CHEMICALLY EVOKED HYPOTHERMIA IN THE MOUSE

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
ANDREW STUART JANOFF
1976

ABSTRACT

CHEMICALLY EVOKED HYPOTHERMIA
IN THE MOUSE

By

Andrew Stuart Janoff

The hypothermic response after parenteral administration of trifluprom azine, promazine, chlorpromazine, L-dopa, reserpine, parachlorophenylalanine, and procaine was investigated in the mouse. Mice injected at 24 hour intervals with trifluprom azine, promazine, or chlorpromazine exhibited tolerance (a diminished response) with repeated administration. The degree of induced hypothermia and resistance to tolerance after chlorpromazine was greater in male mice than in female mice. Repeated injections of trifluprom azine produced less tolerance in 5 week old mice then in adult mice, but this compound failed to induce hypothermia in 5 week old mice after the first injection. Prior administration of trifluprom azine dramatically potentiated the hypothermic effect of promazine.

L-dopa was unable to potentiate chlorpromazine hypothermia but did evoke a nonadapting hypothermic response when administered alone. Reserpine also evoked a nonadapting hypothermia. Further, animals treated with small doses of reserpine exhibited an increasing response with repeated injections. Parachlorophenylalanine induced hypothermy that became intermittent with successive administration. Procaine

did not affect core body temperature at all. The results presented here indicate that of the compounds studied only reserpine and L-dopa would be of value as hypothermic agents in any long term investigation.

CHEMICALLY EVOKED HYPOTHERMIA IN THE MOUSE

By

Andrew Stuart Janoff

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Biophysics

1976

This is for

my Mother

and

my Father

ACKNOWLEDGEMENTS

The author wishes to express sincere and grateful appreciation to Dr. Barnett Rosenberg for his continual encouragement and advice over the course of this work. The author is further indebted to Dr. Gabor Kemeny for many stimulating discussions, to Loretta VanCamp for her valuable suggestions, and to Paula Faber and Steve Wallace for their excellent technical assistance. Deep gratitude is expressed to Julie, whose support and understanding have, in a large way, made this thesis possible.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
Thermodynamics of Ageing and Death	1
Hypothalamic Mechanisms of Thermoregulation	3
Disruption of Thermoregulation by the Systematic Administration of Drugs	6
Phenothiazines	6
Procaine	8
Parachlorophenylalanine	8
Reserpine	9
METHODS	10
General Procedures	10
Phenothiazines	11
Promazine	11
Triflupromazine	11
Chlorpromazine and L-Dopa	11
Determination of cross tolerance between triflupromazine, promazine, and chlorpromazine	12
Procaine	12
Parachlorophenylalanine	12
Reserpine	13

TABLE OF CONTENTS (cont'd.)

Page

RESULTS 14

Phenothiazines 14

Promazine 14

Trifluprom azine 14

Chlorpromazine and L-dopa 17

Determination of cross tolerance between triflupromazine, promazine and chlorpromazine 23

Procaine 26

Parachlorophenylalanine 26

Reserpine 26

DISCUSSION 30

APPENDICES 37

APPENDIX A - Pathways of Monoamine Biosynthesis 37

APPENDIX B - Chemical Structures of the Compounds Investigated 38

LIST OF REFERENCES 40

LIST OF TABLES

Table		Page
1	Mean core body temperature (^{o}C) ± S.E. of mature female mice (n=6) injected i.p. with promazine, 25 mg/kg, at 24 hour intervals for 7 consecutive days	15
2	Mean core body temperature (^{o}C) ± S.E. of mature female mice (n=6) injected i.p. with trifluprom azine, 5 mg/kg, at 24 hr intervals for 6 consecutive days	15
3	Mean core body temperature (^{o}C) ± S.E. of 5 week old female mice (n=6) injected i.p. with trifluprom azine, 5 mg/kg, at 24 hr intervals for 6 consecutive days	15
4	Development of tolerance after i.p. administration of promazine (25 mg/kg) at 24 hour intervals for 7 consecutive days in mature female mice	16
5	Development of tolerance after i.p. administration of trifluprom azine (5 mg/kg) at 24 hour intervals for 6 consecutive days in mature female mice	16
6	Development of tolerance after i.p. administration of trifluprom azine (5 mg/kg) at 24 hour intervals for 6 consecutive days in 5 week old female mice	16
7	Development of tolerance after i.p. administration of chlorprom azine (5 mg/kg) at 24 hour intervals for 10 consecutive days in mature male mice	22
8	Mean core body temperature (^{o}C) ± S.E. of mature female (above) and male mice (below) injected i.p. with procaine at dose levels indicated	22
9	Development of increasing sensitivity in mature female mice after successive i.p. administration of reserpine (.5 mg/kg)	22
10	Mean core body temperature (^{o}C) ± S.E. of mature female mice injected i.p. with reserpine as indicated	28

LIST OF FIGURES

Figure		Page
1	Time vs core body temperature of mature female mice injected with chlorpromazine, chlorpromazine plus L-dopa (above) or L-dopa (below)	18
2	Time vs core body temperature of mature male mice injected with chlorpromazine, chlorpromazine plus L-dopa or L-dopa, and mature female mice injected with chlorpromazine	20
3	Time vs core body temperature of mature female mice injected sequentually with triflupromazine, promazine and chlorpromazine at 24 hour intervals	24
4	Time vs core body temperature of mature female mice injected with promazine 24 hours after prior injection of promazine or triflupromazine	24
5	Time vs core body temperature of mature male and female mice injected with parachlorophenylalanine methyl ester (425 mg/kg) at 24 hour intervals for 6 consecutive days	25
6	Time vs core body temperature of mature female mice injected with reserpine as indicated	27
A1	Biosynthesis of Catecholamines	37
A2	Biosynthesis of 5-hydroxytryptamine	37
B1	Chlorpromazine	38
B2	Promazine	38
B3	Triflupromazine	38
B4	L-dopa	38
B5	Reserpine	39
B6	Procaine	39
B7	Parachlorophenylalanine Methyl Ester	39

INTRODUCTION

Thermodynamics of Ageing and Death

Previous work has suggested that thermodynamics is a valid approach to the study of ageing and death in biological systems (Rosenberg, Kemeny, Switzer, Hamilton, 1971). This approach simplifies the study of ageing in that it avoids a description of complex molecular interactions and poorly understood physiological dysfunctions related to senescence.

Procaryotic organisms, virions and yeast, when exposed to lethal temperatures above their optima for growth tend to die according to pseudo first order kinetics such that:

$$dn/dt = -k_d n \qquad (1)$$

The absolute rate theory equation (Gladstone, Laidler and Eyring, 1941) describes k_d as varying exponentially with temperature so:

$$k_d = \kappa k_B T/h \exp [\Delta S^{\ddagger}/R] \text{ X } \exp [-\Delta H^{\ddagger}/RT] \qquad (2)$$

Rosenberg and Kemeny et al (1971) have shown that data gathered from a large number of investigations involving the thermal killing of unicellular organisms indicates that:

$$\Delta S^{\ddagger} = a\Delta H^{\ddagger} + b \qquad (3)$$

This equation represents a compensation law because changes in

$\Delta S^{\ddagger}$ are partially compensated for by changes in $\Delta H^{\ddagger}$ resulting in a relatively constant value of k_d. At the compensation temperature, $T_c = 1/a = 330^{o}K$, the compensation is exact, $b = -66$ cal/mole^{o}K. The fact that in vitro thermal denaturation of a large number of proteins under different experimental conditions can be described by the above equations (1-3) yielding the same values for a and b in equation 3 ($330^{o}K$, -66 cal/mole^{o}K) has lead Rosenberg and Kemeny et al (1971) to advance the general hypothesis that protein denaturation is the rate limiting step for thermal death in unicellular organisms.

In order to extend a thermodynamic description of ageing and thermal death to multicellular poikilotherms, other methods of analysis are needed due to the nonexponential survivorship curves exhibited by these organisms. Larry Smith (Rosenberg, Kemeny, Smith, Skurnick, Bandurski, 1973) discovered that the square shaped survivorship curves of multicellular organisms are best fit by describing the mortality rate as a power of time such that:

$$\mu(t) = -\frac{1}{N(t)}\frac{dN(t)}{dt} = At^{n} \qquad (4)$$

in its integrated form:

$$\frac{N(t)}{N_o} = e^{-\frac{At^{n+1}}{n+1}} \qquad (5)$$

Therefore by plotting the log log of percent survivors against the log of time, straight lines were obtained with a slope of n + 1 and the extrapolated y intercept yielding the value of A. Survivorship curves of Drosophila melanogaster at different ambient temperatures

showed higher rates of mortality at higher temperatures with A being temperature dependent but n being relatively temperature independent. This suggested that:

$$A = A_o e^{-\Delta H^{\ddagger}/RT} \qquad (6)$$

The Arrhenius plot subsequently obtained showed a good straight line. The slope, $\Delta H^{\ddagger}$ = 190 kcal/mole yielded the activation enthalpy for the mortality rate of Drosophila. The existence of an activation enthalpy for age related death in Drosophila suggests that simple molecular events are involved. Moreover, the high value determined for $\Delta H^{\ddagger}$ suggests that these monomolecular events relate to protein denaturation.

Using the value of $\Delta H^{\ddagger}$ = 190 kcal/mole and equations 4-6 it can be shown theoretically that the temperature dependence of ageing and death in multicellular organisms is such that in humans a decrease of core body temperature of only $2^{o}C$ will extend median survival time from 71.8 years to 100 years (Rosenberg, Kemeny et al, 1973). Any thermodynamic study of ageing in laboratory mammals, however, is obviously dependent on techniques which would allow for the manipulation of the core body temperature of these organisms. Such techniques require an understanding of the neurochemistry of thermoregulation.

Hypothalamic Mechanisms of Thermoregulation

Feldberg and Myers (1964) have suggested that the balance between the release of norepinephrine and serotonin in the anterior hypothalamus of homeotherms is the mechanism whereby body temperature is regulated. Presumably ascending monoaminergic pathways which terminate in the

rostral hypothalamus (Fuxe, 1965; Fuxe, Hokfelt and Ungerstedt, 1970) and carry information from temperature receptors in the skin (Bligh, 1966) deep in the body (Hammel, 1968) and the blood (Hayward and Baker, 1968) synapse with monoaminoceptive cholinergic efferents ultimately involved in the control of thermogenesis. Much evidence has accumulated to indicate that the release of 5-HT in the anterior hypothalamus in mammals acts through a cholinergic pathway to signal heat production, while the release of norepinephrine in this region blocks the cholinergic heat producing pathway (Myers, 1969; Myers and Yaksh, 1969; Myers, 1970; Hall, 1972; Hall and Myers, 1972; Knox and Lomax, 1972; Rudy and Wolf, 1972; Myers, 1974; Chawla, Johri, Saxena, Singhal, 1975; Metcalf, Myers, Redgrave, 1975; Myers, 1975).

A second cholinergic pathway apparently located in the posterior hypothalamus and modulated by the ratio of Na^{+} to Ca^{++} ions in this area functions as a heat loss mechanism (Myers, 1974). Thus the set-point core body temperature for homeotherms is represented physiologically as the Na^{+} to Ca^{++} ratio in the posterior hypothalamus. Increases in the Ca^{++}/Na^{+} ratio in this area depress while decreases elevate the set-point temperature (Myers and Veale, 1970; Veale and Yaksh, 1971; Myers and Veale, 1971; Myers and Yaksh, 1971; Myers and Brophy, 1972; Myers and Buckman, 1972; Myers and Tytell, 1972). Homeotherms, therefore, are able to regulate their core body temperature around an ionically determined set-point by regulating the levels of monoamines released in the anterior hypothalamus.[†] Heat production is generally thought to be associated with cellular oxidation, increased

---

[†]Appendix A details pathways of monoamine biosynthesis.

oxygen consumption and shivering (Prusiner and Poe, 1968) while heat loss is related to peripheral blood flow, body posture and panting (Cremer and Bligh, 1969; Feldberg, 1975).

The importance of this model is that it exhibits distinct species continuity. When injected directly into the anterior hypothalamus 5-HT elevates and norepinephrine lowers the body temperature of the unanesthetized cat (Feldberg and Myers, 1965), monkey (Myers and Yaksh, 1969), rat (Avery, 1971; Crawshaw, 1972), ground squirrel (Beckman and Satinoff, 1972), and dog (Myers, 1975). Systemic administration of 5HTP which is rapidly decarboxylated to form 5-HT in brain tissue results in a hyperthermic response in the rabbit that can be blocked by the serotonin antagonist 2-brom LSD (Horita and Gogerty, 1958), while the blockage of central 5-HT receptors in the rat and mouse results in hypothermia (Grabowska, Michaluk,Antkiewicz, 1973). Further, hyperthermia induced in the rat by systemic administration of drugs is correlated with an increased turnover of 5-HT in the brain (Reid, 1970; Bruinvels and Kemper, 1971) and hypothermia is associated with increased levels of norepinephrine in the hypothalamus (Bruinvels and Kemper, 1971). Finally, peripheral cooling of the monkey elevates the release of 5-HT from the anterior hypothalamus (Myers and Beleslin, 1971) and peripheral warming of the cat evokes the release of norepinephrine from this region (Myers and Chinn, 1973).

While inconsistent effects of biogenic amines on temperature have been reported, these effects are generally obtained in studies not involving the central administration of amines, so that direct peripheral effects cannot be discounted, or in studies involving the

injection into the hypothalamus of a high dose of the amine in a large volume. Myers (1968, 1970) and Avery (1971) have shown that when increasingly high doses or large volumes of a biogenic amine are administered centrally a reversal of the initial effect occurs.

Disruption of Thermoregulation by the Systemic Administration of Drugs

Although it is possible to alter core body temperature in homeotherms by the central administration of biogenic amines, this technique does not seem to be feasible for the kinds of long term studies that would be necessary in the investigation of the temperature dependence of ageing in mammals. Simpler techniques that could nevertheless render homeotherms hypothermic for a part or all of the duration of their lives would be more desirable. The experiments reported here investigate the potential of various drugs to evoke hypothermia in the mouse after systemic administration. The purpose of these investigations was to determine for each of the compounds or combination of compounds tested the existence or nonexistence of a hypothermic response and the development or nondevelopment of a diminished response (tolerance) with repeated trials. The compounds chosen were generally either those previously reported to have produced hypothermia in mammals or those suspected of the capability to interrupt thermoregulation by altering effective hypothalamic concentrations of monoaminergic transmitters. Appendix B shows the chemical structures of the compounds investigated.

Phenothiazines. Chlorpromazine, the prototype phenothiazine, has long been known to produce hypothermia in experimental animals. There is little doubt that its main site of action is on the

hypothalamus (Cremer and Bligh, 1969). Centrally the hypothermia evoked by this compound after systemic administration is correlated with decreased 5-HT turnover (Shore, Pletscher, Tomich, Carlsson, Kuntzman, Brodie, 1957; Cox and Potkonyak, 1967) and increased turnover of norepinephrine and dopamine (Moore, 1971). Peripherally, chlorpromazine induced hypothermia is associated with decreased utilization of calorogenic substrates (Mueller and McDonald, 1968), heat loss due to peripheral vasodilation (Decourt, Brunaud, Brunaud, 1953; Baxter, Bolster, McKecnhie, 1954; Dobkin and Gilbert, 1954; Giaja, J. and Markovic-Giaja, 1954), depressed oxidative processes (Courvoisier, Fournel, Ducrot, Kolsky, Roetschet, 1953) and suppression of shivering (Kollias and Bullard, 1964). Tolerance, however, develops rapidly with respect to the hypothermia induced by this compound upon repeated administration in the mouse (Rosenberg, personal communication).

In an effort to determine whether other phenothiazine congeners also exhibit tolerance related to their ability to evoke hypothermia, the temporal characteristics of promazine and trifluprom azine induced temperature depression were investigated. To determine whether the sequential administration of trifluprom azine, promazine and chlor-promazine would provide a more efficacious method of evoking extended hypothermia than the successive administration of either congener alone, experiments were performed to test the existence of cross tolerance with respect to the hypothermic response evoked by these agents. Finally experiments were designed to determine whether an exogenous source of norepinephrine precursor, L-dopa, would offset the tolerance reaction to chlorpromazine.

Procaine. Systemic administration of procaine leads to a centrally mediated peripheral vasodilation in laboratory animals (Peterson, 1955; 1955a) and possibly humans (Glasgow and Sinclair, 1962; Gordon, Fudema, Snider, Abrams, Tobin, Kraus, 1965; Livingston and Perrin, 1972). Since peripheral vasodilation is generally associated with heat loss this suggests a possible hypothermic effect of procaine.

Aslan (Aslan, Urabiescu, Domilescu, Campeanu, Continiu, Stanescu, (1965) and others (Berger, 1960) have reported that laboratory rodents treated chronically with procaine exhibit an increased survival rate when compared to non-treated controls. Chronic administration of procaine in humans has also been reported to increase longevity (Aslan, 1962). If procaine does in fact have the ability to depress core body temperature this would help to explain these results. Accordingly experiments were performed to test the efficacy of procaine as a hypothermic agent.

Parachlorophenylalanine. Parachlorophenylalanine (PCPA) was chosen for investigation because it inhibits tryptophan hydroxylation (Knapp and Mandell, 1972), and thus reduces brain levels of 5-HT (Preziosi, Scapagnini, Nistico, 1968; Aghajanian, Kohor, and Roth, 1973). PCPA may (Koe and Weissman, 1966; Welch and Welch, 1967; Miller and Maickel, 1969) or may not (McDonald and Mueller, 1969; Voicer, 1969) slightly decrease cerebral norepinephrine levels by inhibiting phenylalanine and tyrosine hydroxylase (Koe and Weissman, 1966). Miller and his co-workers (1969a) have reported a 41 and 22 percent reduction respectively of hypothalamic levels of 5-HT and norepinephrine after intraperitoneal injection of PCPA in the rat.

Since this compound apparently preferentially reduces 5-HT concentrations in the hypothalamus it should theoretically induce a hypothermic response. Experiments were therefore performed to test this hypothesis.

Reserpine. Systemic administration of reserpine in laboratory animals induces a hypothermic response (Shemano and Nickerson, 1958; Fondy, Karker, Calcagnino, Emlich, 1974). Presumably this effect is mediated by the ability of this compound to deplete central monoaminergic stores (Miller, Cox, Snodgrass, Maickel, 1969) especially in the hypothalamus (Shore, Pletscher, Tomich, Carlsson, Kuntzman, Brodie, 1957; Cooper, Cranston, Honour, 1967). The peripheral effects of reserpine which include vasodilation (Nickerson, 1970) and decreased mobilization of calorogenic substrates (Mueller and McDonald, 1968) have also been well described. In view of these reports it was of interest to know the feasibility of using reserpine as a hypothermic agent for an extended period of time. Experiments were therefore conducted to answer this question.

METHODS

General Procedures

The experimental animal used in these studies was the Swiss Webster mouse, male and female, obtained from Spartan Research Animals, Inc., Haslett, Michigan. The animals were marked for individual identification and unless otherwise noted housed six to a cage and kept at ambient temperature (22°C). Food and water were available *ad lib*. To minimize circadian rhythms all experiments were conducted under conditions of 24 hour light. The experimental compounds were administered either intraperitoneally (i.p.) or subcutaneously (s.c.) in .85% saline, sterile double distilled water, or other suitable diluents. The dose schedule was dependent on the properties of the individual compounds being tested. Dose levels chosen were those that in previous trials were shown to produce maximum effects on body temperature with minimum side effects such as sedation, torpor, and extrapyramidal involvement. Core body temperature was determined prior to injection and thereafter monitored at regular intervals with a Yellow Springs Instruments telethermometer and a Fisher Scientific small animal probe. The probe was inserted rectally at a distance of 2.5 cm and allowed to equilibrate before temperature was recorded. Each cohort of animals served as its own control. Data were analyzed by analysis of variance (completely random design), and the Student-Newman-Keuls test for unequal sample size (Sokal and

Rohlf, 1969). The level of significance was chosen as $P<.05$.

Phenothiazines

Promazine. The experimental population consisted of six mature females. Every animal received promazine intraperitoneally at 25 mg/kg in .5 ml volumes carried in .85% saline. Injections were made at regular 24 hour intervals. Core body temperatures were determined at 30 minute intervals for three hours following the injections. The experiment ran for seven days.

Trifluprομazine. The experimental population consisted of six mature females and six 5-week old females. Each animal received trifluprομazine intraperitoneally at 5 mg/kg in .5 ml volumes carried in .85% saline. Injections were made at regular 24 hour intervals. Core body temperatures were determined at 30 minute intervals for three hours following injections. The experiment ran for six days.

Chlorpromazine and L-Dopa. The experimental population consisted of 18 mature females divided into three cages of six animals. The first group received L-dopa subcutaneously in .2 ml volumes as a slurry in .85% saline at 266 mg/kg three times a day at regular eight hour intervals. The second cage received L-dopa via the same route and dosage schedule but also was treated at regular 24 hour intervals with intraperitoneal injections of chlorpromazine in .85% saline, .5 ml volumes at 5 mg/kg. The remaining group received chlorpromazine alone via the same dosage schedule as above. In all cases the administration of chlorpromazine coincided with the administration of L-dopa. Core body temperature was determined in all groups after chlorpromazine administration at 30 minute intervals for 180 minutes. The experiment ran three days. To investigate sex dependent response

differences to chlorpromazine, L-dopa, and the combination, the entire experiment was repeated using male animals. In this case, due to an amplified response, core body temperature was followed at 30 minute intervals for 240 minutes after chlorpromazine. This experiment ran 10 days.

Determination of cross tolerance between trifluprομazine, promazine and chlorpromazine. Trifluprομazine, promazine and chlorpromazine were sequentially administered in effective initial doses (5 mg/kg, 25 mg/kg, and 5 mg/kg respectively) at 24 hour intervals. The experimental population consisted of six mature females. All compounds were carried in .85% saline and injected intraperitoneally in .5 ml volumes. Core body temperatures were recorded at 30 minute intervals for two hours following the injections. The experiment ran three days.

Procaine

The experimental population consisted of six males and six females. The animals were caged individually to avoid any masking of a hypothermic response because of huddling behavior. Half the males received intraperitoneal injections of procaine HCl in .5 ml volumes at 8 mg/kg. The other half received twice this dose via the same route. The same dose schedule was followed for the female population. The procaine HCl was carried in .85 saline. Core body temperature was determined at 30 minute intervals for two hours after the injection.

Parachlorophenylalanine

The experimental population consisted of six mature males and

six mature females. Every animal received parachlorophenylalanine methyl ester intraperitoneally in .15 ml volumes carried in .85% saline, 425 mg/kg at regular 24 hour intervals. The methyl ester was used due to the insolubility of the parent compound in any suitable diluent. Core body temperature was monitored at 30 minute intervals for three hours after injections. The experiment ran six days.

Reserpine

The experimental population consisted of three groups of three mature females, individually housed to avoid huddling, and kept in a constant temperature cabinet at 28.0°C and 45-50% humidity. (Earlier trials had shown that ambient temperatures lower than 28.0°C caused reserpinized animals to experience deep hypothermic reactions which were apparently lethal.) The three experimental groups received reserpine at 0.5 mg/kg, 1.0 mg/kg, and 2 mg/kg, respectively. The reserpine was obtained in a presolubilized form as a commercial preparation (Serpasil, Ciba), diluted to the correct dosage with sterile double distilled water and injected intraperitoneally in .2 ml volumes. The injections were made at intervals of no less than two days and no greater than six days. Core body temperature was determined at regular intervals. To alleviate the anorexia sometimes associated with the administration of this compound, a slurry of powdered mouse pellets and water was made available *ad lib*. The experiment ran for 32 days.

RESULTS

Phenothiazines

Promazine. Promazine evoked a significant hypothermia on each of the seven days it was administered to mature female mice (Table 1). In every case hypothermia appeared within 30 minutes after injection and was still evident at 180 minutes. Normothermia was always restored within 24 hours. Except on days 2 and 5, the return to normothermia began within 180 minutes after injection. Tolerance appeared after three days with respect to the hypothermic response exhibited at 90 minutes following promazine, and after two days with respect to the hypothermic response exhibited at 180 minutes. Table 4 shows the further development of tolerance.

Trifluprom azine. The hypothermic response induced by trifluprom azine was investigated in both mature and 5 week old female mice. In mature mice trifluprom azine evoked a significant hypothermia on each of the six consecutive days it was administered (Table 2). On days 2-6 hypothermia appeared at 30 minutes following injection; on day 1 hypothermia did not appear until 60 minutes after injection. In all cases, however, hypothermia was still evident at 180 minutes and no return towards normothermia was begun over this interval. Normothermia was, nevertheless, always restored within 24 hours after injection. Tolerance appeared after one day with respect to the hypothermic response exhibited at both 90 and 180 minutes following

Time (Min)	0	30	60	90	120	150	180
Day 1	39.17±.11	36.25±.28	35.33±.11	34.67±.25	34.33±.21[b]	35.17±.49	35.75±.50
Day 2	39.00±.13	36.08±.08	35.08±.15[a]	35.42±.44	35.83±.54	35.83±.46	36.58±.54
Day 3	39.33±.11	36.33±.11	35.42±.30[b]	35.58±.45	36.67±.38	37.00±.45	37.50±.45
Day 4	39.08±.15	36.50±.26	36.25±.44	36.33±.48	36.17±.42[b]	37.25±.42	37.83±.21
Day 5	39.25±.11	36.75±.28	36.16±.28[a]	36.33±.40	36.50±.48	37.17±.38	37.50±.34
Day 6	39.25±.17	36.83±.21[b]	36.92±.33	37.08±.30	37.83±.30	38.33±.17	38.42±.15
Day 7	39.50±0.00	37.00±.26	36.83±.31[b]	36.91±.30	37.50±.31	37.58±.35	38.16±.17

Table 1.* Mean core body temperature (°C) ±S.E. of mature female mice (n=6) injected i.p. with promazine, 25 mg/kg, at 24 hr intervals for 7 consecutive days.

Time (Min)	0	30	60	90	120	150	180
Day 1	39.50±0.00	(37.92±.40)	36.33±.48	35.00±.74	34.00±.82	34.08±.88	33.75±1.23
Day 2	39.33±.11	38.17±.17	37.42±.33	37.17±.28	37.67±.38	37.08±.40[a]	37.25±.44
Day 3	39.50±0.00	38.08±.15	36.92±.24	36.42±.43[a]	36.67±.48	36.67±.56	36.83±.65
Day 4	39.41±.08	37.67±.17	37.08±.55	36.92±.49	36.16±.54[a]	36.83±.71	36.92±.71
Day 5	39.58±.08	38.00±.22	37.00±.47	37.00±.53	36.67±.65[a]	36.75±.74	36.92±.72
Day 6	39.50±0.00	37.75±.25	37.00±.26[a]	37.00±.32	37.83±.20	37.33±.33	37.25±.31

Table 2.* Mean core body temperature (°C) ±S.E. of mature female mice (n=6) injected i.p. with trifluprομazine, 5 mg/kg, at 24 hr intervals for 6 consecutive days.

Time (Min)	0	30	60	90	120	150	180
Day 1	38.75±.11	(36.92±.51)	(35.42±.72)	(34.92±.87)	(35.67±1.95)	(35.00±1.37)	(35.42±1.29)
Day 2	38.58±.15	37.00±.18	35.66±.44	35.25±.46[a]	35.50±.51	35.83±.51	36.58±.64
Day 3	38.58±.15	37.17±.24	36.50±.25[b]	36.50±.44	36.67±.35	37.41±.24	37.75±.21
Day 4	39.33±.11[c]	37.00±.26	36.83±.28[b]	36.83±.31	37.67±.21	37.58±.08	37.73±.17
Day 5	39.08±.20	37.17±.17	36.67±.17[a]	37.25±.30	37.83±.43	37.50±.18	37.67±.21
Day 6	39.50±0.00[c]	37.42±.15	37.50±.18	37.50±.37	37.17±.30[a]	38.08±.30	38.08±.30

Table 3.* Mean core body temperature (°C) ±S.E. of 5 week old female mice (n=6) injected i.p. with trifluprομazine, 5 mg/kg, at 24 hr intervals for 6 consecutive days.

---

*The dotted line indicates injection. Parentheses enclose mean core body temperatures not different from the intratrial preinjection value (P>.05). The deepest hypothermia reached after each injection (underlined) was compared to the intratrial mean core body temperature at 180 min. This provided a method of determining whether a return towards normothermia began within the daily experimental interval. The comparison of preinjection temperatures between trials determined whether normothermia was restored prior to each injection. [a]Indicates not different from intratrial value at 180 min (P>.05). [b]Indicates different from intratrial value at 180 min (P<.05). [c]Indicates different from preinjection value on day 1 (P<.05).

Day:	1	2	3	4	5	6	7
°C:	34.67±.25[a]	35.42±.44	35.58±.45	36.33±.48	36.33±.40	37.08±.30	36.91±.30
Day:	1	2	3	4	5	6	7
°C:	35.57±.50[b]	36.58±.54	37.50±.45	37.83±.21	37.50±.34	38.42±.15	38.16±.17

Table 4.* Development of tolerance after i.p. administration of promazine (25 mg/kg) at 24 hour intervals for 7 consecutive days in mature female mice.

Day:	1	2	3	4	5	6
°C:	35.00±.74[a]	37.17±.28	36.42±.43	36.92±.49	37.00±.53	37.00±.32
Day:	1	2	3	4	5	6
°C:	33.75±1.23[b]	37.25±.44	36.83±.65	36.92±.49	36.92±.72	37.25±.31

Table 5.* Development of tolerance after i.p. administration of triflupromazine (5 mg/kg) at 24 hour intervals for 6 consecutive days in mature female mice.

Day:	2	3	4	5	6
°C:	35.25±.46[a]	36.50±.44	36.83±.30	37.25±.30	37.50±.37
Day:	2	3	4	5	6
°C:	36.58±.64[b]	37.75±.21	37.75±.17	37.67±.21	38.08±.30

Table 6.* Development of tolerance after i.p. administration of triflupromazine (5 mg/kg) at 24 hour intervals for 6 consecutive days in 5 week old female mice. Hypothermia did not develop at any time after injection on day 1.

*Days underlined by the same line were not different with respect to the hypothermic response exhibited at 90 minutes (above) or 180 minutes after injection (below). P>.05, n=6.
[a]Indicates core body temperature at 90 minutes after injection. [b]Indicates core body temperature at 180 minutes after injection.

trifluprομazine. Further tolerance did not develop over the course of the remaining trials (Table 5).

Administration of trifluprομazine to 5 week old mice for six consecutive days evoked a significant hypothermia on days 2-6. The hypothermia appeared at 30 minutes after injection and was still evident at 180 minutes. Hypothermia did not develop at any time after injection on day 1 (Table 3). While a return to normothermia was not evidenced within 180 minutes following the administration of trifluprομazine on days 2, 5, and 6, in no case was hypothermia still apparent 24 hours after injection. Tolerance appeared after four days with respect to the hypothermic response exhibited at 90 minutes following trifluprομazine, but further tolerance did not occur. Tolerance never developed with respect to the hypothermic response at 180 minutes following trifluprομazine over the course of this experiment (Table 6).

Chlorprομazine and L-dopa. The hypothermic response evoked by chlroprομazine, L-dopa, and the combination was investigated in both mature male and female mice. In female mice chlorprομazine induced significant hypothermia on each of the three consecutive days it was administered. Hypothermia appeared 60 minutes after injection on day 1, and 30 minutes after injection on days 2 and 3 (Figure 1). In all cases hypothermia was still evident at 180 minutes and no significant return to normothermia was initiated over this interval. Normothermia was, however, always restored within 24 hours. Tolerance developed after one day with respect to the hypothermic response at 90 and 180 minutes following chlorprομazine. Core body temperature at 90 and 180 minutes on day 2 was significantly different than core body

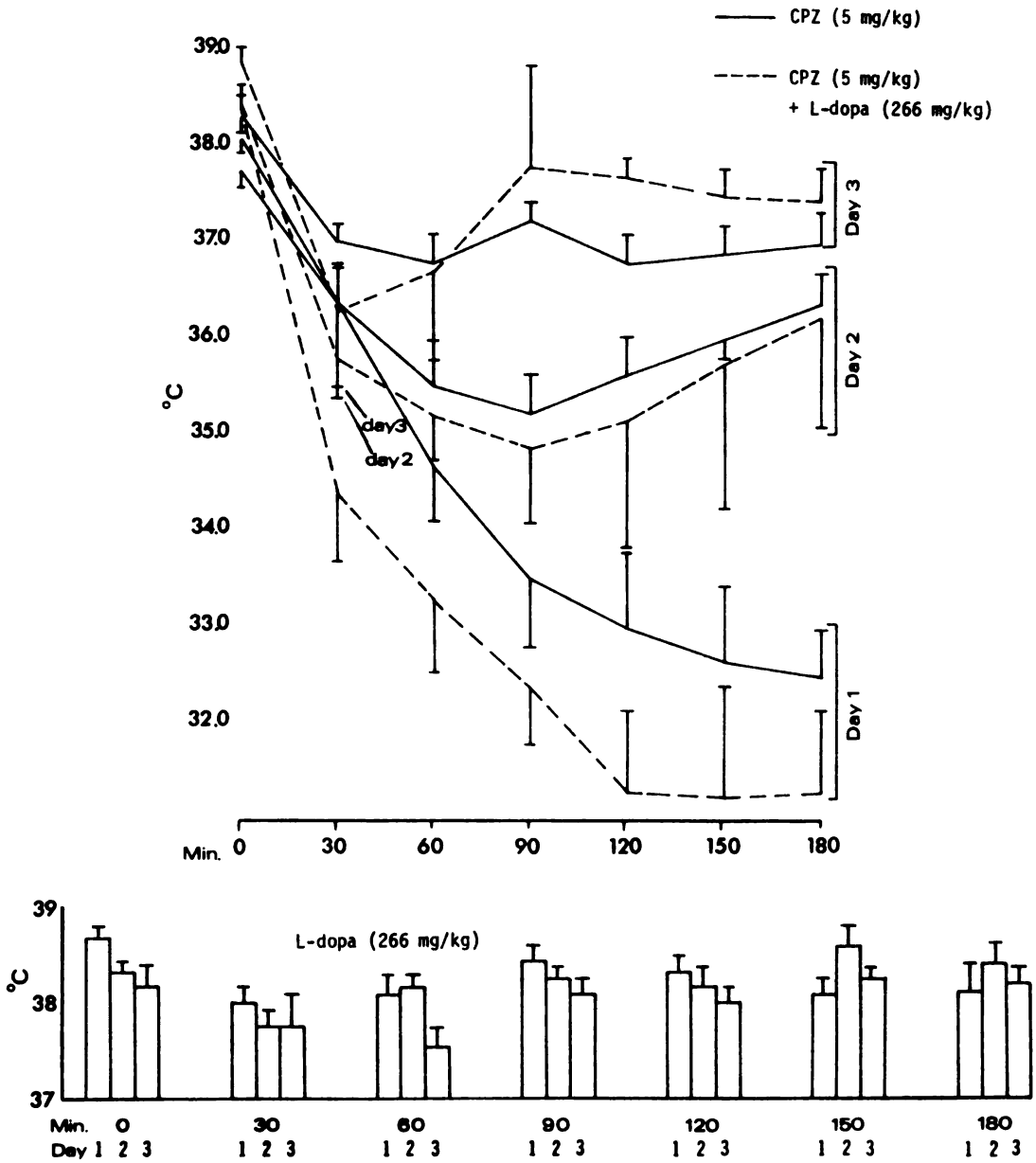

Figure 1. Time vs core body temperature of mature female mice injected with chlorpromazine, chlorpromazine plus L-dopa (above) or L-dopa (below). Injections were made at 24 hour intervals for three successive days. Chlorpromazine was injected i.p.; L-dopa was injected s.c. Each point (above) or bar (below) represents the mean core body temperature ±S.E. of 6 mice.

temperature at these times on days 1 and 3.

The administration of L-dopa did not significantly affect the core body temperature of female mice, nor did L-dopa alleviate tolerance with respect to the hypothermic response evoked by chlorpromazine in these animals. The core body temperature of mice receiving both chlorpromazine and L-dopa was only significantly less than animals receiving chlorpromazine alone at 30 minutes after injection on day 1. Indeed, animals receiving both L-dopa and chlorpromazine exhibited significantly higher mean core body temperatures than animals receiving chlorpromazine alone at time 0 and 120 minutes on day 3.

Figure 2 shows the effects of chlorpromazine, L-dopa and the combination on the core body temperature of male mice. The effects of chlorpromazine in female mice are also plotted on this figure for comparison. In male mice chlorpromazine induced a significant hypothermia on each of the ten consecutive days it was administered except on day 3. In all cases where hypothermia occurred it appeared 30 minutes after injection and was still evident at 180 minutes. The deepest hypothermia reached was never significantly lower than the hypothermic response at 180 minutes suggesting that there was no trend towards normothermia over this interval.

No trend towards normothermia ever developed over the 240 minute daily experimental interval on days 1, 2, 5, 6, and 7. However, normothermia was always restored within 24 hours. A trend towards normothermia did begin after 180 minutes on days 4, 8, and 10. In contrast to parameters previously defined for female mice, in male mice tolerance took six days to develop with respect to the hypothermic

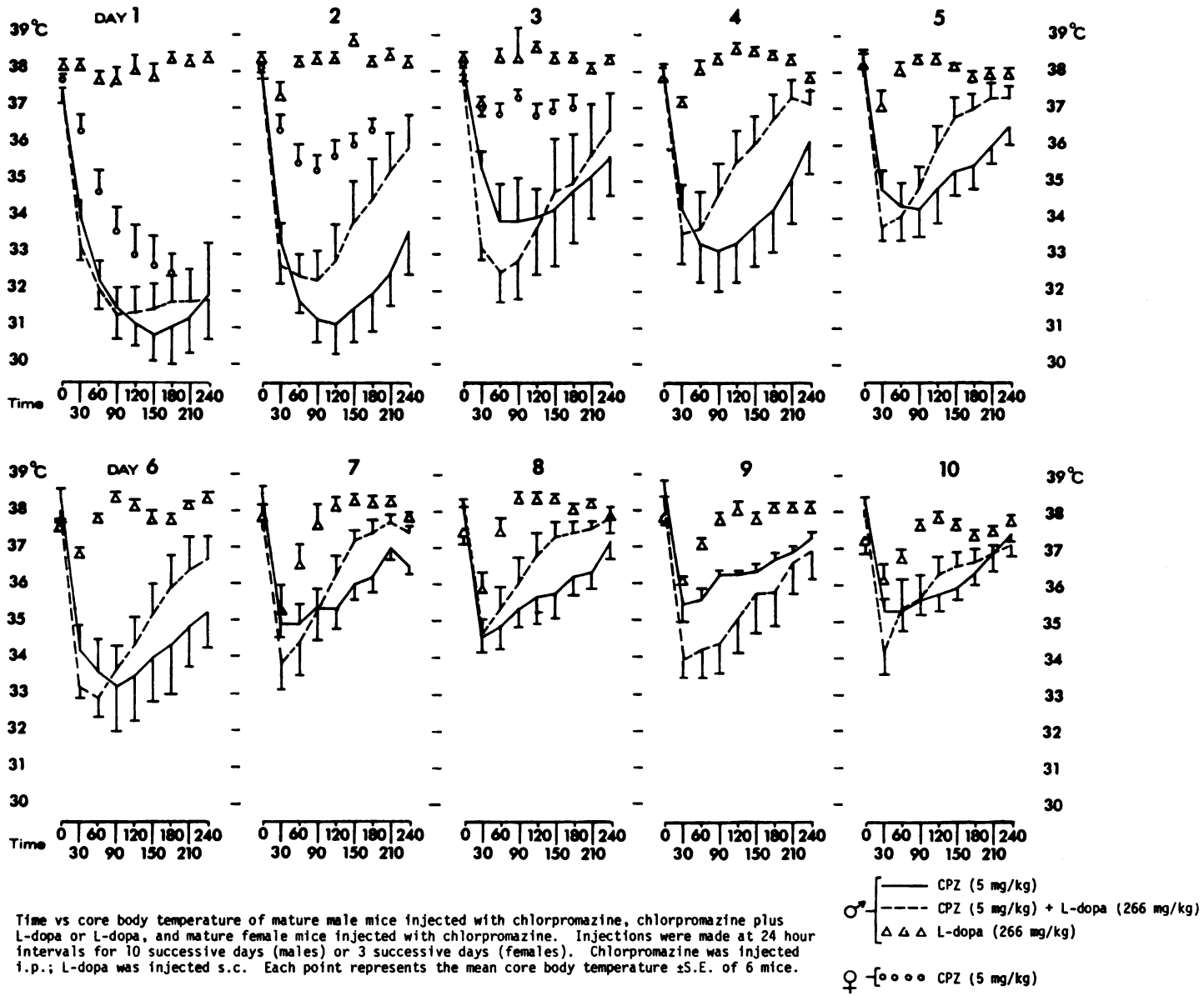

Figure 2. Time vs core body temperature of mature male mice injected with chlorpromazine, chlorpromazine plus L-dopa or L-dopa, and mature female mice injected with chlorpromazine. Injections were made at 24 hour intervals for 10 successive days (males) or 3 successive days (females). Chlorpromazine was injected i.p.; L-dopa was injected s.c. Each point represents the mean core body temperature ±S.E. of 6 mice.

response at 90 and 180 minutes following chlorpromazine (Table 7).

Comparison of hypothermia evoked in male and female mice after chlorpromazine administration for three successive days showed that significantly deeper Hypothermia appeared in males at 30 and 60 minutes after injection on day 1, at all times after injection on day 2 and at 30, 60, and 90 minutes after injection on day 3. Preinjection tempteratures were never different.

In male mice the continual administration of L-dopa at 8 hour intervals for 10 successive days significantly affected core body temperature from day 3 through day 10. On each of these days core body temperature at 30 minutes after injection was significantly depressed. On days 3, 4, 5, 6, 7, 8, and 10 this hypothermia was transient, normothermia was restored at 60 minutes. On day 9 the hypothermia existed for 90 minutes. Hyperthermia was exhibited at 120 and 150 minutes after injection on day 4, and 90 and 120 minutes after injection on day 6. Preinjection temperatures on days 2, 3, and 5 were also significantly elevated. In spite of these fluctuations a comparison of the L-dopa evoked temperature depression at 30 minutes after injection between trials suggested that there was no development of tolerance at this time over the 10 day experimental period ($P<.05$).

Treatment with L-dopa, however, did not alleviate tolerance with respect to the hypothermia evoked by chlorpromazine in male mice. The mean core body temperature of animals receiving both chlorpromazine and L-dopa was only significantly less than animals receiving CPZ alone at 30 minutes after injection on day 3, 0 minutes after injection on day 7 and 30 and 90 minutes after injection on day 9. Indeed animals receiving both L-dopa and chlorpromazine exhibited a significantly

Day:	1	2	3	4	5	6	7	8	9	10
°C:	31.50±.66	31.17±.63	33.92±1.20	33.08±1.13	34.17±.74	33.17±1.15	35.42±.52	35.25±.53	36.25±.11	35.58±.44

Day:	1	2	3	4	5	6	7	8	9	10
°C:	31.00±.97	31.92±1.04	34.67±1.29	34.17±1.12	35.42±.51	34.33±1.20	36.17±.42	36.17±.53	36.67±.21	36.33±.28

Table 7. Development of tolerance after i.p. administration of chlorpromazine (5 mg/kg) at 24 hour intervals for 10 consecutive days in mature male mice. Days underlined by the same line were not different with respect to the hypothermic response exhibited at 90 minutes (above) or 180 minutes after injection (below). P>.05, n=6.

Time (min)	0	30	60	90	120
8 mg/kg	39.33±.17	39.33±.17	39.00±.29	38.67±.33	38.67±.33
16 mg/kg	39.50±0.00	39.50±0.00	39.17±.17	39.17±.17	39.66±.44

Time (min)	0	30	60	90	120
8 mg/kg	39.00±0.00	38.83±.44	39.00±.29	38.67±.17	38.67±.17
16 mg/kg	38.50±.58	38.83±.60	38.67±.44	38.83±.44	38.83±.44

Table 8. Mean core body temperature (°C) ±S.E. of mature female (above) and male mice (below) injected i.p. with procaine at dose levels indicated. The dotted line signifies injection. In no case did procaine effect core body temperature. P>.05, n=3.

Day:	4	5	6	7	8
°C:	35.25±.75	34.25±.25	33.50±1.00	32.50±.50	30.80±.25

Table 9. Development of increasing sensitivity in mature female mice after successive i.p. administration of reserpine (.5 mg/kg). Days underlined by the same line were not different with respect to the hypothermic response exhibited at 6 hours after injection. P>.05, n=2,3.

higher mean core body temperature than animals receiving chlorpromazine alone at 180 and 210 minutes after injection on day 5; 150, 180, and 240 minutes after injection on day 7 and at 150 and 210 minutes after injection on day 8.

Determination of cross tolerance between trifluprom azine, promazine and chlorpromazine. The existence of cross tolerance with respect to the hypothermic response evoked by trifluprom azine, promazine and chlorpromazine was investigated to determine if the sequential administration of these compounds would provide a more efficacious method of inducing extended hypothermia than the successive administration of either compound alone. Figure 3 shows the hypothermic response evoked by the sequential administration of trifluprom azine, promazine and chlorpromazine at 24 hour intervals to mature female mice. Significant hypothermia developed after each compound was injected. The hypothermic response produced by trifluprom azine was not significantly different than that produced in previous trials (Table 2) under the same conditions. Similarly the hypothermia evoked by chlorpromazine after pretreatment with trifluprom azine and promazine did not significantly differ from the temperature depression produced in previous trials (Figure 1) involving the administration of three doses of chlorpromazine 24 hours apart. In contrast the hypothermic response produced by promazine 24 hours after trifluprom azine (Figure 4) was significantly greater than that produced in previous trials involving the administration of two doses of promazine 24 hours apart.

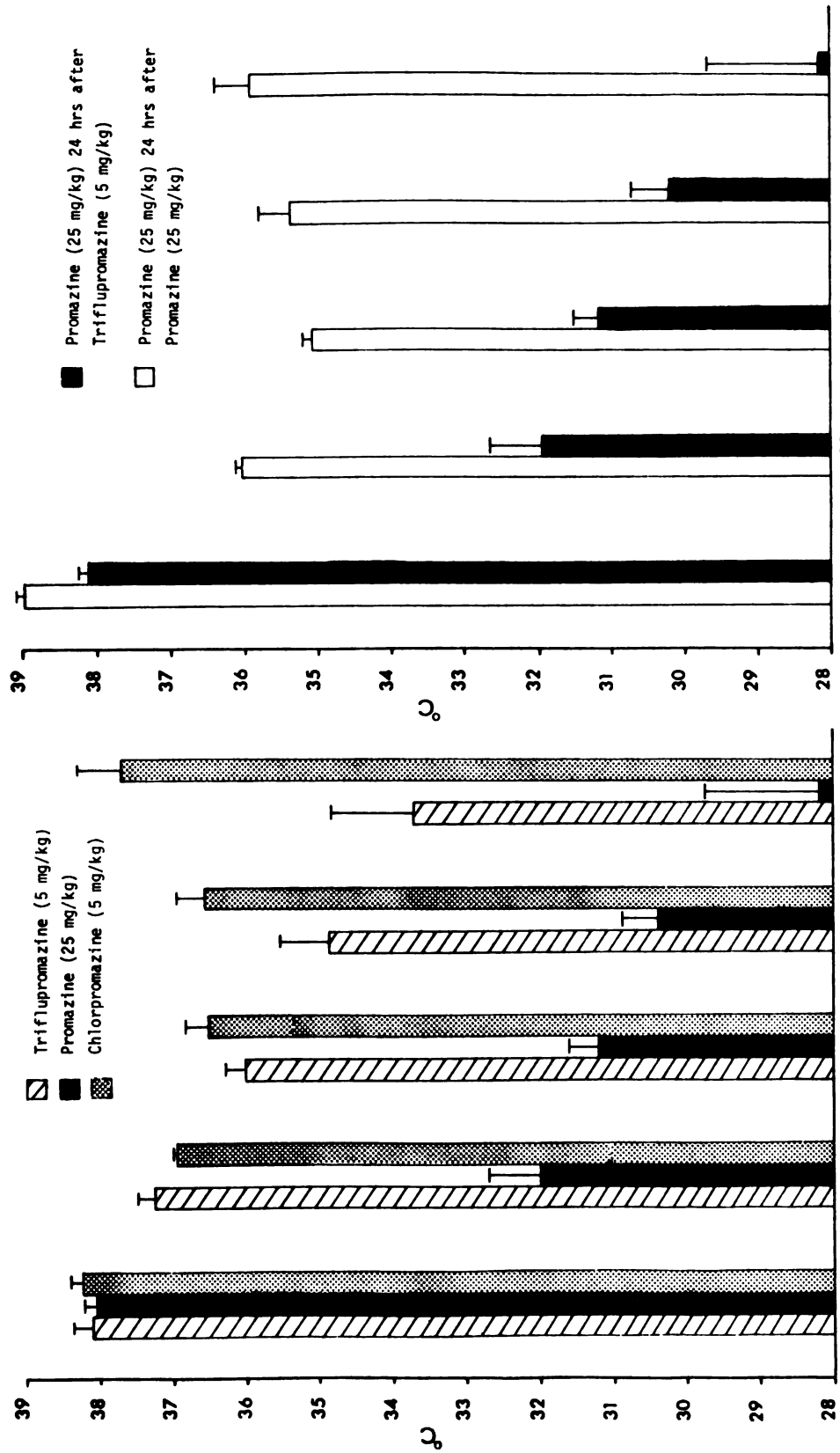

Figure 3. Time vs core body temperature of mature female mice injected sequentially with triflupromazine, promazine and chlorpromazine at 24 hour intervals. All injections were i.p. Each bar represents the mean core body temperature ±S.E. of 6 mice.

Figure 4. Time vs core body temperature of mature female mice injected with promazine 24 hours after prior injection of promazine or triflupromazine. All injections were i.p. Each bar represents the mean core body temperature ±S.E. of 6 mice.

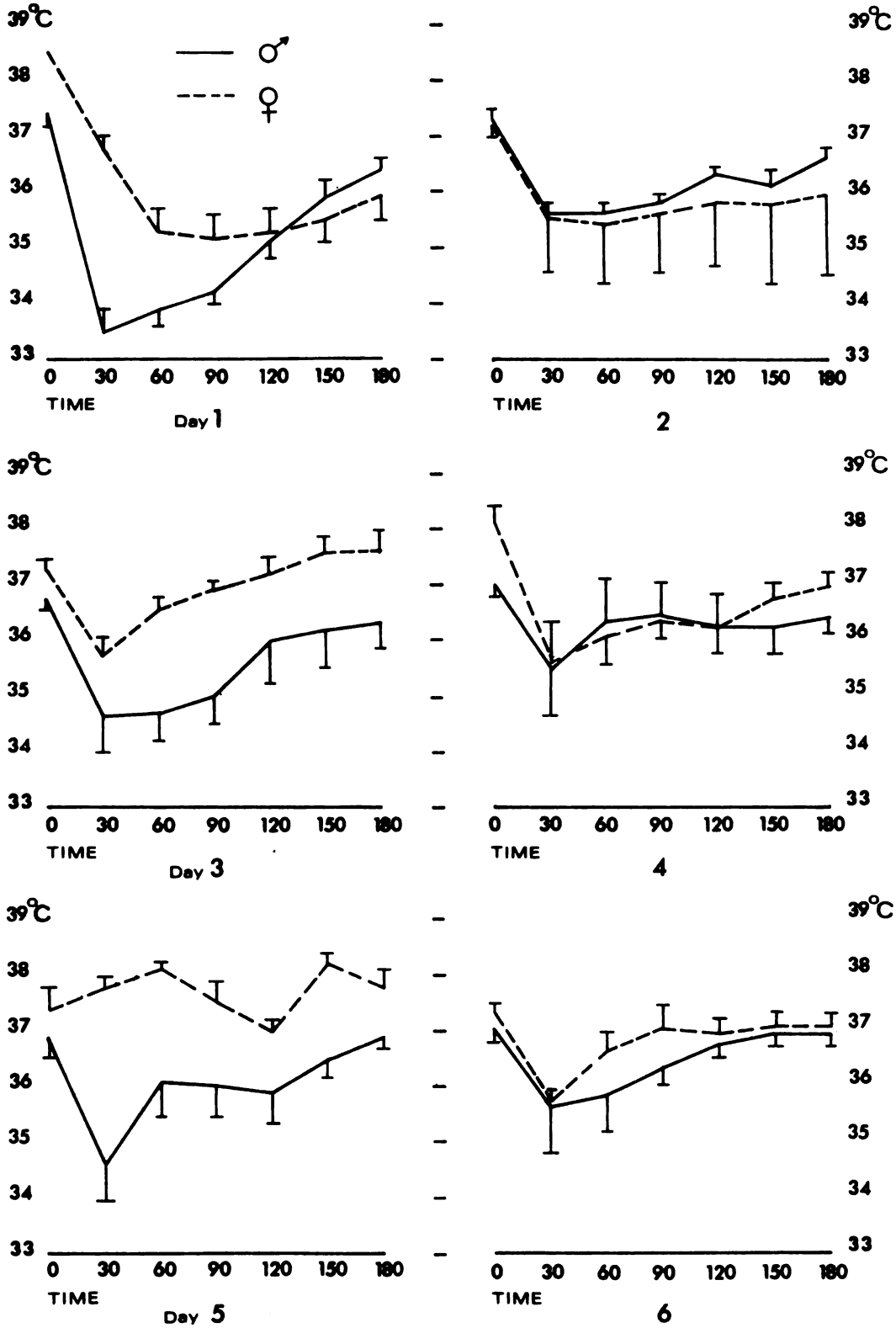

Figure 5. Time vs core body temperature of mature male and female mice injected with parachlorophenylalanine methyl ester (425 mg/kg) at 24 hour intervals for 6 consecutive days. All injections were i.p. Each point represents the mean core body temperature ±S.E. of 6 mice.

Procaine

Procaine did not in any way affect core body temperature of male or female mice at either dose level administered (Table 8).

Parachlorophenylalanine

Figure 5 shows the effects of the sequential administration of PCPA for six consecutive days on the core body temperature of both male and female mice. PCPA evoked a hypothermic response in female mice on days 1 and 4, and in male mice on days 1 and 2, which appeared at 30 minutes after injection and was still evident at 180 minutes. In females the deepest hypothermia reached on these days was not significantly different from the hypothermic response at 180 minutes suggesting that there was no trend towards normothermia over this interval. In males this method of analysis revealed that there was a trend towards normothermia during this period. On days 3 and 6 in females and days 3 and 5 in males PCPA evoked a hypothermia which was only significant at 30 minutes after injection. And on days 2 and 5 in females and 4 and 6 in males no hypothermia occurred at all after PCPA. In females preinjection core body temperatures on days 2, 3, 5, and 6 were significantly depressed with respect to these values on days 1 and 4. In males preinjection core body temperatures did not differ significantly over the course of this experiment.

Reserpine

Figure 6 shows the effects of the sequential administration of reserpine on the core body temperature of mature female mice. A total of eight injections were made over a 32 day period. Table 10

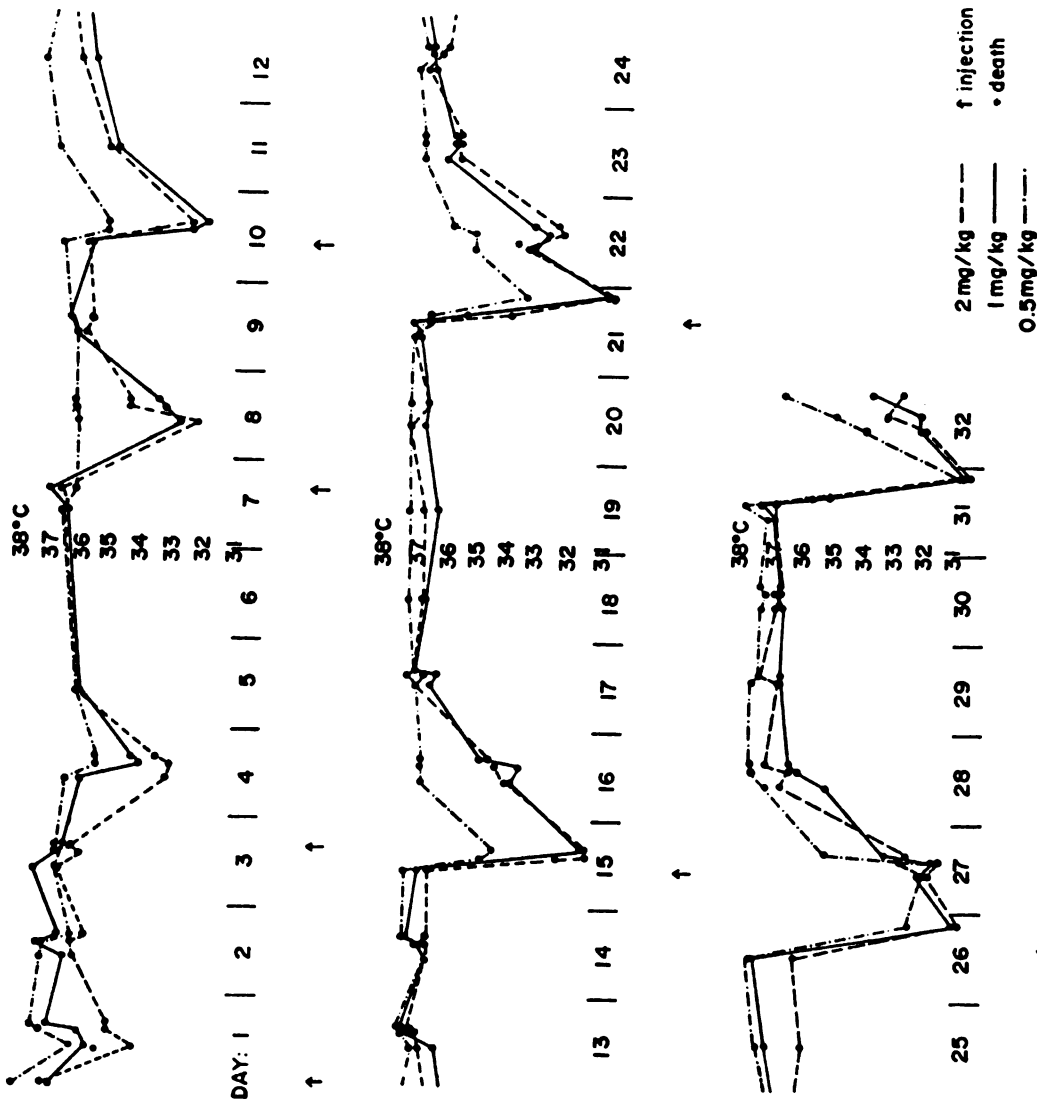

Figure 6. Time vs core body temperature of mature female mice injected with reserpine as indicated. All injections were i.p. Each point represents the mean core body temperature of 2 or 3 mice.

	Day 1				Day 2			Day 3		
Time (hr)	0 ↓	18	22	24	42	46	48	66	70	72 ↓
.5 mg/kg	38.2±.33	36.3±.17*	37.3±.44	37.6±.17	37.3±.33	37.2±.17	36.3±.17*	37.2±.33	36.7±.17*	36.8±.33*
1.0 mg/kg	37.0±0.00	35.8±.25*	36.0±0.00*	37.0±.50*	36.5±.50	37.3±.25	36.7±.75	37.5±.50	36.8±.25	36.5±.50
2.0 mg/kg	37.2±.17	34.2±.33*	35.0±0.00*	35.0±.29*	36.2±.44	36.3±.33	35.8±.33	36.8±.44	36.0±.29	36.3±.33

	Day 4			Day 5	Day 7		Day 8			Day 9
Time (hr)	90	94	96	114	162	168 ↓	186	190	192	210
.5 mg/kg	36.5±.29	35.5±.29*	35.5±.29*	36.2±.17	36.7±.17	36.2±.33	36.2±.17*	36.3±.44*	36.2±.44*	36.3±.17
1.0 mg/kg	36.0±.50*	34.0±.50*	34.3±.25*	36.0±0.00	36.5±.50	37.0±0.00	32.8±.25*	33.3±.25*	33.5±0.00*	36.3±.25
2.0 mg/kg	33.2±.33*	33.0±.76*	33.5±.29*	36.2±.17	36.5±0.00	36.7±.44	32.3±.60*	34.5±.28*	34.5±.29*	36.0±0.00

	Day 10				Day 11	Day 12		Day 13		
Time (hr)	214	234 ↓	238	240	262	286	306	310	312	330
.5 mg/kg	36.5±0.00	36.8±.25*	35.3±.75*	35.3±.75*	37.0±0.00	37.5±0.00	37.0±0.00	37.3±.25	37.5±0.00	36.8±.25
1.0 mg/kg	36.5±0.00	35.8±.17	32.5±.29*	32.0±.76*	35.0±.76	35.8±.17	36.2±.44	37.0±.29	37.3±.17	36.5±.29
2.0 mg/kg	35.8±.60	36.0±.58	33.7±.17*	32.5±.58*	35.3±.73	36.3±.33	36.7±.17	36.8±.17	37.0±0.00	36.5±.29

	Day 14		Day 15			Day 16			Day 17	
Time (hr)	334	336	354 ↓	358	360	378	382	384	402	406
.5 mg/kg	36.5±0.00	37.3±.25	37.3±.50	34.8±.25*	34.3±.25*	36.8±.25*	36.8±.25*	36.8±.25*	37.0±0.00	37.3±.25
1.0 mg/kg	36.8±.11	37.2±.17	36.8±.17	32.2±.73*	31.3±.60*	33.8±.93*	33.5±.44*	34.7±.33*	36.3±.29	36.0±.33
2.0 mg/kg	36.7±.17	36.5±0.00	36.5±0.00	31.2±.17*	31.2±.33*	34.0±.87	34.3±1.09*	34.5±.76*	37.0±0.00	36.7±.33

	Day 18	Day 19	Day 20		Day 21				
Time (hr)	408	426	450	474	480	498	502 ↓	504	508	522
.5 mg/kg	37.0±0.00	37.3±.25	37.3±.25	37.3±.25	37.3±.25	37.3±.25	37.3±.25	36.7±.25	33.5±1.00*	35.3±.25*
1.0 mg/kg	37.0±0.00	36.7±.33	36.3±.44	36.8±.17	36.8±.17	37.0±0.00	37.3±.33	35.5±.50*	30.7±.33*	33.3±.17*
2.0 mg/kg	37.0±.29	36.8±.73	36.8±.60	37.3±.33	36.7±.83	37.3±.33	36.7±.33	34.0±1.26*	30.5±.50*	33.5±1.00*

	Day 22		Day 23			Day 24			Day 25	Day 26
Time (hr)	526	528	546	550	552	570	574	576	598	622 ↓
.5 mg/kg	35.3±.75*	36.0±0.00*	37.0±0.00*	37.0±0.00	37.0±0.00	37.3±.25	36.8±.25	37.0±0.00	37.5±0.00	37.8±.25
1.0 mg/kg	32.8±.33*	33.3±.73*	36.3±.44	35.8±.60	36.0±.58	36.7±.33	36.8±.17	36.8±.33	37.2±.33	37.7±.17
2.0 mg/kg	32.3±.25*	32.5±.50*	35.8±.25	36.0±0.00	35.8±.75	37.0±.50	36.5±0.00	36.3±.25	36.0±0.00	36.3±0.00

	Day 27				Day 28			Day 29		
Time (hr)	628	642	646	648	666	670	672	694	696	714
.5 mg/kg	32.5±.50*	32.0±.50*	31.5±0.00*	35.3±.25*	37.3±.25	37.8±.25	37.8±.25	37.8±.25	37.5±0.00	37.5±0.00
1.0 mg/kg	31.0±0.00*	32.2±.66*	31.7±.17*	33.3±.66*	35.3±1.42	36.2±.83	36.5±.29	36.7±.33	36.8±.17	36.7±.33
2.0 mg/kg	30.8±.25*	31.8±.25*	32.8±.25*	32.5±.50*	36.8±.25	36.5±0.00	37.3±.25	36.8±.25	37.5±0.00†	37.0±0.00

	Day 30		Day 31				Day 32		
Time (hr)	718	720	738	742 ↓	744	748	762	766	768
.5 mg/kg	37.3±.25	37.5±0.00	37.3±.25	38.0±.50	35.8±.75*	30.8±.25*	34.0±.50*	35.0±.50*	36.8±.25*
1.0 mg/kg	37.0±.29	36.8±.17	37.0±0.00	37.5±0.00	35.2±.93*	30.7±.17*	32.2±.73*	32.2±.17*	33.8±.73*
2.0 mg/kg	36.8±.25	36.8±.25	37.0±0.00	37.0±0.00	35.8±.75	30.5±0.00*	32.0±.50*	33.3±.25*	32.5±1.25*

Table 10. Mean core body temperature (°C) ±S.E. of mature female mice injected i.p. with reserpine as indicated. Arrow signifies injection. *Indicates core body temperature significantly lower than intratrial preinjection value. †Indicates core body temperature significantly greater than intratrial preinjection value. α=.05, n=2,3.

shows that the hypothermic response following reserpine was extended and in many cases was still apparent at 24 hours after injection independent of dose. At 1 and 2 mg/kg no significance was exhibited with respect to the degree of hypothermia produced six hours after injection on trials 4-8. At .5 mg/kg the temperature depression at six hours after injection on trials 4-7 was significantly less than that produced by higher doses but became increasingly more pronounced with repeated administrations (Table 9) so that no significant difference appeared across doses at six hours after injection on the final trial.

Over the course of this experiment two animals died. One animal in the 1.0 mg/kg group was found dead 18 hours after the first injection. This death was attributed to an unhealthy precondition. The second animal, which was in the .5 mg/kg group, was found drowned on the 522nd hour of the experiment in a dish containing a slurry of powdered mouse pellets and water placed in its cage to compensate for anorexia.

DISCUSSION

To the extent that the effects of the drugs investigated in these studies were mediated through an alteration of cerebral amine levels, the evoked changes in core body temperature reported here support the monoamine theory of thermoregulation proposed by Feldberg and Myers (1965). Thus phenothiazines which decrease 5-HT turnover and increase catecholamine turnover in the brain, reserpine which depletes central monoamine stores, and PCPA which preferentially depletes cerebral 5-HT, all produced significant hypothermy in the mouse.

The investigations regarding phenothiazines suggested that these compounds would be largely inadequate as hypothermic agents due to the development of tolerance with repeated administration. Mature female mice treated with chlorpromazine, trifluprom azine or promazine all exhibited an adaptive response to evoked hypothermia with repeated injections, although trifluprom azine produced the least striking tachyphylaxis. While mature mice made hypothermic by administration of trifluprom azine and chlorpromazine never exhibited a significant return to normothermia for at least 180 minutes following injection, a significant return to normothermia did occur within this interval in mice treated with promazine on 5 out of 7 trials.

An evaluation of the abilities of these compounds to produce a

long lasting hypothermia concomitant with a minimal adaptive response, therefore, suggested trifluprom azine>chlorpromazine>promazine. Davis and Brody (1965) have found that the ability of phenothiazines to inhibit Na^+K^+ ATPase in the brain and therefore to stabilize neuronal membranes to Na^+ and K^+ flux also follows this same potency ratio. If phenothiazines limit Na^+ flux in the posterior hypothalamus it is possible that their hypothermic effects may be, in part, due to their ability to effectively increase the Ca^{++}/Na^+ ratio in this area.

The duration and intensity of action of many drugs are determined by the speed at which they are metabolized in the body by enzymes in the liver microsomes (Conney, 1967). Since chlorpromazine is well known to stimulate liver growth and liver microsomal enzyme levels (Silverstini, Catanese, Del Basso, 1966) and phenothiazine metabolites are largely inactive (Johri and Biachetti, 1966) it is probable that this mechanism accounted for the adaptive responses after phenothiazines seen in these studies.

The induction of the liver microsomal enzyme fraction by foreign compounds is usually more marked in the adult female rodent than in the adult male. Westfall (Westfall, Boules, Shields, 1964) has shown that male mice are affected by pentobarbitol for a longer time than females, and the duration of action can be decreased by treating the males with stilbesterol and increased by treating the females with testosterone. Likewise chronic treatment of male mice with testosterone or methyltestosterone prolongs the action of hexobarbitol and coincidentally decreases the activity of the corresponding enzyme system (Norvick, Stohler, Swagzkis, 1966).

Although both male and female mice, in the studies presented here,

exhibited a tolerance to repeated administration of chlorpromazine, the degree of induced hypothermia and resistance to tolerance was strikingly greater in the males than in the females, presumably as a result of the greater efficacy of chlorpromazine to induce hepatic microsomal enzymes in females. Similarly repeated injections of trifluprom azine produced statistically less tolerance in 5 week old female mice than in adult females. These results are consistent with the findings of Jondorf and his co-workers (Jondorf, Maickel, Brodie, 1959) who have shown that liver microsomes are poorly developed in immature mice. It is conceivable that administration of trifluprom azine may also involve the induction of an enzyme system necessary for the production of active phenothiazine metabolites. This would explain the failure of this compound to evoke a hypothermic response in immature mice when first administered. And it would also explain the ability of prior administration of trifluprom azine to dramatically potentiate the hypothermic effect of promazine. If such a quality exists in trifluprom azine it could possibly be advantageously exploited in future studies.

An alternate but not mutually exclusive explanation of the tolerance produced by repeated administration of phenothiazines concerns the finding by Moore (1971) that the increased turnover of central catecholamines produced by these compounds is correlated with a decreased receptor availability. It is plausible, therefore, that tolerance results as the catecholamine receptors become increasingly less vulnerable. This mechanism would explain why in these studies L-dopa did not reliably potentiate the hypothermic effects of chlorpromazine in male or female mice but when

administered alone was capable of producing hypothermy at least in males.

Despite its reported ability to produce rapidly appearing centrally mediated hypotension in experimental animals (Peterson, 1955a), procaine, under the conditions of these investigations, did not produce any significant hypothermy in male or female mice. PCPA, however, which has been reported by numerous investigators to potentiate the effects of other hypothermic agents (Bruinvels and Kemper, 1971; Grabowska et al, 1973) but to lack the ability to produce hypothermia when administered alone to rodents (Bruinvels and Kember, 1971; Grabowska, 1973; Maj and Pawlowska, 1973) did produce statistically significant hypothermia under the conditions of the experiments reported here when administered to mice in comparable doses. Even though destruction of central serotonergic neurons and blockage of central 5-HT receptors has been shown to produce hypothermia (Yehuda and Wurtman, 1972) it is doubtful that PCPA produced its hypothermic effects solely by depletion of hypothalamic 5-HT. Miller et al (1969) has shown that central depletion of 5-HT in rats after PCPA is significant for about eight days, yet the results presented here indicate that animals treated daily with this compound exhibited only intermittent hypothermy, although a slight hypothermia may have been prolonged for 24 hours or more in female but not male mice. It is possible, therefore, that a direct action on peripheral vasculature as has been suggested by McDonald and Mueller (1969), played a role in the hypothermia generated by this drug.

Of all the compounds tested in these studies, only two, reserpine and L-dopa, exhibited the ability to produce consistent hypothermia

with repeated administration. Reserpine has been shown to lower the concentration of monoamines in the hypothalamic area of experimental animals for approximately 30 hours (Brodie, Finger, Orlong, Quinn, Sulser, 1960; Shellenberger and Elder, 1967), a time course which correlates well with the temporal characteristics of the hypothermic responses seen in mice injected with comparable doses of reserpine under the conditions of the experiments presented here. It is likely that the effects of this compound are mediated by the central release of catecholamines since prior depletion of these transmitters blocks or reverses reserpine induced hypothermia (Shellenberger, et al, 1967). In this respect reserpine acts in much the same way to depress temperature as does chlorpromazine. Reserpine, however, unlike chlorpromazine, does not exhibit an adaptive response. Indeed, mice treated with .5 mg/kg of reserpine exhibited an increasing sensitivity to this compound with repeated trials, eventually reaching the same temperature depression profile as was seen in mice treated with higher doses. The finding that mice injected with elevated doses of reserpine did not show an increasing response to repeated injections but did not show tolerance could possibly suggest that the central catecholamine receptors in reserpinized mice, unlike those in chlorpromazine treated mice, reach a constant level of availability. Although it is generally acknowledged that reserpine exerts a "hit and run" effect (Brodie, Olin, Kuntzman, Shore, 1957) it is certainly possible that the profound hypothermia produced by this compound acts to inhibit the induction of specific enzyme systems and to slow its elimination from the body (Sjoqvist and Hammer, 1968; Cagen, Janoff, Bus, Gibson, 1976).

Like reserpine, L-dopa also produced a nonadapting hypothermic response probably mediated through a central mechanism. Butcher and his co-workers (Butcher, Engel, Fuxe, 1972) have shown that systematic administration of L-dopa in rats at doses comparable to those used in the studies reported here, reduced central 5-HT levels by 30%, increased dopamine levels by 300%, but had no effect on norepinephrine levels. In addition, histochemical techniques revealed the presence of catecholamines within serotonergic terminals after L-dopa. Brittain and Handley (1967) have shown that intraventricular administration of dopamine in mice evokes hypothermia. Further, when L-dopa was administered together with a peripheral decarboxylase inhibitor on a single trial basis in rats, significant hypothermia was induced but did not occur when L-dopa was administered alone (Maj and Pawlowski, 1973). Other workers have demonstrated that systemic administration of apomorphine produces hypothermia in rats and mice which can be blocked by various dopamine antagonists (Barnett, Goldstein, Taber, 1972; Grabowska et al, 1973).

In the investigations presented here, L-dopa evoked hypothermia appeared in male mice after two days of prior administration and did not exhibit a diminishing magnitude with repeated injections over the remaining trials. It is likely that in these mice two days of L-dopa loading were required before central concentrations of catecholamines were effectively increased. Similarly, female mice which were treated with L-dopa as in males failed to show a hypothermic response within three days and may have required a longer period for central catecholamine concentrations to saturate monoaminergic neurons. Iverson (1970) has demonstrated that the ability of central

monoaminergic neurons to concentrate monoamines is large, and only when the concentration mechanism is saturated are monoamines released.

In an intriguing experiment Cantzias and his co-workers (Cantzias, Miller, Nicholson, Maston, Tang, 1974) showed that rats fed large amounts of L-dopa in their diet (4% w/w) exhibited a 73% increase in life span and a concomitant prolongation of vitality when compared to nontreated controls. Since experiments reported here indicate that L-dopa depresses core body temperature and may even exhibit increased potency with long term administration, it seems highly likely that these animals were hypothermic. Thermodynamically a small decrease in body temperature could account for this increase in life span.

In summary, L-dopa and reserpine both induced nonadapting hypothermy in mice under the conditions of these investigations, probably by evoking the release of catecholamines in the anterior hypothalamus. When L-dopa was administered systemically to rats, together with RO4-4602, to prevent peripheral decarboxylation, nilamide (a MAO inhibitor), and reserpine, histochemical techniques revealed a bright fluorescence indicative of catecholamines both within and around the terminals of central dopaminergic and serotonergic neurons particularly in the anterior hypothalamus (Butcher et al, 1972). Neurochemically this correlated with a 700% increase in whole brain dopamine and a 86% decrease in whole brain 5-HT. In light of the body of information presented here, it is highly probable that this L-dopa/RO4-4602/nilamide/reserpine "cocktail" represents an extremely effective nonadapting hypothermic agent.

APPENDICES

APPENDIX A

Pathways of Monoamine Biosynthesis

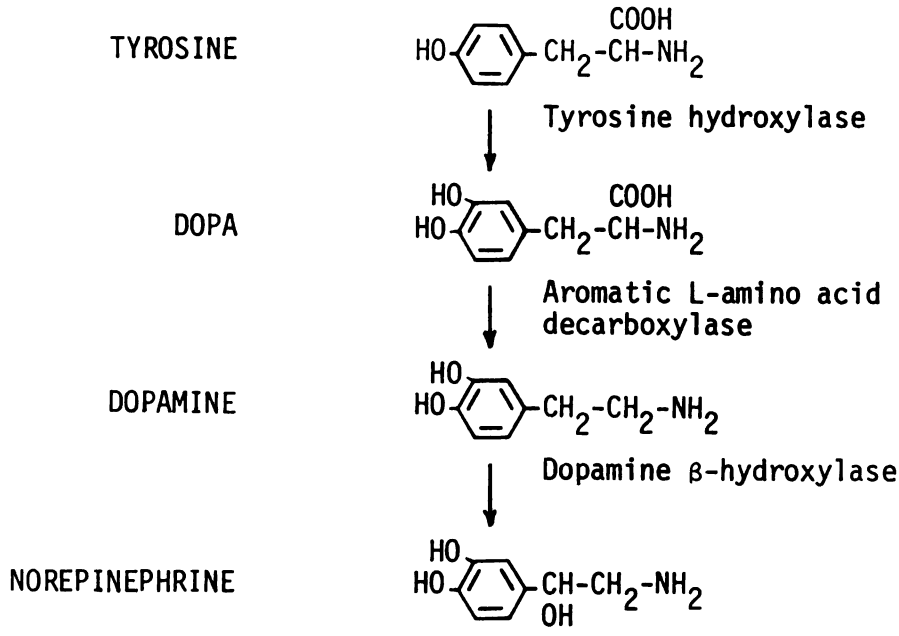

Figure A1. Biosynthesis of Catecholamines

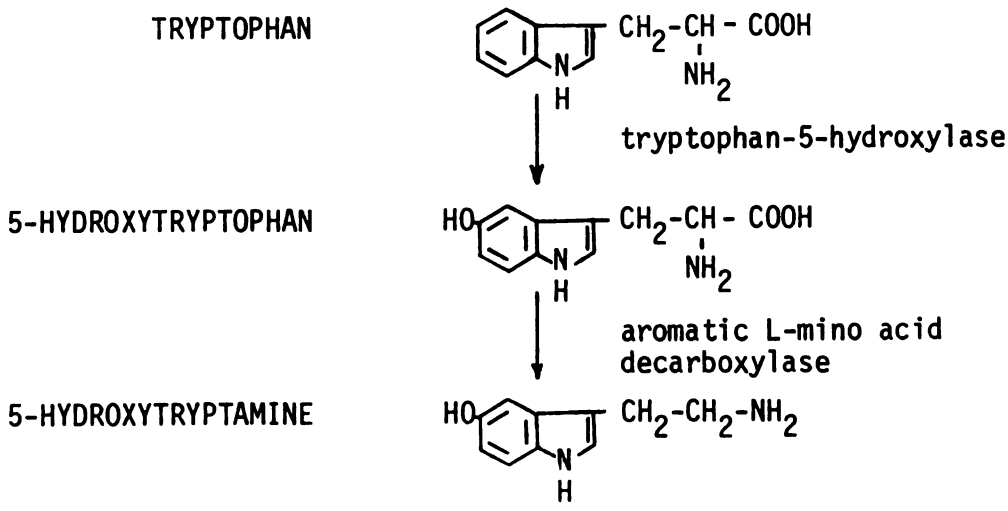

Figure A2. Biosynthesis of 5-hydroxytryptamine

APPENDIX B

Chemical Structures of the Compounds Investigated

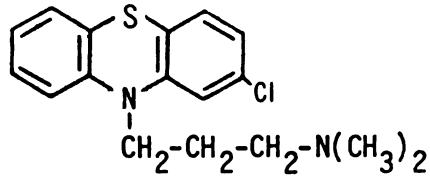

Figure B1. Chlorpromazine

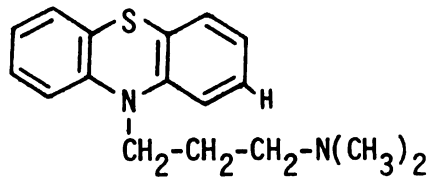

Figure B2. Promazine

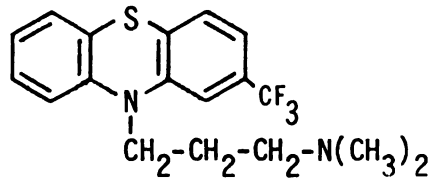

Figure B3. Triflupromazine

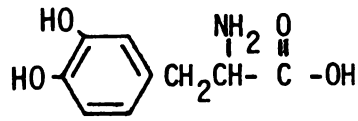

Figure B4. L-dopa

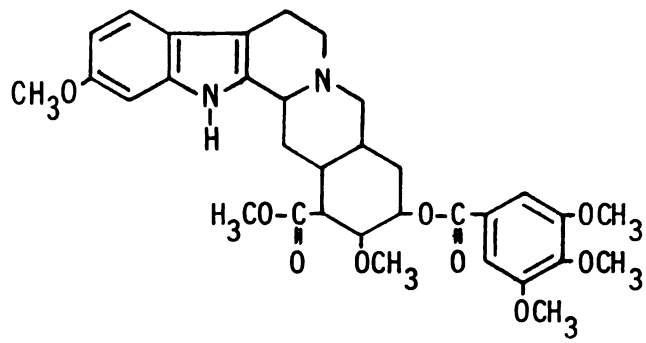

Figure B5. Reserpine

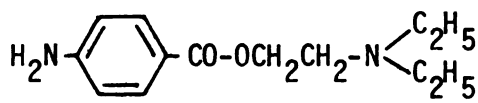

Figure B6. Procaine

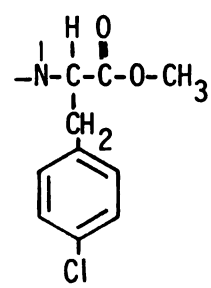

Figure B7. Parachlorophenylalanine Methyl Ester

LIST OF REFERENCES

LIST OF REFERENCES

Aghajanian, G. K., Kuhor, M. J., Roth, R. H. (1973). Serotonin-containing neuronal perikarya and terminals: differential effects of p-chlorophenylalanine. Brain Res., 54, 85-101.

Aslan, A. (1956). A new method for prophylaxis and treatment of aging with novocain - eutrophic and rejuvenating effects. Therapiewoche, 7, 14-22.

Aslan, A., Urabiescu, A., Domilescu, C., Campeanu, L., Continiu, A., Stanescu, S. (1965). Long-term treatment with procaine in albino rats. J. Geront., 20, 1-8.

Avery, D. D. (1971). Intrahypothalamic adrenergic and cholinergic injection effects on temperature and ingestive behavior in the rat. Neuropharmacology, 10, 753-763.

Barnett, A., Goldstein, J. and Taber, R. I. (1972). Apomorphine-induced hypothermia in mice: a possible dopaminergic effect. Arch. Int. Pharmacodyn., 198, 242-247.

Baxter, R. W., Bolster, J. A. and McKechnie, S. (1954). Three phenothiazine derivatives in anaesthesia. Anaesthesia, 9, 79-87.

Beckman, A. L. and Satinoff, E. (1972). Arousal from hibernation by intrahypothalamic injections of biogenic amines in ground squirrels. Am. J. Physiol., 222, 875-879.

Berger, P. (1960). Les effets du traitement chronique a la procaine sur la senescence du rat blanc. Path. Biol., Paris, 8, 1163-1166.

Bligh, R. (1966). Effects on temperature of monoamines injected into the lateral ventricles of sheep. J. Physiol., 185, 46-47.

Brittain, R. T. and Handley, S. L. (1967). Temperature changes produced by the injection of catecholamines and 5-hydroxytryptamine into the cerebral ventricles of the conscious mouse. J. Physiol., 192, 805-813.

Brodie, B. B., Olin, J. S., Kuntzman, R. G., Shore, P. A. (1957). Possible interrelationship between release of brain norepinephrine and serotonin by reserpine. Science, 125, 1293-1294.

Brodie, B. B., Finger, K. F., Orlons, F. B., Quinn, G. P., and Sulser, F. (1960). Evidence that tranquilizing action of reserpine is associated with change in brain serotonin and not in brain norepinephrine. J. Pharm. Exp. Ther., 129, 250-256.

Bruinvels, J. and Kemper, G. C. M. (1971). Role of noradrenaline and 5-hydroxytryptamine in tetrahydronaphthylamine induced temperature changes in the rat. Br. J. Pharmacol., 43, 1-9.

Butcher, L. L., Engel, J., Fuxe, K. (1972). Behavioral, biochemical, and histochemical analyses of the central effects of monoamine precursors after peripheral decarboxylase inhibition. Brain Res., 41, 387-411.

Cagen, S. Z., Janoff, A. S., Bus, J. S., and Gibson, J. E. (1976). Effect of paraquat (methyl viologen) on liver function in mice. J. Pharm. Exp. Ther., 198, 222-228.

Catzias, G. C., Miller, S. T., Nicholson, A. R., Maston, W. H., Tang, L. C. (1974). Prolongation of the life span in mice adapted to large amounts of L-dopa. Proc. Nat. Acad. Sci., 71, 2466-2469.

Chawla, N., Johri, M. B. L., Saxena, P. N., and Singhal, H. T. (1975). Cholinergic mechanisms in central thermoregulation in pigeons. Br. J. Pharmacol., 53, 317-372.

Conney, A. H. (1967). Pharmacological implications of microsomal enzyme induction. Pharmac. Revs., 19, 317-366.

Cooper, K. E., Cranston, W. I., Honour, A. J. (1967). Observations on the site and mode of action of pyrogens in the rabbit brain. J. Physiol., 191, 325-337.

Courvoisier, S., Fournel, J., Ducrat, R., Kolsky, M., Koetschet, P. (1953). Proprietes pharmacodynamiques du chlorhydrate de chloro-3 (dimethylamino 3 propyl)-10 phenothiazine. Arch. Int. Pharmacodyn., 92, 305-361.

Cox, B. and Potkonjak, D. (1967). The effect of ambient temperature on the actions of tremorine on body temperature and on the concentration of noradrenaline, dopamine, 5-hydroxytryptamine and acetylcholine in rat brain. Br. J. Pharmacol. Chemother., 31, 356-366.

Crawshaw, L. I. (1972). Effects of intracerebral 5-HT injection on thermoregulation in rat. Physiol. Behav., 9, 133-140.

Cremer, J. E. and Bligh, J. (1969). Body temperature and responses to drugs. Brit. Med. Bull., 25, 299-306.

Davis, P. W. and Brody, T. M. (1965). Inhibition of Na^+K^+-activated adenosine triphosphatase activity in rat brain by substituted phenothiazines. Biochem. Pharmacol. 15, 703-710.

Decourt, P., Brunaud, M. and Brunaud, M. (1953). Action d'un narcobiotique (chlorpromazine) sur la temperature centrale des animaux homeotherms soumis a des temperatures ambiantes superieures, egales ou interieures a leur temperature centrale normale. C.R. Soc. Biol. Paris, 147, 1605-1609.

Dobkin, A. B., Gilbert, R. G. B., Lamoureaux, L. (1954). Physiological effects of chlorpromazine. Anaesthesia, 9, 157-174.

Feldberg, W. (1975). Body temperature and fever: Changes in our views during the last decade. Proc. R. Soc., 191, 199-229.

Feldberg, W., and Myers, R. D. (1964). Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. J. Physiol., 173, 226-237.

Feldberg, W. and Myers, R. D. (1965). Changes in temperature produced by micro-injections of amines into the anterior hypothalamus of cats. J. Physiol., 177, 239-245.

Fondy, T. P., Karker, K. L., Calcagnino, C., Emlich, C. A. (1974). Effect of reserpine (NSC-59272)-induced hypothermia on life span in mouse L1210 leukemia. Cancer Chemother. Report, 58, 317-324.

Fuxe, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. IV. The distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand., Suppl. 247, 64, 37-85.

Fuxe, K., Hakfelt, T., and Ungerstedt, U. (1970). Morphological and function aspects of central monoamine neurons. Int. Rev. Neurobiol., 13, 93-126.

Giaja, J. and Markovic-Giaja (1954). La chlorpromazine et la thermoregulation. Soc. Biol., Paris, 148, 842-844.

Glasgow, E. F. and Sinclair, P. C. (1962). Dissociation of thermal sensibility in procaine nerve block. Brain, 85, 791-798.

Glasstone, S., Laidler, K. J. and Eyring, H. (1941). The Theory of Rate Processes. McGraw-Hill Book Company, Inc., New York.

Gordon, P., Fudema, J. J., Snider, G. C., Abrams, A., Tobin, S. S., Kraus, J. D. (1965). The effects of a European procaine preparation in an aged population II. Physiological effects. J. Geront., 20, 144-150.

Grabowska, M., Michaluk, J., Antkiewicz, L. (1973). Possible involvement of brain serotonin in apomorphine-induced hypothermia. Eur. J. Pharmacol., 23, 82-89.

Hall, G. H. (1972). Changes in body temperature produced by cholinomemetic substances injected into the cerebral ventricles of unanaesthetized cats. Br. J. Pharmacol., 44, 634-641.

Hall, G. H. and Myers, R. D. (1972). Temperature changes produced by nicotine injected into the hypothalamus of the conscious monkey. Brain Res., 37, 241-251.

Hammel, H. T. (1968). Regulation of internal body temperature. Ann. Rev. Physiol., 30, 641-710.

Hayward, J. N. and Baker, M. A. (1968). Diuretic and thermoregulatory responses to preoptic cooling in the monkey. Amer. J. Physiol., 214, 843-850.

Horita and Gogerty (1958). The pyretogenic effect of 5-hydroxytryptophan and its comparison with that of LSD. J. Pharmacol. Exptl. Ther., 122, 195-200.

Iverson, L. L. (1970). Neuronal uptake processes for amines and amino acids. In E. Costa and E. Giacolini (eds), Biochemistry of Simple Neuronal Models, Advances in Biochemical Psychopharmacology, Vol. 2, Raven Press, New York.

Johnson, G. A., Kim, E. G., Boukma, S. J. (1968). Mechanism of norepinephrine depletion by 5-hydroxytryptophan, Proc. Soc. Exp. Med. Biol., 128, 509-512.

Jondorf, W. R., Maickel, R. P., and Brodie, B. B. (1959). Inability of newborn mice and guinea pigs to metabolize drugs. Biochem. Pharmacol., 1, 352-354.

Jori, A., and Bianchetti, A. (1966). Effect of chlorpromazine and of its metabolites on blood glucose and glucose tolerance. Int. J. Neuropharmacol., 5, 435-440.

Kirkpatrick, W. E. and Lomax, P. (1971). Temperature changes induced by chlorpromazine and N-methylchlorpromazine in the rat. Neuropharmacol., 10, 61-66.

Knapp, S. and Mandell, A. J. (1972). Parachlorophenylalanine - its three phase sequence of interactions with the two forms of brain tryptophan hydroxylase. Life Sci., 11, 761-771.

Knox, G. V. and Lomax, P. (1972). The effect of nicotine on thermosensitive units in the rostral hypothalamus. Proc. West. Pharmacol. Soc., 15, 179-183.

Koe, K. B. and Weissman, A. (1966). P-chlorophenylalanine, a specific depletor of brain serotonin. J. Pharmacol. Exptl. Ther., 154, 499-516.

Kollias, J. and Bullard, R. W. (1964). The influence of chlorpromazine on physiological and chemical mechanisms of temperature regulation in the rat. J. Pharmacol. Exptl. Ther., 145, 373-381.

Livingston, K. E. and Perrin, R. G. (1972). Central physiological effects of experimental intravenous procaine hydrochloride. J. Neurosurg., 37, 188-194.

Maj, J. and Pawlowski, L. (1973). The hypothermic effect of L-dopa in the rat. Life Sci., 13, 141-149.

McDonald, R. K., Mueller, P. S. (1969). Effect of P-chlorophenylalanine administration on thermoregulation in the rat. Proc. Soc. Int. Congr. Coll. Int. Neuro-Psychopharmacol., 6, 317-319.

Metcalf, G., Myers, R. D., Redgrave, P. C. (1975). Temperature and behavioral responses induced in the unanaesthetized cat by the central administration of RX72601, a new anticholinesterase. Br. J. Pharmacol., 55, 9-15.

Miller, F. P., Cox, R. H., Snodgrass, W. R. and Maickel, R. P. (1969). Comparative effects of p-chlorophenylalanine, p-chloroamphetamine, and p-chloro-N-methylamphetamine on rat brain norepinephrine, serotonin, and 5-hydroxyindole-3-acetic acid. Biochem. Pharmacol., 19, 435-442.

Miller, F. P. and Maickel, R. P. (1969a). Role of brain amines in the behavioral depression produced by a benzoquinolizine. Life Sci., 8, 435-442.

Moore, K. E. (1971). Biochemical correlates of the behavioral effects of drugs. In Rech, R. H. and Moore, K. E. (eds). An Introduction to Psychopharmacology. Raven Press, New York.

Mueller, P. S. and McDonald, R. K. (1968). Hypothermia induced by reserpine and chlorpromazine. Proc. Soc. Exp. Biol. Med., 128, 826-30.

Myers, R. D. (1968). Discussion of serotonin, norepinephrine and fever. Adv. Pharmacol., 6, 318-321.

Myers, R. D. (1969). Temperature regulation: neurochemical systems in the hypothalamus. In W. Nauta, W. Haymaker, and E. Anderson (eds) The Hypothalamus. Charles Thomas, Springfield.

Myers, R. D. (1970). The role of hypothalamic transmitter factors in the control of body temperature. In J. D. Hardy (ed) Physiological and Behavioral Temperature Regulation. Charles Thomas, Springfield.

Myers, R. D. (1974). Handbook of Drug and Chemical Stimulation of the Brain. Van Nostrand Reinhold Company, New York.

Myers, R. D. (1975). Species continuity in the thermoregulatory responses of the pigtailed macaque to monoamines injected into the hypothalamus. Comp. Biochem. Physiol., 51A, 639-645.

Myers, R. D. and Yaksh, T. L. (1969). Control of body temperature in the unaesthetized monkey by cholinergic and aminergic systems in the hypothalamus. J. Physiol., 202, 483-500.

Myers, R. D. and Veale, W. L. (1970). Body temperature: possible ionic mechanism in the hypothalamus controlling the set point. Science, 170, 95-97.

Myers, R. D. and Beleslin, D. (1971). Changes in serotonin release in hypothalamus during cooling or warming of the monkey. Am. J. Physiol., 220, 1746-1754.

Myers, R. D. and Veale, W. L. (1971). The role of sodium and calcium ions in the hypothalamus in the control of body temperature of the unanaesthetized cat. J. Physiol., 212, 411-430.

Myers, R. D., Veale, W. L., and Yaksh, T. L. (1971). Changes in body temperature of the unanaesthetized monkey produced by sodium and calcium ions perfused through the cerebral ventricles. J. Physiol., 217, 381-392.

Myers, R. D. and Yaksh, T. L. (1971). Thermoregulation around a new set point established in the monkey by altering the ratio of sodium to calcium ions within the hypothalamus. J. Physiol., 218, 609-633.

Myers, R. D. and Brophy, P. D. (1972). Temperature changes in the rat produced by altering the sodium-calcium ratio in the cerebral ventricles. Neuropharmacol, 11, 351-361.

Myers, R. D. and Buckman, J. E. (1972). Deep hypothermia induced in the golden hamster by altering cerebral calcium levels. Amer. J. Physiol., 223, 1313-1318.

Myers, R. D. and Tytell, M. (1972). Fever: reciprocal shift in the brain sodium to calcium ratio as the set-point temperature rises. Science, 178, 765-767.

Myers, R. D. and Chinn, C. (1973). Evoked release of hypothalamic norepinephrine during thermoregulation in the cat. Am. J. Physiol., 224, 230-236.

Nickerson, M. (1970). Antihypertensive agents and the drug therapy of hypertension. In, The Pharmacological Basis of Therapeutics, Goodman, L. and Gilman, A. (eds). The MacMillan Company, New York.

Norwick. W. J., Stohler, L. M. and Swagzdis, J. (1966). The influence of steroids on drug metabolism in the mouse. J. Pharmacol. Exp. Ther., 151, 139-142.

Peterson, C. G. (1955). Neuropharmacology of Procaine I. Peripheral nervous actions. Anesthesiology, 16, 678-698.

Peterson, C. G. (1955a). Neuropharmacology of Procaine II. Central nervous actions. Anesthesiology, 16, 976-993.

Preziosi, P., Scapagnini, U., Nistico, G. (1968). Brain serotonin depletors and adrenocortical activation. Biochem. Pharmacol., 17, 1309-1313.

Prusiner, S. and Poe, M. (1968). Thermodynamic considerations of mammalian thermogenesis. Nature, 220, 235-236.

Reid, W. D. (1970). Turnover rate of brain 5-hydroxytryptamine increased by D-amphetamine. Br. J. Pharmacol., 40, 483-491.

Rosenberg, B., Kemeny, G., Switzer, R. C., and Hamilton, T. L. (1971). Quantitative evidence for protein denaturation as the cause of thermal death. Nature, 232, 471-473.

Rosenberg, B., Kemeny, G., Smith, L. G., Skurnick, I. D. and Bandurski, M. J. (1973). The kinetics and thermodynamics of death in multicellular organisms. Mech. Age. Dev., 2, 275-293.

Rudy, R. A. and Wolf, H. H. (1972). Effect of intracerebral injections of carbamylcholine and acetylcholine on temperature regulation in the cat. Brain Res., 38, 117-130.

Shellenberger, M. S. and Elder, J. T. (1967). Changes in rabbit core temperature accompanying alterations in brainstem monoamine concentrations. J. Pharmacol. Exptl. Ther., 158, 219-226.

Shemano, I. and Nickerson, M. (1958). Effect of ambient temperature on thermal responses to drugs. Can. J. Biochem. Physiol., 36, 1243-1249.

Shore, P. A., Pletscher, A., Tomich, E. G., Carlsson, A., Kuntzman, R., and Brodie, B. B. (1957). Role of brain serotonin in reserpine action. Ann. N.Y. Acad. Sci., 66, 609-617.

Silverstrini, B., Cotanese, B. and Del Basso, P. (1956). Researches on the increase of liver weight produced by some drugs. Biochem. Pharmacol., 15, 249-254.

Sjoqvist, F. and Hammer, W. (1968). The effect of desmethylimipromine and other "anti-tremorine" drugs in the metabolism of tremorine and oxytremorine in rats and mice. Biochem. Pharmacol., 17, 915-934.

Sokal, R. R. and Rohlf, F. J. (1969). Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, San Francisco.

Volicer, L. (1969). Correlation between behavioral and biochemical effects of P-chlorophenylalanine in mice and rats. Int. J. Neuropharmacol., 8, 361-364.

Welch, A. S. and Welch, B. L. (1967). Effect of P-chlorophenylalanine on brain noradrenaline in mice. J. Pharm. Pharmacol., 19, 632.

Westfall, B. A., Boulos, B. M., Shields, J. L. and Garb, S. (1964). Sex differences in pentobarbitol sensitivity in mice. Proc. Soc. Exp. Biol. Med., 115, 509-510.

Yehuda, S. and Wurtman, R. J. (1972). Release of brain dopamine as the probable mechanism for the hypothermic effect of D-amphetamine. Nature, 240, 477-478.