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MEANS OF A SIMPLE COOLING TEST

SCHOOL OF AVIATION MEDICINE
RANDOLPH AIR FORCE BASE, TEXAS

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**ESTIMATION OF THYROXINE OUTPUT BY THE THYROID GLANDS OF NORMAL AND
ADRENALECTOMIZED RATS BY MEANS OF A SIMPLE COOLING TEST**

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ESTIMATION OF THYROXINE OUTPUT BY THE THYROID GLANDS OF NORMAL AND ADRENALECTOMIZED RATS BY MEANS OF A SIMPLE COOLING TEST

Adrenalectomy, administration of propylthiouracil, or a combination of these two treatments increased significantly the rate of cooling of rats restrained and subjected to air at 5° C. Propylthiouracil administration increased rate of cooling 55 percent above that of untreated control rats while adrenalectomy increased rate of cooling 84 percent. The combination of these two treatments increased rate of cooling 99 percent. Dead rats cooled 201 percent faster than control rats. Administration of graded doses of thyroxine to both adrenalectomized-PTU-treated rats and to normal-PTU-treated rats indicated that 1.9 and 4.4 gamma per day of thyroxine, respectively, were required to return rate of cooling to that of comparable controls. These doses are, therefore, the best estimates of thyroxine outputs by thyroids of these animals. The results suggest that adrenalectomy is accompanied by diminished thyroid function. This fact makes difficult any evaluation of the relative roles of thyroid gland and adrenal glands in the maintenance of body temperature of rats exposed to cold.

Many separate studies have shown that rats without adrenals survive only a few hours when subjected to cold air (1-3). Surgically thyroidectomized rats may survive 4 or 5 days at similar temperatures (1, 4). Despite these facts, little is known of the relative contributions of each of these glands to the maintenance of body temperature of rats subjected to cold air. Previous studies had shown that adrenalectomy increased rate of cooling of rats subjected to cold air approximately 56 percent above that of normal rats subjected to cold air in the same fashion (3). An objective of the experiments reported here was to determine the effect of thyroidectomy and of thyroidectomy plus adrenalectomy on cooling rate in order to assess the relative contributions of each organ to maintenance of body temperature during exposure to cold air. It also seemed possible that this simple cooling procedure might be used to estimate daily output of thyroxine by thyroid glands of normal and adrenalectomized rats. The results of the experiment support this possibility and suggest that the thyroxine output of adrenalectomized rats is less than half that of normal rats.

METHODS AND RESULTS

Male rats of the Holtzman strain were used in all experiments. Body weights of the rats ranged from 200 to 250 gm. This range was strictly maintained because of the known effects of body weight on cooling rate (3). Large rats have been shown to cool more slowly than smaller rats. The effect of body weight on cooling rate within the range of body weights from 200 to 250 gm. was corrected by means of the formula given previously (3). By this means cooling rates were corrected to a standard weight of 240 gm.

Three separate experiments were performed. The materials and methods which were common to all three experiments are described below. The rats were kept 2 or 3 per cage in a room maintained at $24^{\circ} \pm 1^{\circ}$ C. and illuminated from 8 a.m. to 6 p.m. All rats were given Purina laboratory chow as food and were allowed ad libitum choice between tap water and 0.15 molar NaCl solution as drinking fluids until the cooling test took place.

Adrenalectomy was performed by the dorsal approach under ether anesthesia one week prior to the cooling test. Control rats were sham-operated to assess the effects of the operation.

Sham-operation consisted of removal of a piece of fatty tissue from the region of the adrenal gland.

To effect chemical "thyroidectomy" propylthiouracil, at a level of 0.1 percent, was mixed thoroughly into the diet. Treatment was begun two weeks prior to the cooling test.

Colonic temperature was measured using a copper-constantan thermocouple which was inserted 5 cm. into the colon of the rat. The thermocouples were led off to a potentiometer which continuously recorded the colonic temperature. The rats were restrained prior to cooling by taping each foot with adhesive tape and leaving a free tail, which was tacked to a board. After restraint for 15 or 20 minutes at room temperature, the rats were placed in a thermoregulated room maintained at $5^{\circ} \pm 2^{\circ} \text{C}$. The relative humidity of the room varied from 80 to 85 percent. Each rat was kept in cold air until its colonic temperature fell to 26°C . at which time it was removed from the cold; it was then freed and rewarmed artificially. Since repeated exposures to air at 5°C . have been shown to adapt rats to cold (i.e., reduce rate of cooling), each rat in these experiments was used in only one test. Over the range of colonic temperatures from 38° to 26°C ., the relationship between colonic temperature and duration of exposure to cold air was found, in previous studies, to be linear for both sham-operated and adrenalectomized rats (3, 5). Therefore, colonic cooling rate (CCR) measured in degrees centigrade per hour was

subsequently used to assess response of rats to cold. Statistical analysis of the difference between means was performed by the use of the t-test for the 95 percent confidence limit (6).

Experiment 1

One hundred and three rats were used in this experiment. Thirty-two rats were given propylthiouracil (PTU); 21 were adrenalectomized; 14 were treated with PTU and adrenalectomized; 7 were sacrificed by ether inhalation just prior to the cooling experiment; and 29 were sham-operated controls.

Details of this experiment—including number of rats per group, resting colonic temperature, and colonic cooling rate—are given in table I. In the order of increasing cooling rates the groups may be arranged as follows: sham-operated, PTU-treated, adrenalectomized, adrenalectomized + PTU-treated, and dead rats. Thus, PTU treatment increased CCR 54.9 percent above that of control rats; adrenalectomy, 84.0 percent; adrenalectomy + PTU treatment, 99.2 percent; dead rats were found to cool 200.7 percent faster than control rats. It appears that the effects of adrenalectomy and PTU-treatment are not quantitatively additive. Treatment of adrenalectomized rats with PTU did not increase CCR significantly above that of adrenalectomized-untreated rats ($P = .14$). Each experimental group listed in table I cooled significantly ($P < .01$) faster than the sham-operated, control rats.

TABLE I
The effect of thyroid and adrenal glands on colonic cooling rate and resting colonic temperature of rats

Group	Number of rats	Mean body weight (gm.)	Resting colonic temp. ($^{\circ}\text{C}$.)	Colonic cooling rate ($^{\circ}\text{C}$. hr.)	P*	Increase above control group
Sham-operated	29	248	$37.4 \pm 0.1^{\dagger}$	$3.93 \pm 0.31^{\dagger}$	—	—
PTU-treated	32	224	36.5 ± 0.2	6.09 ± 0.28	< .01	54.9
Adrenalectomy	21	238	36.7 ± 0.2	7.23 ± 0.26	< .01	84.0
Adx + PTU	14	215	34.9 ± 0.3	7.85 ± 0.27	< .01	99.2
Dead	7	252	36.3 ± 0.1	11.82 ± 0.52	< .01	200.7

*Probability value (comparison with CCR of sham-operated group).

$^{\dagger} \pm 1$ standard error of mean.

Table I also lists the initial mean colonic temperature of each group of rats in air at 25° C. Each of the treatments listed reduced resting colonic temperature significantly ($F < .01$) below that of control rats.

Experiment 2

Forty-four rats were used in this experiment. Fifteen rats were treated with PTU for two weeks prior to the cooling test and were given an intraperitoneal injection of 2.5 gamma thyroxine per day (in 0.25 ml. normal saline) for two days before and on the day of the cooling test (one hour before cold exposure). Nine other rats were also treated with PTU but were given 3 gamma thyroxine per day (in 0.25 ml. saline), while a third PTU-treated group of 8 rats received 4 gamma thyroxine per day. Control rats were injected with 0.25 ml. saline. A fifth group of 12 rats was given PTU + 0.2 percent desiccated thyroid powder, mixed thoroughly into the diet, for two weeks preceding the test. All rats were subjected to the standard cooling test described above.

The results of this experiment are shown in table II and figure 1. Administration of 2.5 gamma thyroxine per day to PTU-treated rats did not counteract completely the effect of propylthiouracil on colonic cooling rate. These rats cooled 39.6 percent faster than sham-operated

controls. Administration of 3 gamma thyroxine per day to PTU-treated rats returned CCR to 12.7 percent of that of control rats while administration of 4 gamma returned rate of cooling nearly to normal (7.6 percent). Differences between mean CCR's of sham-operated rats and rats receiving 3 or 4 gamma thyroxine were not significant. Addition of 0.2 percent desiccated thyroid powder to the food appeared to produce an effect on CCR equivalent to daily injection of 3.2 gamma thyroxine per day. Administration of even the lowest dose of thyroxine (2.5 gamma per day) returned to normal the initial resting colonic temperature as measured prior to cooling (table II). Further increase in dose level to 3 and 4 gamma per day caused an elevation in colonic temperature significantly above that of sham-operated rats.

It may be observed in figure 1 that a smooth curve connecting the mean cooling rates of PTU-treated, normal rats given 0, 2.5, 3, or 4 gamma thyroxine per day would cross the line representing mean cooling rate of sham-operated rats at 4.4 gamma thyroxine per day. If it is assumed that PTU inhibits completely only thyroid function, this value would appear to be an estimate of the daily thyroxine output of the thyroid gland of the normal rat since it is this dose of thyroxine which returns rate of cooling of PTU-treated rats to that of untreated, normal rats.

TABLE II
Effects of propylthiouracil and thyroxine treatment on colonic cooling rate of sham-operated rats

Group	Number of rats	Mean body weight (gm.)	Resting colonic temp. (°C.)	Colonic cooling rate (°C./hr.)	P*	Percent increase above control group
Sham-operated	29	248	37.4 ± 0.1 [†]	3.97 ± 0.31 [†]	—	—
PTU-treated	32	224	36.5 ± 0.2	6.09 ± 0.28	< .01	54.9
PTU + 2.5 y thyroxine	15	231	37.4 ± 0.1	5.49 ± 0.25	< .01	39.6
PTU + 3.0 y thyroxine	9	241	38.1 ± 0.1	4.43 ± 0.56	.44	12.7
PTU + 4.0 y thyroxine	8	243	38.3 ± 0.1	4.23 ± 0.44	.64	7.6
PTU + 0.2% desiccated thyroid powder	12	233	37.9 ± 0.2	4.92 ± 0.25	.06	25.2

*Probability value (comparison with CCR of sham-operated group).

[†] ± 1 standard error of mean.

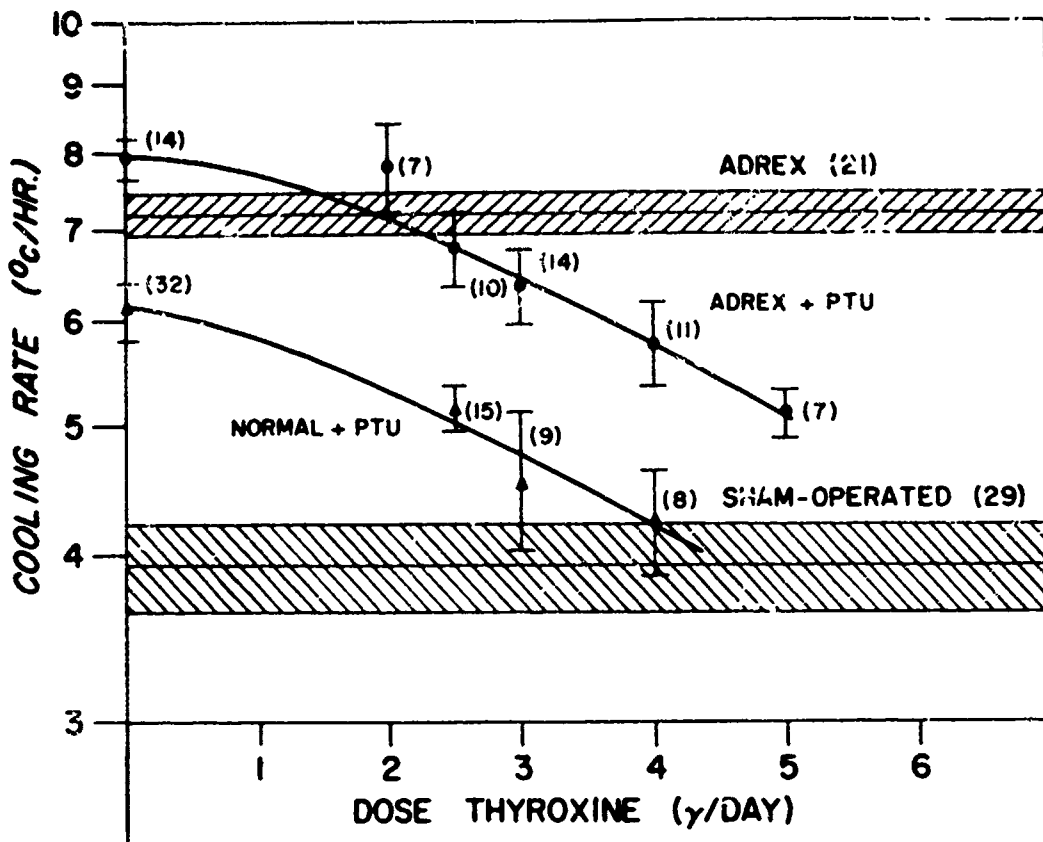


FIGURE 1

Colonic cooling rate (°C./hr.) graphed against the dose of thyroxine administered (gamma/day) to adrenalectomized (●) and normal (▲) rats receiving PTU. ± 1 standard error is set off at each mean. The mean colonic cooling rate of adrenalectomized untreated rats ± 1 standard error is shown as a line across the top of the figure; that for sham-operated rats is represented by a line across the bottom of the figure. The number in parenthesis represents number of rats used.

Experiment 3

Sixty-three rats were used in this experiment. All rats were adrenalectomized one week prior to the cooling test. When PTU was used, treatment began two weeks prior to the cooling test while thyroxine treatment began two days prior to the cooling test with the final dose being given on the day of the cooling test, one hour before cold exposure. The various treatments, number of rats in each group, mean body weight, initial body temperature prior to cold exposure, and colonic cooling rate are given in table III. The probability values shown in table III indicate that treatment with PTU and 4 or 5 gamma thyroxine per day decreased significantly the CCR.

In figure 1 it may be observed that 1.9 gamma

thyroxine per day returned CCR of PTU-treated, adrenalectomized rats to that of adrenalectomized rats. Higher doses of thyroxine decreased rate of cooling still further. Administration of 5.0 gamma per day of thyroxine to PTU-treated, adrenalectomized rats reduced CCR significantly below that of adrenalectomized rats and within 29 percent of that of sham-operated rats.

Table III indicates that administration of 3.0 gamma or more of thyroxine per day to adrenalectomized-PTU-treated rats returned resting body temperature to that of sham-operated rats. Lower doses were ineffective in this regard. None of the doses used increased body temperature of adrenalectomized rats above that of sham-operated rats as was observed for normal PTU-treated rats (table II).

DISCUSSION

The rate of fall of colonic temperature of restrained rats exposed to air at 5° C. is influenced by both adrenal and thyroid glands. Removal of either increases rate of cooling. It is interesting that the combination of adrenalectomy with propylthiouracil treatment did not make the effects of each additive. However, rate of cooling of such rats was greater than that of either treatment alone. The relative contribution to heat production by muscular activity of adrenalectomized rats may be different from that of thyroidectomized or normal rats. This possibility could help to explain the differences in cooling rate of these three groups of rats. Ring (7) has shown that total body heat production of adrenalectomized rats at any given body temperature during cooling was less than that of normal rats while the change of metabolism (heat production) per change of body temperature was slightly higher. It is likely that the lower metabolism of adrenalectomized rats during cooling is due either to muscular fatigue (less shivering) or to depletion of carbohydrate stores or both. Other experimental evidence exists which supports the fact that muscles of adrenalectomized rats

fatigue more quickly than those of normal rats (8). The relative fatigability of thyroidectomized rats apparently has not been tested.

These experiments suggest, but do not prove, that adrenalectomy, in addition to abolishing adrenal function, also appears to diminish thyroid function. Administration of PTU to adrenalectomized rats did not increase significantly the cooling rate above that of adrenalectomized rats (table III). Measurement of cooling rates of PTU-treated-adrenalectomized rats given graded doses of thyroxine suggests that the daily output of thyroxine by thyroid glands of adrenalectomized rats is less than half that of thyroid glands of normal rats. This interpretation of the data agrees with the findings of Flückiger and Verzar (9), who observed that tracer doses of radioactive iodine were picked up normally by thyroid glands of adrenalectomized rats but were released much more slowly. These authors suggested that the decrease in release of radioactive iodine by the thyroid gland following adrenalectomy was a consequence of the reduced metabolic rate accompanying this condition. The reduced thyroid function of adrenalectomized rats is an important consideration in attempting

TABLE III

The effect of thyroxine and propylthiouracil on the colonic cooling rate and resting body temperature of adrenalectomized rats.

Group	Number of rats	Mean body weight (gm.)	Resting body temperature (°C.)	Colonic cooling rate (°C./hr.)	P*	P†
Sham-operated	29	248	37.4 ± 0.1‡	3.93 ± 0.31*	—	—
Adrenalectomized	21	238	36.7 ± 0.2	7.23 ± 0.26	—	< .01
Adx. + PTU	14	215	34.9 ± 0.3	7.85 ± 0.27	.14	< .01
Adx. + PTU + 2 γ thyroxine	7	253	36.3 ± 0.3	7.81 ± 0.61	.32	< .01
Adx. + PTU + 2.5 γ thyroxine	10	231	35.7 ± 0.1	6.77 ± 0.35	.11	< .01
Adx. + PTU + 3.0 γ thyroxine	14	241	37.3 ± 0.2	6.36 ± 0.41	.07	< .01
Adx. + PTU + 4.0 γ thyroxine	11	243	37.5 ± 0.2	5.77 ± 0.41	< .01	< .01
Adx. + PTU + 5.0 γ thyroxine	7	223	37.3 ± 0.2	5.08 ± 0.19	< .01	.09

*Probability value (comparison of CCR with that of adrenalectomized group).

†Probability value (comparison of CCR with that of sham-operated group)

‡± 1 standard error of mean.

to assess the relative contributions of adrenal glands and thyroid gland to maintenance of body temperature in the cold. This finding suggests another possible explanation for the faster cooling of adrenalectomized rats as compared with PTU-treated rats.

The estimated thyroxine output of the thyroid gland of normal rats in this experiment was 4.4 gamma per day. This value is similar to that observed by Dempsey and Astwood (5.2 gamma per day) to be necessary for reduction of thyroid size to normal after treatment with thiouracil and maintenance at an air temperature of 25° C. (10). Similar experiments were not performed by these investigators on adrenalectomized rats.

It is interesting to compare the data of experiment 1 with that observed in experiments 2 and 3. The combination of adrenalectomy and thyroidectomy increased rate of cooling 99 percent above that of sham-operated, control rats, while thyroidectomy (PTU treatment) alone increased cooling rate 55 percent above that of controls. The difference between these two should be due to lack of adrenal glands (44 percent). But adrenalectomy alone was observed to increase rate of cooling 84 percent above that of control rats. The difference between this value and that calculated by difference above (44 percent) must be that resulting from lack of thyroid function (45 percent). If it is assumed that PTU-treatment causes complete inhibition of thyroxine formation by the thyroid gland, then the thyroid gland of adrenalectomized rats must be functioning at $(55-45)/55 \times 100$ or 19 percent of its normal activity. Assuming a normal daily thyroxine output of 4.4 gamma per day, the cooling rate data above would suggest that the daily thyroxine output of adrenalectomized rats was about 0.8 gamma per day. This is about half of that calculated from experiment 3 (1.9 gamma per day). Qualitatively, this rough calculation also indicates a decreased thyroid function in adrenalectomized rats. The quantitative differences between the daily output of thyroxine calculated above and that observed in experiment 3 may be due to the implicit assumption used above—viz, that thyroidectomy inhibits

only thyroid function and adrenalectomy inhibits only adrenal function. This assumption is probably incorrect. For example, experiment 3 has demonstrated that adrenalectomy is accompanied by decreased thyroid function. On the other hand, surgical thyroidectomy (11) or treatment with thiouracil (12-14) appear to produce adrenal atrophy and diminished adrenal function, although propylthiouracil does not (15, 16).

It may be observed in figure 1 that linear extrapolation of the curve for adrenalectomized rats treated with PTU to the line representing the mean colonic cooling rate of sham-operated rats would give an intersection at approximately 7.1 gamma thyroxine per day. This is the amount of thyroxine required theoretically to return rate of cooling of PTU-treated—adrenalectomized rats to that of sham-operated rats. This value is considerably higher than that necessary to return rate of cooling of PTU-treated normal rats to that of sham-operated rats (4.4 gamma per day). It is possible that this difference in dose levels between adrenalectomized and sham-operated rats may represent a difference in utilization of thyroxine at the cellular level. Indeed, Hoffman et al. (17) observed that the oxygen consumption of adrenalectomized thyroidectomized rats given thyroxine did not increase by the same percentage as that of thyroidectomized rats given the same dose of thyroxine. Administration of adrenal cortical extract to the adrenalectomized-thyroidectomized rats resulted in a normal response to injections of thyroxine.

Although it has been observed that adrenalectomized rats cool faster than thyroidectomized rats, it cannot be stated unequivocally that adrenal glands are more important in maintenance of body temperature during cold exposure under these conditions. The more rapid cooling rate of adrenalectomized rats could be due to: (a) decreased thyroid function; (b) decreased utilization of thyroxine at the cellular level; (c) decreased muscular activity and shivering; (d) a combination of all these factors and (e) other factors. Items (a) and (b) above have been observed experimentally; the other possibilities must await further experimentation.

SUMMARY

Adrenalectomy, administration of propylthiouracil, or a combination of these two treatments increased significantly the rate of cooling of rats restrained and subjected to air at 5° C. Propylthiouracil administration increased rate of cooling 55 percent above that of untreated, control rats while adrenalectomy increased rate of cooling 84 percent. The combination of these two treatments increased rate of cooling 99 percent. Dead rats cooled 201 percent faster than control rats. Administration of graded doses of thyroxine to both adrenalectomized - PTU-treated rats and to normal - PTU-treated rats indicated that 1.9 and 4.4 gamma per day of thyroxine,

respectively, were required to return rate of cooling to that of comparable controls. These doses are therefore the best estimates of thyroxine outputs by thyroids of these animals. The results suggest that adrenalectomy is accompanied by diminished thyroid function. This fact makes difficult any evaluation of the relative roles of thyroid gland and adrenal glands in the maintenance of body temperature of rats exposed to cold.

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