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URINARY EXCRETION OF CATECHOLAMINES IN VARIOUS SITUATIONS

BY

AYAKO SUDOH*1

ABSTRACT

Urinary excretion of adrenaline and noradrenaline was measured in human subjects in various situations. It was found that the catecholamine excretion increased in some of these situations. The increase of adrenaline excretion was about 400% during giving birth, 130% in a mental work 80% in tilting of posture, and 30% in monotonous light work, and the increase of noradrenaline excretion was about 130% in tilting of posture, 60% in heavy physical work, and 20% on the first day of mental work, compared to each control value. From these findings, it was concluded that adrenaline excretion is elevated in mental or emotional strains and that the quantity of increased adrenaline might be roughly proportional to the degree of the strain. Noradrenaline excretion was also thought to have a connection with emotion though to a less extent than adrenaline excretion. It was tentatively considered that passive emotion or mental strains induce the increase of adrenaline excretion and active emotion or physical strains are accompanied by the increase of noradrenaline output.

INTRODUCTION

The sympathetic nervous system and the adrenal medulla were found to be activated by emotional stimuli in animals in the classical investigation of Cannon[1)], and then many studies have since been made to clarify the effect of emotional or mental strains on the sympathico-adrenomedullary system in human beings.

In the early period of this century, adrenaline was isolated from the adrenal gland by Takamine[2)] and it was found to be a physiologically active substance. Later, noradrenaline was identified by Euler[3)] with a sympathetic nerve transmitter in the nerve endings, and then the pathway of catecholamine biosynthesis and metabolism was revealed using radioactive substrates. On the other hand, adrenaline and noradrenaline were found to be excreted into urine in a constant proportion after administration of these amines[4)], and it was found that free radioactive catecholamines

*1 須藤綾子: Department of Hygiene (Chief: Prof. H. KITA), School of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
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in urine are reduced within 30 min after their injection[5]. Therefore, quantitative information about the activity of the sympathico-adrenomedullary system would be obtained by measuring the amount of adrenaline and noradrenaline in urine collected during a certain period of time.

In the present work, urinary excretion of adrenaline and noradrenaline of human subjects was measured in various situations as follows: (a) passive tilting of posture, (b) a continuous arithmetic calculation, (c) monotonous light work, (d) heavy physical work, and (e) giving birth. For these studies, a semi-automated procedure was introduced to determine the amount of adrenaline and noradrenaline in human urine accurately and speedily.

Method

The trihydroxyindole method of Euler and Lishajko[6] seems to be suitable for the determination of urinary catecholamines, because of its specificity and sensitivity. However, many of the errors in this manual method are considered to be due to incorrect timing of the addition of reagents and of the reading of the fluorescence intensity, accidental contamination from glassware, and instability of some reagents. These disadvantages should be eliminated if the method is carried out by an auto-

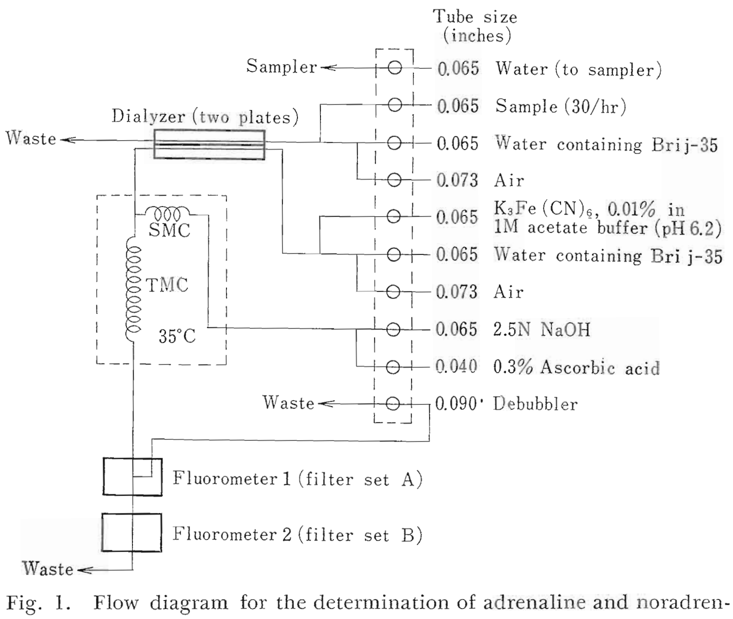

Fig. 1. Flow diagram for the determination of adrenaline and noradrenaline with Technicon AutoAnalyzer.
Filter set A. 1°: 405 nm, 2°: over 485 nm.
Filter set B. 1°: 436 nm, 2°: over 510 nm.

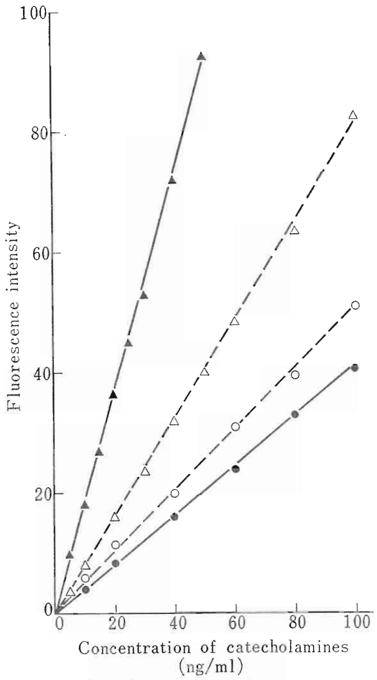

Fig. 2. Calibration curves for adrenaline and noradrenaline.

········△······· adrenaline with filter set A.
········○······· noradrenaline with filter set A.
——▲—— adrenaline with filter set B.
——●—— noradrenaline with filter set B.

mated analysis.

After purification of catecholamines in human urine with alumina by the method of Euler and Lishajko[6], citric acid was added to the alumina eluate to prevent the interference of aluminum hydroxide produced at neutral pH for the determination. These samples were assayed with Technicon Auto Analyzer. The flow diagram of the method is shown in Fig. 1. For the differential estimation of the two catecholamines, two filter sets were used.

Linear relationship between concentration of catecholamine and its fluorescence intensity by this method is shown in Fig. 2. The recovery of 20 ng/ml of adrenaline or 40 ng/ml of noradrenaline added to the alumina eluate of urine was 93% on an average in both.

To study the relation between the present automated method (y, y′) and the manual method of Euler and Lishajko[6] (x, x′), adrenaline and noradrenaline in fifty urine eluates were determined by these two methods.

A significant correlation between the data obtained by the two methods was found on each amine ($r=0.941$ and 0.978, $y=0.86x+4.4$ and $y'=1.05x'-2.4$ for adrenaline and noradrenaline, respectively).

The advantage of this automated method is rapidity of determination and 30 samples of alumina eluate can be assayed in 1 hr.

EXPERIMENTS

Experiments 2 and 3-i in this paper were partially described in other reports[7,8].

Experiment 1. Adrenaline and noradrenaline in normal human urine.

Urine specimens from three healthy subjects, 24, 29, and 33 years old, were collected during 24 hr at 6-hr intervals starting at noon. The subjects were research workers and they did their usual work in the experimental day. Free adrenaline and noradrenaline, and creatinine[9] were determined on each urine. The excretion was expressed as ng per mg of creatinine (ng/mg creatinine), considering the difficulty of punctuality in urine collection.

Diurnal variation of adrenaline and noradrenaline excretion in normal human life is shown in Fig. 3. The catecholamine values were high in daytime and low at night, and there was no remarkable variation in the average values of catecholamine excretion during daytime.

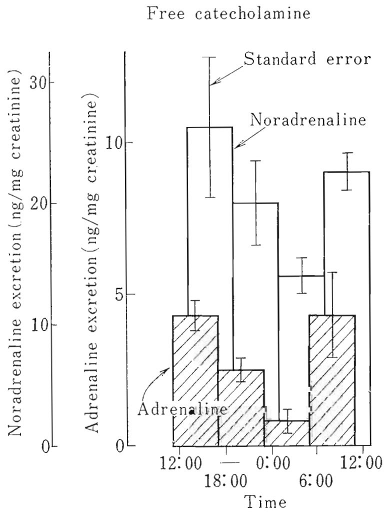

Fig. 3. Diurnal variation of adrenaline and noradrenaline excretion in normal human life.

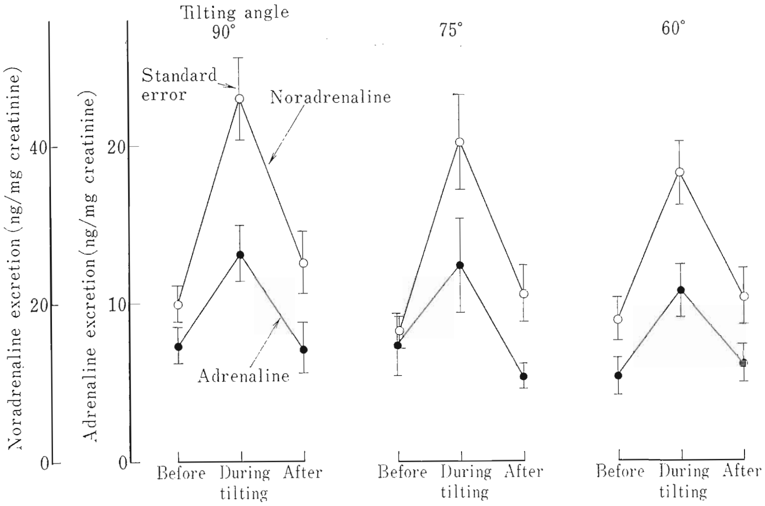

Fig. 4. Adrenaline and noradrenaline excretion before, during, and after tilting of posture.

Experiment 2. Postural change.

Seven healthy students, 21 to 26 years of age, were tilted passively in the supine position on a tilt board for 90 min, from 11:00 to 12:30, after 60 min in a recumbent posture. Thereafter, the subjects took recumbency again during 90 min. This was carried out over three days, and tilting angle was 75° on the first experimental day, 90° on the second day, and 60° on the last day. Three urine specimens were collected from each subject on each experimental day at 10:00–11:00, 11:00–13:00, and 13:00–14:00. Free adrenaline and noradrenaline, and creatinine[9] were measured on each urine specimen.

As seen in Fig. 4, tilting from recumbency to 60°, 75°, or 90° induced a rise in noradrenaline excretion from the preceding value of 18.0, 16.6, and 20.0 to 36.4, 40.6, and 45.9 ng/mg creatinine, respectively ($p<0.01$). Adrenaline output also increased significantly during the tilting ($p<0.05$), from 5.3 to 10.7, 7.4 to 12.4, and 7.4 to 13.1 ng/mg creatinine at 60°, 75°, and 90°, respectively. After tilting, the value of each amine returned to the level similar to that in the control period from every angle.

The heart rate increased by about 30/min during the tilting. The pulse pressure was found to be maintained in a reduced value during tilting compared with that in recumbency, and this was due to the decreased systolic pressure (about 15 mm Hg) and the increased diastolic pressure (about 10 mm Hg). These were observed for every angle.

Experiment 3. Continuous arithmetic calculation.

3-i. Sixteen healthy males, 20 to 55 years, performed Kraepelin-Uchida's test (a continuous arithmetic calculation) from 10:30 to 11:30. They were previously instructed to carry out this test speedily and exactly. 'Test' urine was collected from 10:30 to 12:00, 'control' urine from 9:30 to 10:30, and 'recovery' urine from 12:00 to 13:00. In the latter two periods, the subjects were sitting quietly on a chair.

Average adrenaline excretion was 6.6 ng/mg creatinine for the control period, and significantly increased to 14.0 during the mental load ($p<0.05$) (Fig. 5). The increase of adrenaline showed a tendency to be more remarkable in the ten younger subjects (average increase of adrenaline 130%) than in the older six subjects (70%), but did not differ significantly between the two groups. Average number of figures calculated, that is, work performance rate, was 57 and 42/min in the younger and the older, respectively. A slight increase of noradrenaline excretion was observed during the test, though not significant.

3-ii. In order to elucidate whether this increase of adrenaline excretion is found in the repeating of the task, two healthy males, 22 and 23 years, performed the same test from 10:30 to 11:30 every day for five consecutive days. They were also asked to do the test speedily and exactly as possible. Urine was collected from 9:30 to 10:30, and 10:30 to 12:00. The increase of adrenaline excretion by the test was recognized in all experimental days (Table 1), which indicate that they did not adapt to such a stress within five days, judging from adrenaline excretion. Work performance rate was 62, 71, 74, 78, and 83/min on the first, second, third, fourth,

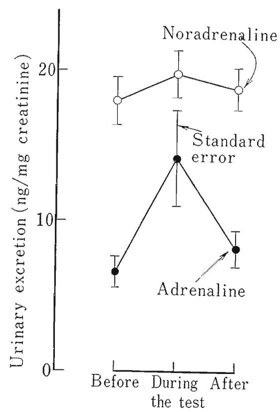

Fig. 5. Adrenaline and noradrenaline excretion before, during, and after an arithmetic calculation test in male subjects.

Table. 1. Adrenaline excretion before and during the calculation test carried out for five consecutive days

Subject	Day	Adrenaline excretion (ng/mg creatinine)		Relative working capacity
		Before test	During test	
M	1	4.7	10.8	72
	2	6.9	13.9	78
	3	4.0	11.3	84
	4	6.4	12.5	87
	5	4.3	12.8	92
H	1	2.8	8.1	51
	2	3.1	7.0	63
	3	0.4	4.4	64
	4	2.5	6.4	68
	5	1.2	5.8	73

and fifth days, respectively.

3-iii. The effect of calculation speed on catecholamine excretion was studied on another group. Eleven healthy males, average 25 years, were directed to carry out the test as much as possible, similarly as above, from 10:30 to 11:30 on two consecutive days, and next day they were requested to perform the calculation at usual speed without any other instruction. About 150% increase in adrenaline output was observed in the test period

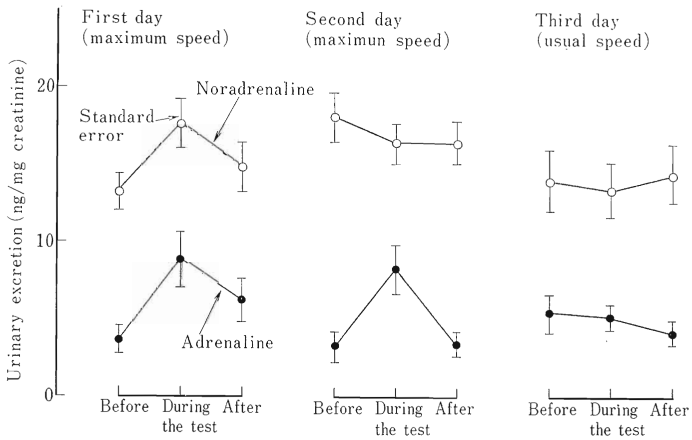

Fig. 6. Adrenaline and noradrenaline excretion before, during, and after the calculation test with maximum speed and usual speed.

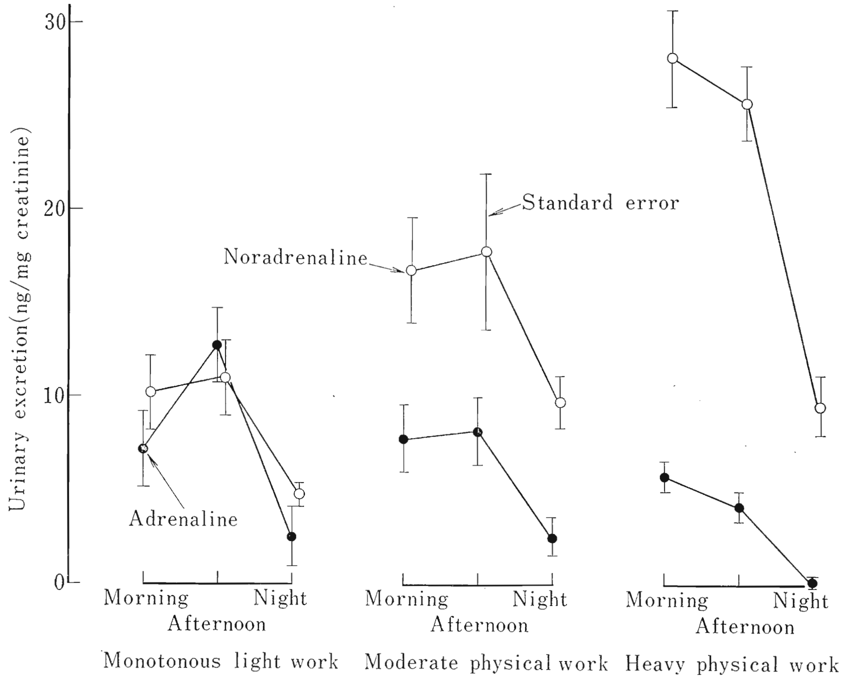

Fig. 7. Adrenaline and noradrenaline excretion in monotonous light work, moderate physical work, and heavy physical work.

of the first two days of maximum speed ($p<0.01$), but any significant increase of adrenaline output was not found during the task on the third day of usual speed (Fig. 6). In the first day, noradrenaline level significantly increased during the test ($p<0.05$), but not on the other two days. Average number of figures calculated was 70 and 75/min on the first and second days of maximum speed, respectively, and 63/min on the third day of usual speed.

Experiment 4. Monotonous light work and heavy physical work.

Subjects investigated were four assemblers (monotonous light work, A group), four machine tool operators (moderate physical work, B group), and four forging men (heavy physical work, C group). The working time was from 8:00 to 16:00. Their urine was collected during 22:00 to 6:00 (night urine), 6:00 to 12:00 (morning urine), and 12:00 to 16:00 (afternoon urine) on two working days.

The results are presented in Fig. 7. The level of adrenaline excretion in monotonous light work was higher in the afternoon than in the morning ($p<0.05$), but, on the contrary, the level in heavy physical work was lower in the afternoon than in the morning ($p<0.05$). The value between 6:00 and 16:00 was 10.0, 8.0, and 5.0 in adrenaline, and 10.6, 17.2, and 26.9 ng/mg creatinine in noradrenaline, in A, B, and C groups, respectively. This value of noradrenaline of the forging men (26.9) was significantly higher than that of the assemblers (10.6) ($p<0.05$). The value of noradrenaline, obtained by subtracting the night value from the value between 6:00 and 16:00, was significantly higher in the forging men than those in the other two groups ($p<0.05$). There was a tendency for increase in adrenaline and decrease in noradrenaline in monotonous light work compared with moderate physical one.

Experiment 5. Delivery.

Eight pregnant women, 26 to 29 years old, were studied during and after delivery. The subjects were all hospitalized when they felt some degree of pains or recognized the water, and they had normal term pregnancies and deliveries with normal babies. They stayed in the hospital nearly one week after delivery. These were the first childbirths in four subjects, the second childbirths in three subjects, and the third in one subject. The first urine collection was made at the time of hospitalization,

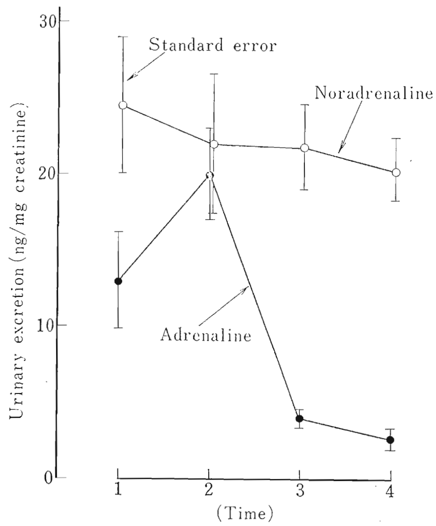

Fig. 8. Adrenaline and noradrenaline excretion during and after delivery.
Time: 1. Before hospitalization.
2. From hospitalization to delivery.
3. About 24 hr (first day) after delivery.
4. Next 24 hr (second day).

the second collection was about 30 min after delivery, and then voluntarily, almost at several-hour intervals, during about 48 hr.

Adrenaline level was markedly elevated in the period between hospitalization and delivery (average 19.9 ng/mg creatinine) in all the subjects compared to that on the first day after delivery (4.0) ($p<0.01$), and the level before hospitalization (13.1) was also higher than that after delivery ($p<0.05$) (Fig. 8). Adrenaline value on the first day after delivery was higher than that on the second day (2.6) ($p<0.01$). Noradrenaline excretion was about 30% higher on the first day after delivery than during delivery in six subjects, but not in two subjects.

Discussion

On carrying out the experiment lasting for several hours to investigate the influence of a stress upon the excretion of a certain hormone, it is necessary to ascertain the usual excretion of this hormone. Catecholamine values in urine were high in daytime and low at night in normal human life, but there was no remarkable variation in the average values of catecholamine excretion during daytime. In the study of daytime sleep[10], it was found that the catecholamine level between 9:00 and 15:00 was reduced near the low level of the night time. Therefore, the diurnal rhythm of catecholamine excretion is not so persistent as the rhythm of 17-hydroxycorticosteroid excretion[11] and it does not play an important role in the evaluation of the effect of a stress on catecholamine excretion.

In the present study, adrenaline and noradrenaline excretion was found to increase in several situations. The increase of adrenaline output was about 400% during delivery, 130% in mental work, 80% in tilting of posture, and 30% in monotonous work, compared with each control value.

As popularly known, labor and delivery are very painful. It is very interesting to clarify whether such pains have any effect on the physiological function of pregnant women. Zuspan[12] found a significant 150% increase of noradrenaline content in 24-hr urine following delivery and 70% increase of adrenaline in the same urine specimen though not significant, and he supposed that it may be due to emotional and physical activity of the delivery process. However, labor pains generally last for half a day at the most. Since it is speculated that catecholamines are metabolized or inactivated very rapidly after secretion[5], urine should better be collected at intervals of as shorter a period of time as possible, in order to clarify the effect on catecholamine secretion. Twenty-four hr are probably too long for urine collection in this case. In the present experiment, urine collection was made at several-hour intervals and significant increase of adrenaline excretion was found in the period between hospitalization and delivery.

Oxytocin from the posterior pituitary is accepted as causing extreme contractility of the uterine musculature during delivery[13], and there seems no report showing that adrenaline participates in it. It is assumed that the increase of adrenaline excretion would be attributed to mental or emotional strain by labor pains. The high adrenaline excretion in most subjects before hospitalization is also assumed to be due to the pains. Mental and physical stresses caused by bleeding during delivery are also considered as a reason for the increase of adrenaline excretion. This volume of blood was 50 to 300 ml, average of 194 ml, in the present subjects. The average of the difference in adrenaline excretion between the labor period and the first day after delivery was 11.8 ± 3.7 ng/mg creatinine in the first childbirth and 20.3 ± 3.5 in the others. This may indicate that the second childbirth is not always easy for mothers compared with the first one. Either way, the mothers said that the pain during labor and delivery cannot be compared with that of some mental work. According to Zuspan[12], the values before and after delivery, except the first 24 hr following delivery, and nonpregnant value were essentially the same for both adrenaline and noradrenaline excretion.

Mental strains caused by performing some intellectual work is also widely known. Frankenhaeuser and Post[14] showed that the excretion of adrenaline increased by 70% during the performance of intellectual work, whereas noradrenaline excretion was not affected. Jones et al.[15] studied the effect of a university examination and recognized about 70% increase of noradrenaline excretion as well as 130% increase of adrenaline. In the present experiment 3-i, the calculation test caused about 110% increase of adrenaline excretion in the male subjects, and there was found a significant correlation between the relative increase of adrenaline output (y) and the number of figures calculated (x): $r=0.65$, $p<0.01$, and $y=3.4x-64.7$. This is consistent with the fact that no significant increase of adrenaline was found in the test with usual calculating speed. Therefore, the increase of adrenaline excretion would be correlated to the degree of mental strains if the latter depends on the work performance. The increase of adrenaline excretion was higher in the younger than in the older in experiment 3-i. This is probably due to the difference of work performance between the two groups. Moderate increase of noradrenaline excretion was found on the first day of the experiment 3-iii, and a similar tendency was observed in the experiment 3-i. It should be noted that increased excretion occurred only on the first day, not on the second or third day. The reason for the noradrenaline increase is not clear, but the problem of adaptation to performing an intellectual work must be considered in its evaluation.

Recently, immobilization or restraint was studied, particularly on animals. Kvetnensky and Mikulaj[16] measured adrenal and urinary catecholamine levels in the rats subjected to immobilization stress during 240 min, and they showed apparent decrease of adrenal adrenaline and gradual increase of urinary adrenaline (about 400%) and noradrenaline (about 90%). They could not find any change in adrenal noradrenaline. Zubek and Schutte[17] determined catecholamine excretion in human males subjected to perceptual deprivation for one week. They found that isolation quitters, i.e., those who failed to complete the prescribed period, showed 70% increased excretion of adrenaline in the experiment while successful subjects showed no change in catecholamine excretion. Zubek[18] observed strikingly similar results in prolonged immobilization. Noradrenaline excretion scarcely changed in either of the experiments. The present author[19] reported that increase of adrenaline (about 70%) and decrease of noradrenaline (about 30%) in urine of human subjects were observed 3 to 4 hr after the start of sitting on a chair or lying on a bed quietly keeping awake, and that many of the subjects told they felt fatigue particularly 3 to 4 hr after the beginning. From these findings, it is considered that restraint or immobilization is a stressful situation for rats, and that it is probably true for men if the condition is severe. It seems interesting that increase of adrenaline and decrease of noradrenaline in urine were observed in the monotonous light work of the present study. This may indicate that the worker engaged in this kind of work is in a similar psychological state as restraint.

First experience and anticipation have also been found to induce the increase of adrenaline excretion. Euler and Lundberg[20] found a significant increase of 250% in adrenaline excretion both in pilots during routine training flights and in unexperienced air-transported passengers. Goodall and Berman[21] found 50% increase of adrenaline excretion in a mock centrifugation of man and the increase of both adrenaline (60%) and noradrenaline (50%) output in real centrifugation, and they supposed that this adrenaline increase was largely related to the emotion in anticipatory state of being centrifuged. The present author and colleagues[22] showed that adrenaline excretion was significantly higher by about 60% in new workers in the first month of their employment than in the seniors in the same working place, but, in the third month, the level of the new workers was almost equal to that of the seniors. Noradrenaline excretion also showed a similar tendency though the change was not significant. The adrenaline increase of the new workers may be related to the emotion in anticipatory state in the first experience. The reason for noradrenaline increase in the new workers is not clear, but might be considered as similar to that in the first day of the calculation test in experiment 3-iii.

Several investigators[23,24] studied the changes of adrenaline and noradrenaline in the urine of human subjects exposed to "film stress." Euler *et al.*[24] showed 70% increase of adrenaline and 35% rise of noradrenaline excretion during the viewing of motion pictures showing a variety of murders, fights, torture, executions, and cruelty to animals. According to Levi's report[23], a bland natural-scenery film lowered the urinary catecholamines significantly (50% in adrenaline and 30% in noradrenaline), an aggression-provoking film and an amusing one were accompanied by significant increase of 30% in adrenaline excretion, and an axiety-provoking film induced adrenaline increase (40%) and noradrenaline rise (30%).

These findings about adrenaline excretion in various emotional states seem to disagree with each other in some respects, which is perhaps attributed to individual difference in the psychological response to the stimuli. On the other hand, it is very difficult to measure the subjective degree of various emotions or mental strains. From our experiences, however, it would be agreed that the degree of strains is reduced in the order of delivery, mental work, tilting of posture, restraint, new environment, and monotonous light work. It may be concluded that adrenaline secretion is elevated in emotional or mental strains, and that the quantity of increase in adrenaline might be roughly proportional to the degree of the strain.

In the present experiments, the increase of noradrenaline excretion was very remarkable in tilting of posture and heavy physical work, and slight increase of noradrenaline was observed in the first day of the experiment 3-iii. Noradrenaline output decreased in monotonous light work.

The marked increase in catecholamine excretion on tilting of posture, particularly in noradrenaline, was described by Sundin[25]. He reported that the rise of urinary output of noradrenaline was most remarkable in the case of 75° tilting, and that smaller but still significant increase was also observed at 25° and 50° tiltings. In the present experiment, in which the tilting angles of 60°, 75°, and 90° were chosen, a significant increase in urinary noradrenaline excretion was observed in every angle. Noradrenaline level was the highest in 90° tilting, though the difference was insignificant among these three angles. The reduction of blood pressure was observed in each case. Remarkable increase of noradrenaline excretion perhaps indicates a strong activation of the vasomotor system by the blood pressure homeostatic reflexes. There is some doubt whether adrenaline increase is related to the emotions caused by tilting or to the change in circulatory system.

Kärki[26] demonstrated a marked increase of noradrenaline excretion and a moderate adrenaline increase in athletes, marathon runners, and skiers, and during muscular work in a wood-cutting competition. In the present study, the forging men excreted larger quantity of noradrenaline during the working time. It is considered that the secretion of catecholamines may be influenced by metabolic and cardiovascular factors in muscular exertion.

Elmadjian *et al.*[4] determined the urinary excretion of adrenaline and noradrenaline in athletes, normal subjects in anticipatory states, and psychiatric patients, and they concluded that active, aggressive emotional displays are related to increased excretion of noradrenaline, with or without increased excretion of adrenaline, whereas tense, anxious but passive emotional displays are related to increased excretion of adrenaline in association with normal excretion of noradrenaline. However, great increase of noradrenaline output observed in the Elmadjian's study seems not to have been related with the emotions but caused by physical exercise in the sport competitions, judging from the above findings.

However, a moderate increase of noradrenaline excretion, besides a large elevation of adrenaline excretion, was often observed in some intellectual work and in viewing some movies which are considered to be accompanied with little physical exercise, as described before. Therefore, noradrenaline excretion may also be related to emotions in various situations, though to a less extent than adrenaline excretion. It is tentatively considered that passive emotion or mental strains induce increased adrenaline excretion and active emotion or physical strains are accompanied with elevated noradrenaline output.

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