE NICOTINAMIDE METHYLATING ENZYME

Component preincubated with enzyme*	Enzyme activity (mmole/kg protein/hr)	Protection of enzyme during preincubation (%)
None	4·6	0
Nicotinamide	5·0	17
S-Adenosylmethionine	6·8	95
Chlorpromazine, $1·25 \times 10^{-5}$ M	6·9	100
Chlorpromazine, $1·25 \times 10^{-5}$ M, and S-Adenosylmethionine	7·3	117
Chlorpromazine, $1·25 \times 10^{-3}$ M	19·9†	
Chlorpromazine, $1·25 \times 10^{-3}$ M, and S-adenosylmethionine	21·5†	
Control (no preincubation‡)	6·9	

* The enzyme was preincubated in buffer 30 min at 38° with component indicated. Missing components were then added, and the complete reaction mixture was reincubated 2 hr at 38°. The complete reaction mixture contained rat liver enzyme (0·3 mg protein, III, Table 1), $1·3 \times 10^{-2}$ M nicotinamide, $6·7 \times 10^{-3}$ M S-adenosylmethionine, and mixed buffer (pH 8). Chlorpromazine was added as indicated. The enzyme activity obtained in absence of preincubation was 6·9 mmoles/kg protein per hr.

† The stimulatory effect of this concentration of chlorpromazine on enzyme activity is significantly higher than that due to protection of the enzyme by $1·25 \times 10^{-3}$ M chlorpromazine during the preincubation period.

‡ The complete reaction mixture was incubated 2 hr at 38° without any preincubation. Enzyme activity increased from 6·9 to 21·5 mmoles/kg protein per hr with addition of $1·25 \times 10^{-3}$ M chlorpromazine to the reaction mixture.

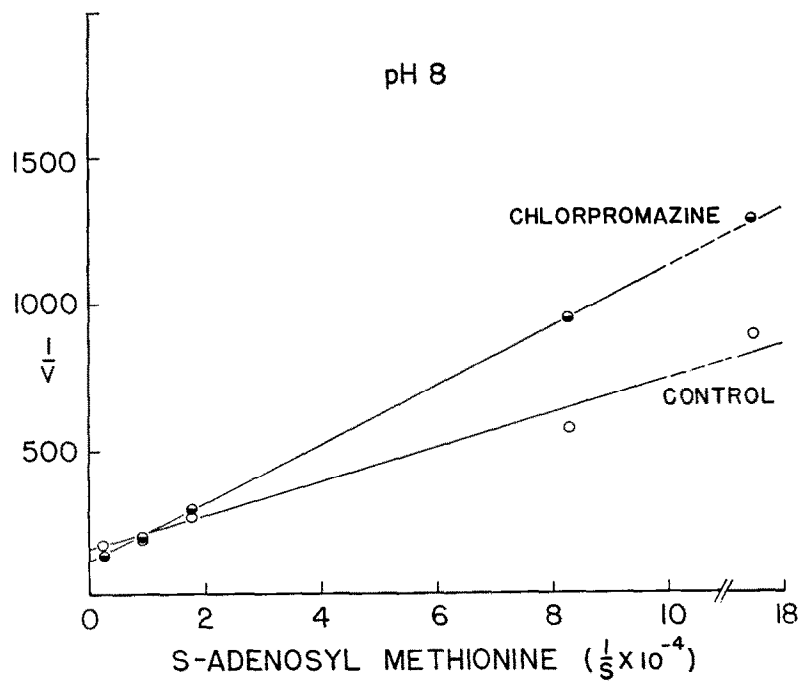

FIG. 4. Effect of S-adenosylmethionine concentration on reaction velocity at pH 8 in the presence and absence of chlorpromazine. Incubations contained $1·3 \times 10^{-6}$ M to $1·3 \times 10^{-3}$ M S-adenosylmethionine, 6×10^{-3} M nicotinamide, rat liver enzyme (50 μg D, Table 2), $7·5 \times 10^{-2}$ M Tris·HCl buffer (pH 8), and $1·2 \times 10^{-3}$ M chlorpromazine where indicated.

Preincubation of the enzyme with a concentration of chlorpromazine ($1{\cdot}25 \times 10^{-3}$ M) known to stimulate enzyme activity resulted in a threefold increase in specific activity, whereas the addition of $1{\cdot}25 \times 10^{-5}$ M chlorpromazine prevented the decrease in enzyme activity caused by the preincubation but did not stimulate the activity of the methylating system. Nicotinamide levels as high as 1×10^{-2} M had slight effect in preventing the loss of enzyme activity during the preincubation period.

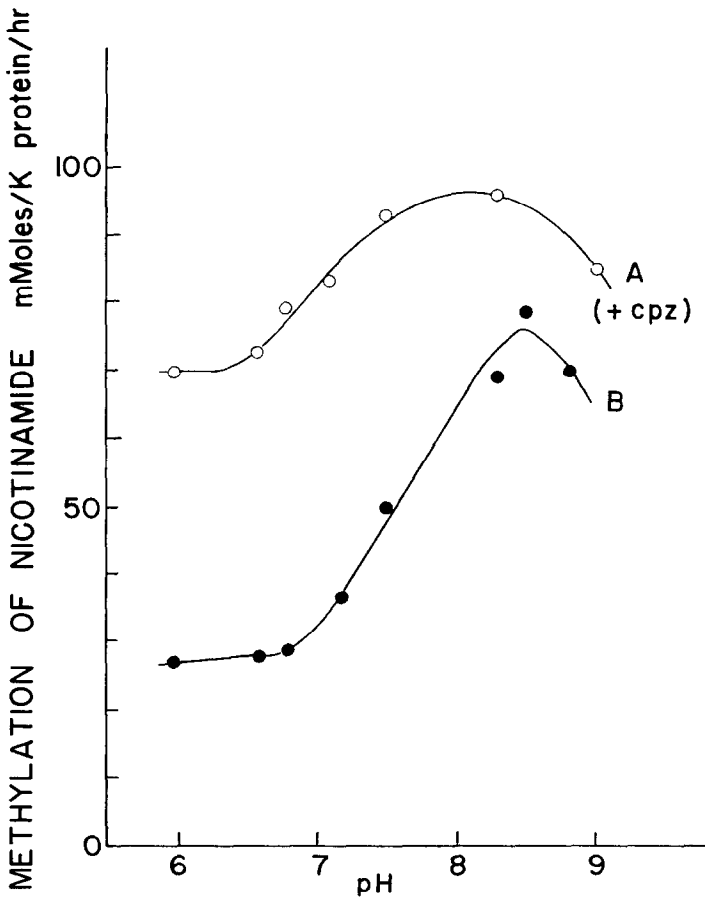

FIG. 5. pH Optimum of nicotinamide methylpherase. Incubations contained $7{\cdot}6 \times 10^{-2}$ M nicotinamide, $7{\cdot}1 \times 10^{-3}$ M S-adenosylmethionine, 4×10^{-2} M mixed buffer (0·1 M KH_2PO_4, 0·1 M Tris and acetic acid), rat liver enzyme (50 μg D, Table 2), and $1{\cdot}5 \times 10^{-3}$ M chlropromazine where indicated (curve A).

Time course of nicotinamide methylation. Rat liver enzyme preparations (25,000 *g* supernatant fluid) were assayed for their ability to catalyze the formation of N^1-methylnicotinamide during a 4-hour incubation period in the presence and absence of chlorpromazine (1×10^{-3} M). A high concentration of nicotinamide (1×10^{-2} M) was added to the reaction mixture. Enzyme activity began to fall after 2 hr and was markedly reduced at the end of the incubation period (Fig. 6). However, both the initial and subsequent rates of the enzyme reaction in the presence of chlorpromazine were at least twice those seen in its absence.

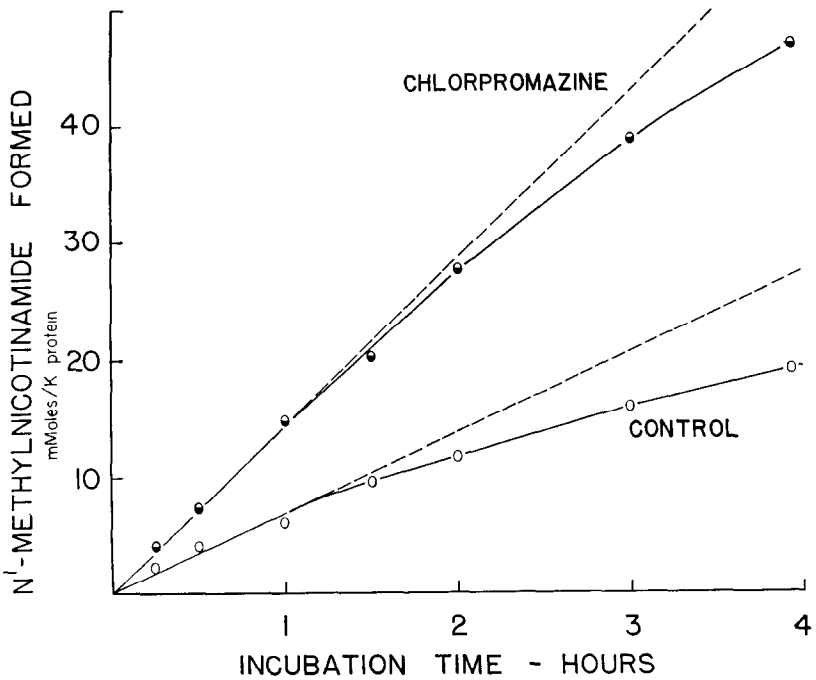

FIG. 6. Time course of nicotinamide methylation. Reaction mixtures contained $1{\cdot}3 \times 10^{-2}$ M nicotinamide, $7{\cdot}1 \times 10^{-3}$ M S-adenosylmethionine, rat liver enzyme (1·4 mg of 25,000 *g* supernatant fluid) 0·2 M Tris (pH 8). Chlorpromazine (1×10^{-3} M) was added to one reaction mixture. N^1-Methylnicotinamide was determined on aliquots of reaction mixture taken at the indicated times.

DISCUSSION

Administration of nicotinamide to the mouse results in large increases in the NAD content of a number of tissues.[1] Chlorpromazine given prior to nicotinamide prolongs the period that tissue NAD concentration remains elevated.[2, 3] These findings suggested the possibility that chlorpromazine interfered in some way with the degradation of nicotinamide or nicotinamide intermediates involved in the formation of NAD, resulting in a high tissue concentration of NAD precursor material persisting for some time. This would be expected to favor a high rate of NAD biosynthesis for a prolonged period.[4, 5] The formation of one of the excretory products of nicotinamide (N^1-methylnicotinamide) was examined therefore, to determine if chlorpromazine interfered with the methylation of nicotinamide. Administration of chlorpromazine to mice was found to inhibit catalysis *in vitro* of the conversion of nicotinamide to N^1-methylnicotinamide in the presence of S-adenosylmethionine by liver enzyme preparations from these animals. Nicotinamide methylation was inhibited 32% to 48% in these experiments.

Inhibition of nicotinamide methylation also occurred when chlorpromazine was added to a reaction mixture containing a partially purified nicotinamide-methylating enzyme from rat liver. The enzyme demonstrated instability when incubated 30 min at 38° in buffer, and this could be prevented by the addition of S-adenosylmethionine or chlorpromazine (1×10^{-5} M). Nicotinamide was unable to prevent this loss of enzyme activity.

The partially pure enzyme preparation was also used to study the effect of substrate concentration on reaction rate in the presence and absence of chlorpromazine. The results of these experiments indicate that at low nicotinamide concentrations there is an inhibition of enzyme activity by chlorpromazine, owing to a reduction of the affinity constant for nicotinamide. The inhibition of the methylating enzyme appears to be competitive. This is shown by a change in the slopes of the Lineweaver-Burk plots and by the increase in the apparent K_m for nicotinamide calculated from these plots.* The K_m for nicotinamide increases 50-fold in the presence of chlorpromazine. However, in the presence of a saturating concentration of nicotinamide (1×10^{-2} M), chlorpromazine increases rather than decreases the maximal velocity of the methylation reaction. It is doubtful that conditions resulting in an increase of enzyme activity are ever attained *in vivo*.

The maximal concentration of chlorpromazine found in rat liver† after a s.c. injection of 100 mg/kg body weight is approximately 3×10^{-3} M and after i.p. injection of 500 mg nicotinamide/kg, the liver concentration of nicotinamide reaches 3 to 4×10^{-3} M.[15] This concentration of nicotinamide would exceed greatly the enzyme saturation level determined *in vitro* in the absence of chlorpromazine (Table 4). Therefore the methylation of nicotinamide would be expected to proceed at maximal velocity. However, in the presence of chlorpromazine, the K_m would be expected to change (Table 4) in such a way that the nicotinamide concentration would be less than half that required to saturate the enzyme. As a result, the methylation of nicotinamide would proceed at a slower rate in chlorpromazine-treated animals. These findings suggest that the administration *in vivo* of chlorpromazine could prolong the period that nicotinamide serves as a precursor for NAD synthesis by inhibiting the nicotinamide-methylating enzymes.

Chlorpromazine and certain other tranquilizers prolong the elevation of liver NAD when given prior to nicotinamide, which suggests that these drugs may exert a common biochemical effect. It has been proposed that while NAD levels may not be directly involved in the tranquilization process, the changes may reflect a basic mechanism of action of these drugs.[4]

* A more detailed discussion of the kinetic data has been presented.[16]

† Smith, Kline and French Co., unpublished observations.

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