only 6% of TS was converted into hydroxylated polar steroids. Jacobson, *et al.*[15] had reported that testosterone was more readily hydroxylated by the microsomal fraction of male rat liver than that of the female. Possible interpretation of these striking discrepancies observed between testosterone and TS in both sexes is not available at present. In view of the fact that some steroid sulfates can serve as an active intermediate in the metabolism,[2] these results may be of any physiological significance of TS or related steroid sulfates in the female rat, but await further elucidation.

Formation of 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone), a potent androgen, was demonstrated only when TS was incubated with the microsomal fraction of female rat liver. The catabolic sequence of 3-oxo-Δ^4-steroids is well established to proceed mainly *via* 4,5-dihydro-3-oxosteroids to saturated 3-hydroxysteroids by the consecutive action of Δ^4-hydrogenases and 3-oxosteroid oxidoreductases.[5] Thus, in most cases, 3-oxo-5α-steroids were preferentially reduced to 3-hydroxy-5α-steroids in our incubation condition.

The sex difference in the Δ^4-5α-hydrogenase activity was clearly demonstrated further by the fact that the 5α-enzyme of female rat liver produced 5α-steroid from TGA though in a small amount, whereas the 5α-enzyme from male rat liver did not yield any 5α-metabolite.[3] Finally, metabolism of testosterone also indicated sex-specific patterns of microsomal 3-oxosteroid oxidoreductase activities, since testosterone was transformed to 3α-hydroxy-5α-steroid by the female, while it was converted to 3β-hydroxy-5α-steroid by the male.[3] These results are in good agreement with the perfusion experiment of testosterone with the rat liver.[16]

15) M. Jacobson and R. Kuntzman, *Steroids*, **13**, 327 (1969).
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Changes in Catecholamine Levels of Mouse Brain during Oscillation-stress

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It has been demonstrated that increased sympathetic activity associated with stressful conditions increases synthesis of norepinephrine (NE) and epinephrine (Epi) in various tissues.[2,3] These conditions include: intense muscular exercise,[3] immobilization, [4,5] revolving drum,[6] changes in environmental temperature[2,7,8] and chronic electroshock sessions.[9,10]

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2) R. Gordon, S. Spector, A. Sjoerdsma, and S. Udenfriend, *J. Pharmac. Exp. Ther.*, **153**, 440 (1966).
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Early studies with these situations had suggested that the increased turnover rates of NE and Epi were the results of the stressful conditions applied to the animals. Gordon, *et al.*,[2] for example, demonstrated that both exercise and cold, though they had little effect on NE and Epi levels, produced marked depletion of NE and Epi when catecholamine synthesis was blocked. This experiment was designed to investigate what would follow if the stress was prolonged.

Result and Discussion

It was found that NE level of the mouse brain was lowered gradually and significantly after the animals were oscillated for more than 2 hr (Table I). These findings, however, are not in agreement with the data reported by other authours.[2-4,6,10] They demonstrated that stress alone did not induce large decrease in the level of brain NE unless the synthesis was blocked by some means. In other words, increased nerve activity induced by stress may activate NE turnover, but have no influence upon NE level in the brain.

When the αMMT treated mice were oscillated, NE level decreased significantly after 1/2 hr of oscillation, compared with the αMMT treated non-oscillated group. After 1 or 2 hr of oscillation, the NE level in the αMMT treated oscillated group was found to be significantly higher than that of the αMMT treated non-oscillated one (Table I). In our previous study on oscillation fatigue,[11] it was found that motor activity of mice was first activated after a 1/2 hr oscillation, decreased sharply after that for 2 hr and completely fatigued by 4 hr of oscillation. It would seem plausible to assume that the turnover rate of NE in the mouse brain might be elevated after 1/2 hr of oscillation. When αMMT inhibited NE synthesis of activated mice, NE level was decreased further by the oscillation because of the increased turnover rate.

Table I. Norepinephrine in Mouse Brain in Per Cent of Normal Values
(0.36±0.006 μg/g; $n=13$)

Treatment	NE in % of control, oscillated for hours of			
	1/2	1	2	4
Untreated	100 (9)	100 (6)	100 (9)[a]	100 (8)[b]
Oscillation	99.1±3.2(8)	99.0±2.9(6)	91.5±2.1(9)[c]	83.0±2.6(7)[d]
α-MMT	70.2±1.9(10)[e]	49.9±3.6(6)	35.2±1.5(9)	14.9±1.1(8)[f]
α-MMT+oscillation	64.0±2.0(10)[g]	50.4±3.6(6)	36.0±1.5(8)	23.0±0.9(7)[h]

statistical significance: *a*) $P<0.01$, *b*) $P<0.001$, *c*) $P<0.01$, *d*) $P<0.001$, *e*) $P<0.05$, *f*) $P<0.01$, *g*) $P<0.05$, *h*) $P<0.01$
Means±s.e.m. 100 mg/kg of α-methyl-meta-tyrosine was given *i.p.* immediately before the animals were put into the cage in a group of 12 and oscillated for 1/2, 1, 2, or 4 hours. Figures in the parentheses indicate number of experiments.

Table II. Dopamine in Mouse Brain in Per Cent of Normal Values
(0.89±0.023 μg/g; $n=13$)

Treatment	DA in % of control, oscillated for hours of			
	1/2	1	2	4
Untreated	100 (10)	100 (6)	100 (9)	100 (8)
Oscillation	109.9±5.8(8)	103.5±1.2(6)	105.3±3.9(9)	101.3±3.3(7)
α-MMT	76.0±1.8(10)	56.1±4.3(6)	39.5±3.6(9)	28.5±1.6(8)
α-MMT+oscillation	76.3±3.5(10)	56.8±3.0(6)	47.9±2.2(8)	33.9±3.1(7)

Means±s.e.m. 100 mg/kg of α-methyl-meta-tyrosine was given *i.p.* immediately before the animals were put into the cage in a group of 12 and oscillated for 1/2, 1, 2, or 4 hours. Figures in the parentheses indicate number of experiments.

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The trends of NE level after the continuation of oscillation for more than one hr with or without αMMT treatment are difficult to interpret.

Brain dopamine levels were not decreased by oscillation (Table II). However, there were consistent slight increases of DA in every oscillated groups. This is in a good agreement with the report of Goldberg and Salama.[6] The mechanisms of these changes are open to question.

Material and Method

Female ddy-strain mice (20—24 g) were used throughout the study. One week prior to each study, the thirteen animals were housed in a light-tight room maintained at 23+1°. The room was illuminated artificially from 7:00 am to 7:00 pm and darkened from 7:00 pm to 7:00 am. Commercial rat chow (CH-2, Clea Japan Inc.) and water were available ad libitum. On the day the animals were sacrificed, they were brought into the laboratory at 7: 30 am and food was removed. Great care was taken during the transfer.

α-Methyl-*meta*-tyrosine (αMMT, Mann Research Laboratories) was dissolved in a small amount of 4 N NaOH, and the solution was adjusted to a pH of 7 with 4 N HCl and diluted with saline to form an 10 mg/ml solution. The αMMT or vehicle solutions were intraperitoneally administered immediately before the oscillation began.

Oscillation-stress was conducted at 20+1° in a stainless cage previously spread with 7 g polyethylene chips, as previously reported.[11] The system permitted a to- and fro operation at 129 excursions per minute. Mice were stressed in groups of 12 animals, for 1/2, 1, 2, or 4 hr. All the animals were killed by decapitation at 11: 30 am, when all the oscillations were scheduled to end. The brain was rapidly exposed and removed, following the guidelines given by Welch and Welch,[12] and assayed on the same day. Three brains were pooled together for each measurement. Brain NE and dopamine (DA) were analyzed fluorometrically according to the method of Shellenberger and Gordon.[13] Vehicle administered groups served as controls in relating to the αMMT administered ones.

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Studies on Indole Derivatives. XXIII.[1] Diels-Alder Reaction of 3-Indoledithiocarboxylic Acid Derivatives and Dimethyl Acetylenedicarboxylate and Reactions of Their Products

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We reported previously that the reaction of indoles with carbon disulfide in tetrahydrofuran, using sodium hydride, and methylation of its products with dimethyl sulfate afforded methyl 3-indoledithiocarboxylates, which were derivatives of an enamino dithiocarboxylate, and the replacement reaction of these dithiocarboxylates with nucleophilic reagents gave the

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2) Location: *1-14, Bunkyo-machi, Nagasaki.*