CHAPTER IV

RESULTS

General Apperances

All of experimental male monkeys were healthy throughout the study period. Every time after drug injection, monkeys were rewarded by food. Thereafter, approximately 1 week, monkey exhibited a learning behavior how to recieve the drug particularly when morphine was injected (Figure 14). They turned their buttocks close to the cage-door and called for the drug injection. However, they always denied to receive ketamine hydrochloride due to some incidences of adversed effect; for instance, nausea, vomitting, body tembling and unpleasant symptoms were occurred after anaesthetization. During morphine treatment, some monkeys (no. 522, 523, 509, 511 and 704) exhibited a threatening, nervous and aggressive behaviors, particularly in pubertal monkey no. 523 bit his own knees. Dramatically, monkeys injected high dose of morphine (6.0 mg/kg/day) showed a significant weight loss and anorexia symptom begining approximately day-15 and not latter than day-44 of treatment period.

Morphine cessation was correlated with withdrawal symptoms. They usually commemces about 20 hours after the last dose of morphine injection with yawning, drowsiness and lessened physical activity. Then followed a typical symptom of loss of appetite,

nervous and rather aggressive and some monkeys (no.522 and 523) injured themself around their knees. Of interest, monkey no.504 treated with 1.5 mg/kg/day morphine showed an excessive hair loss around back and front leg regions. The previous signs and symptoms reached their peak at 1-4 days and they declined over the next 7-10 days. As expected, the violent of withdrawal symptoms was depended upon the dose of morphine treated. After successful withdrawal, monkeys could be able to eat normally, regained their weights and presented the picture of well being. Considerable gain of weight was found within 1 month after terminating morphine injection.

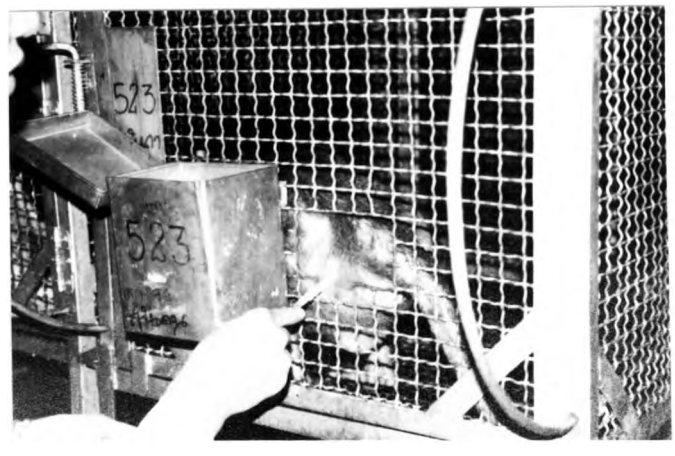

Figure 14 Learning behavior of monkey no.523. He presented his left buttock in front of the cage ready for accepting morphine injection at day-62 of treatment period.

The Study of Acute Effect of Morphine Hydrochloride

1. The effect on serum PRL levels

Result from figure 15 and 16 showed that serum prolactin levels increased significantly in a dose-dependent manner after morphine injection compared to pretreatment baseline values. The higher dose of morphine administration produced a greater degree in PRL release. In all animals, a prompt rise of serum PRL levels occurred as early as 15 minutes after morphine injection and reached a maximum value of 4144.25 $\pm$ 657.59 mIU/L within 30 minutes. Serum PRL levels remained higher than 1,000 mIU/L over 4 hours in all treatment groups. The same dose of morphine treatment(3.0 mg/kg/day) caused a distinct difference in response of PRL levels between adult and pubertal monkeys. The response in pubertal monkeys was similar to that evoked by the lower morphine dose (1.5 mg/kg/day) treated in adults.

The maximal PRL response , at least in part, depended upon the basal PRL levels. As in monkey no.509 and no.704, the pretreatment values were higher than the other monkeys compatible with a greater degree of PRL response to morphine injection. In these monkeys, serum PRL levels during 10 hours after morphine injection were still exceeded baseline levels.

2. The effect on serum testosterone levels

The effect of single injection of morphine on serum testosterone levels are shown in figure 17 and 18. As can be seen, morphine significantly reduced serum testosterone levels in a

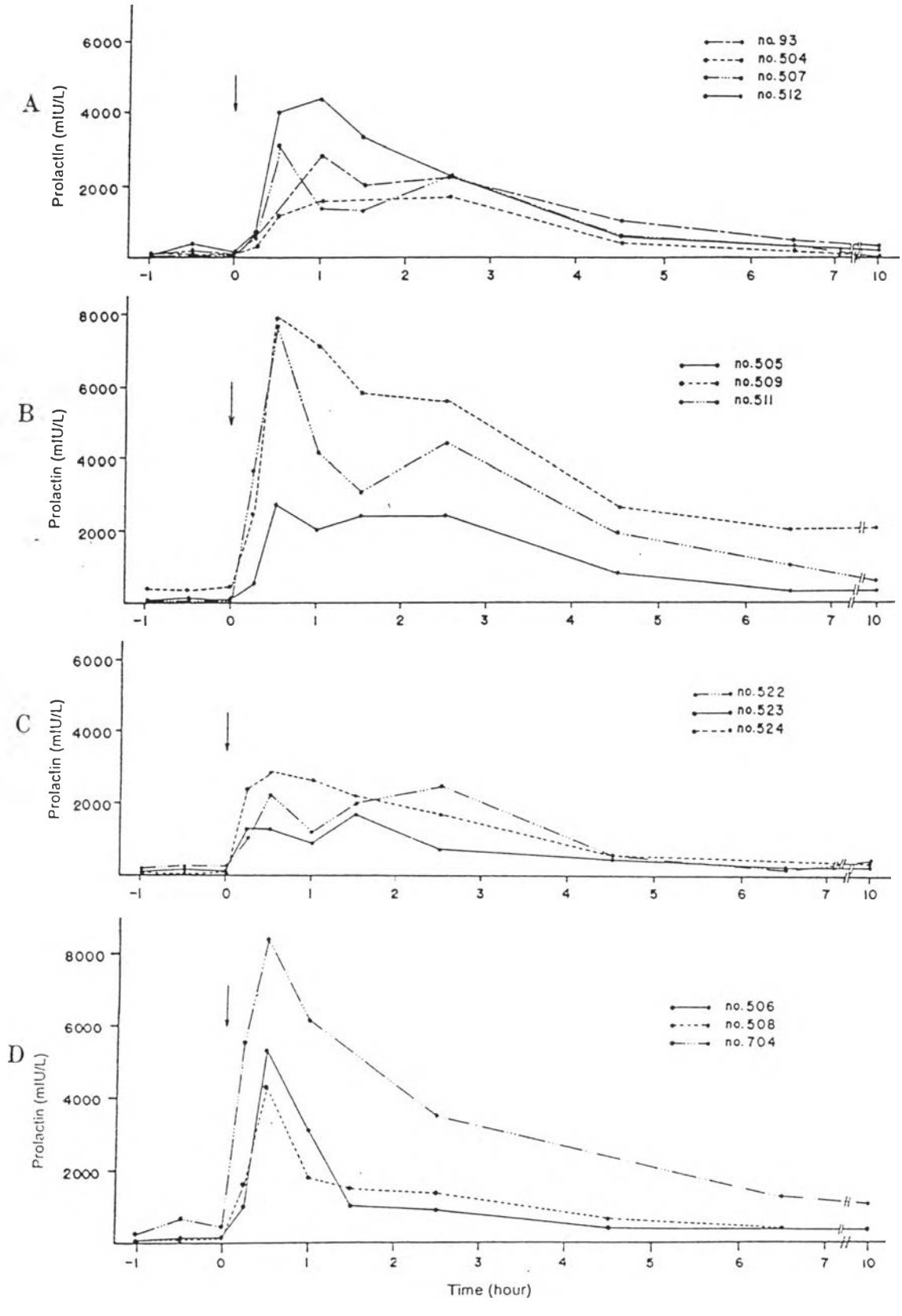

Figure 15 Serum prolactin levels in each monkey after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B.C) and 6.0(D) mg/kg, respectively at time 0.
A.B and D : adult male monkeys; C : pubertal male monkeys.

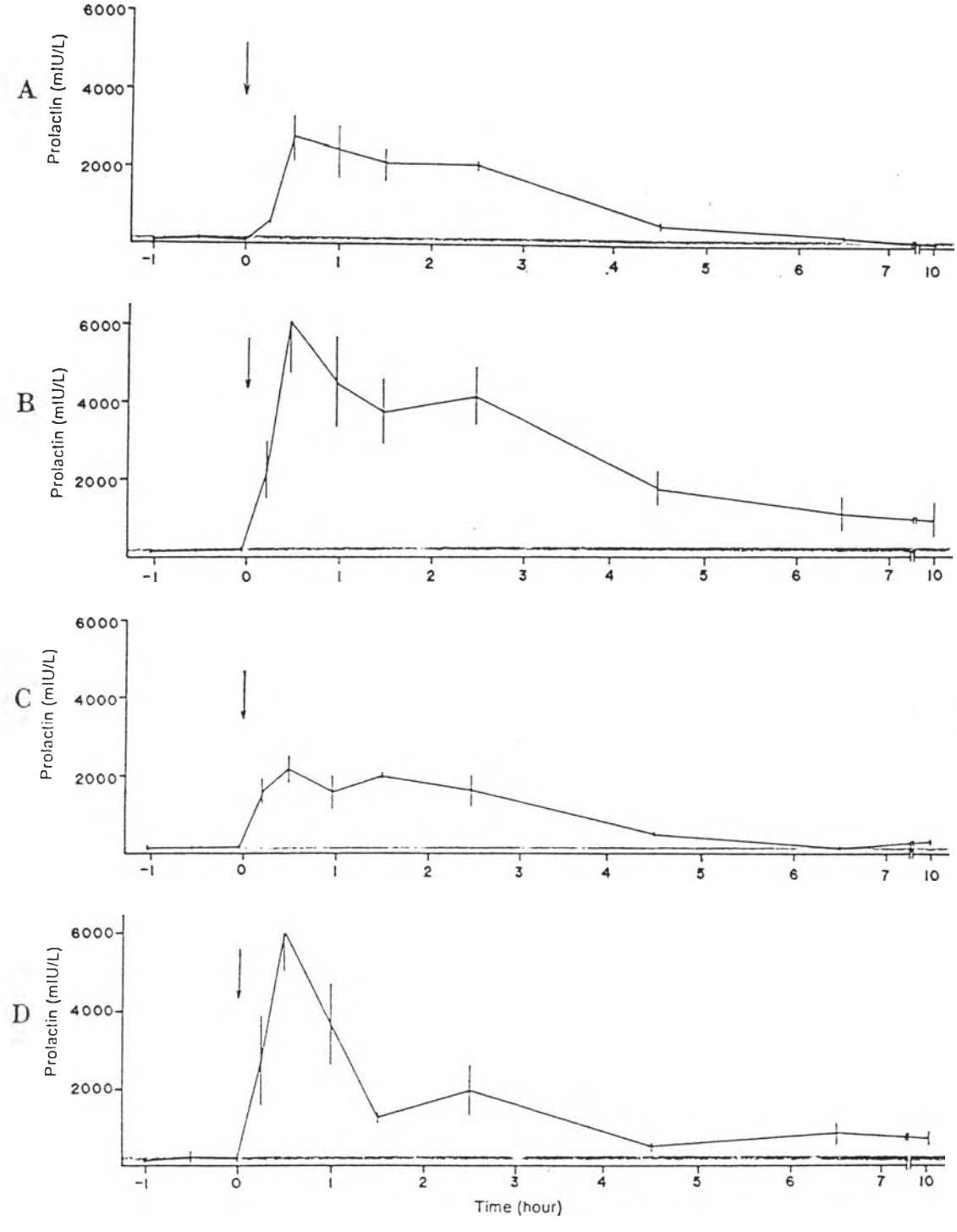

Figure 16 Mean(±SE) serum prolactin levels in each monkey group after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg, respectively at time 0. A,B and D : adult male monkeys; C : pubertal male monkeys. ■ : $\bar{X}$±SE of pretreatment values.

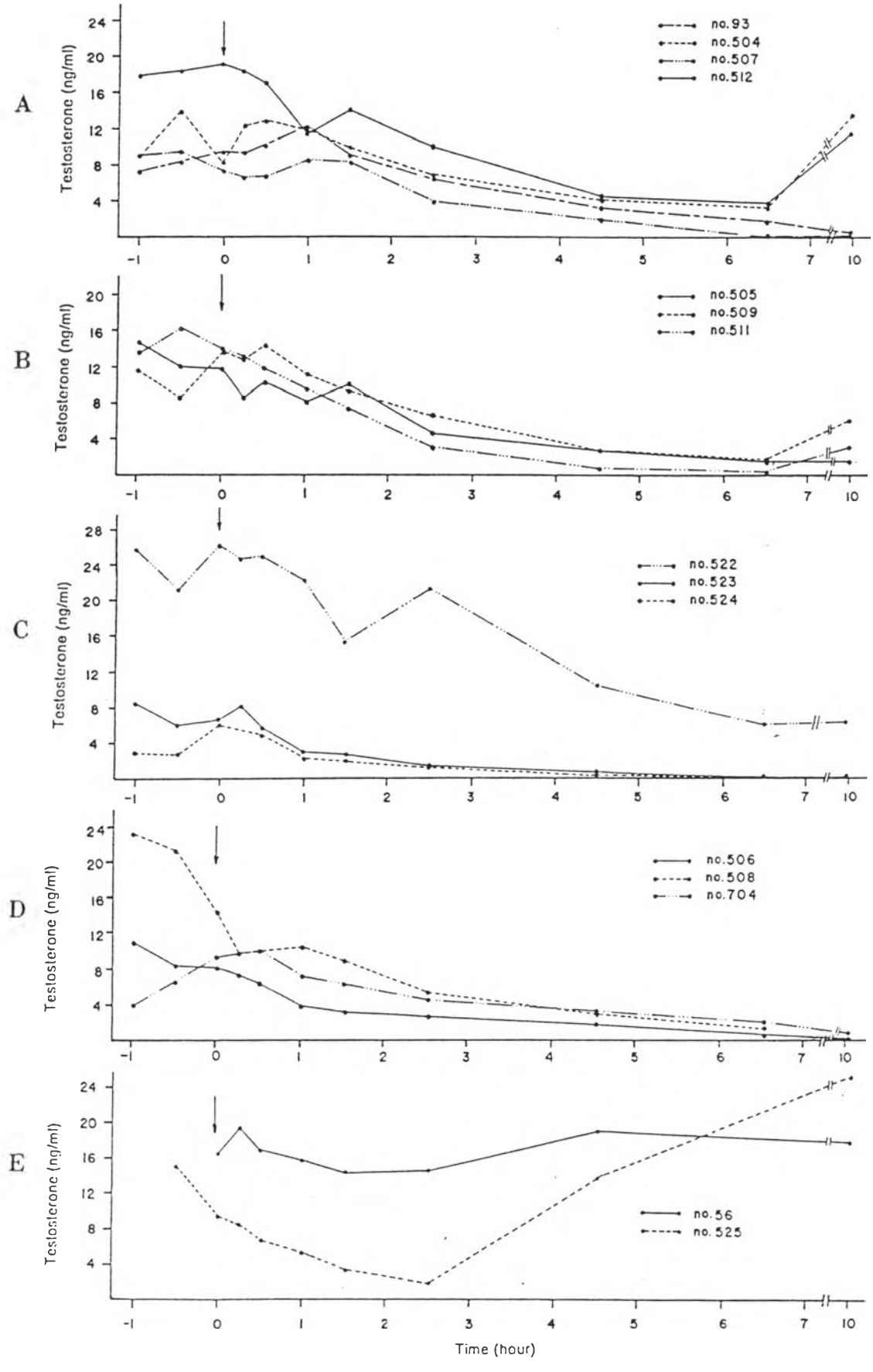

Figure 17 Serum testosterone levels in each monkey after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0 (B,C) and 6.0 (D) mg/kg, respectively at time 0 compared to saline-injected control(E).
A,B and D : adult male monkeys; C : pubertal male monkeys.

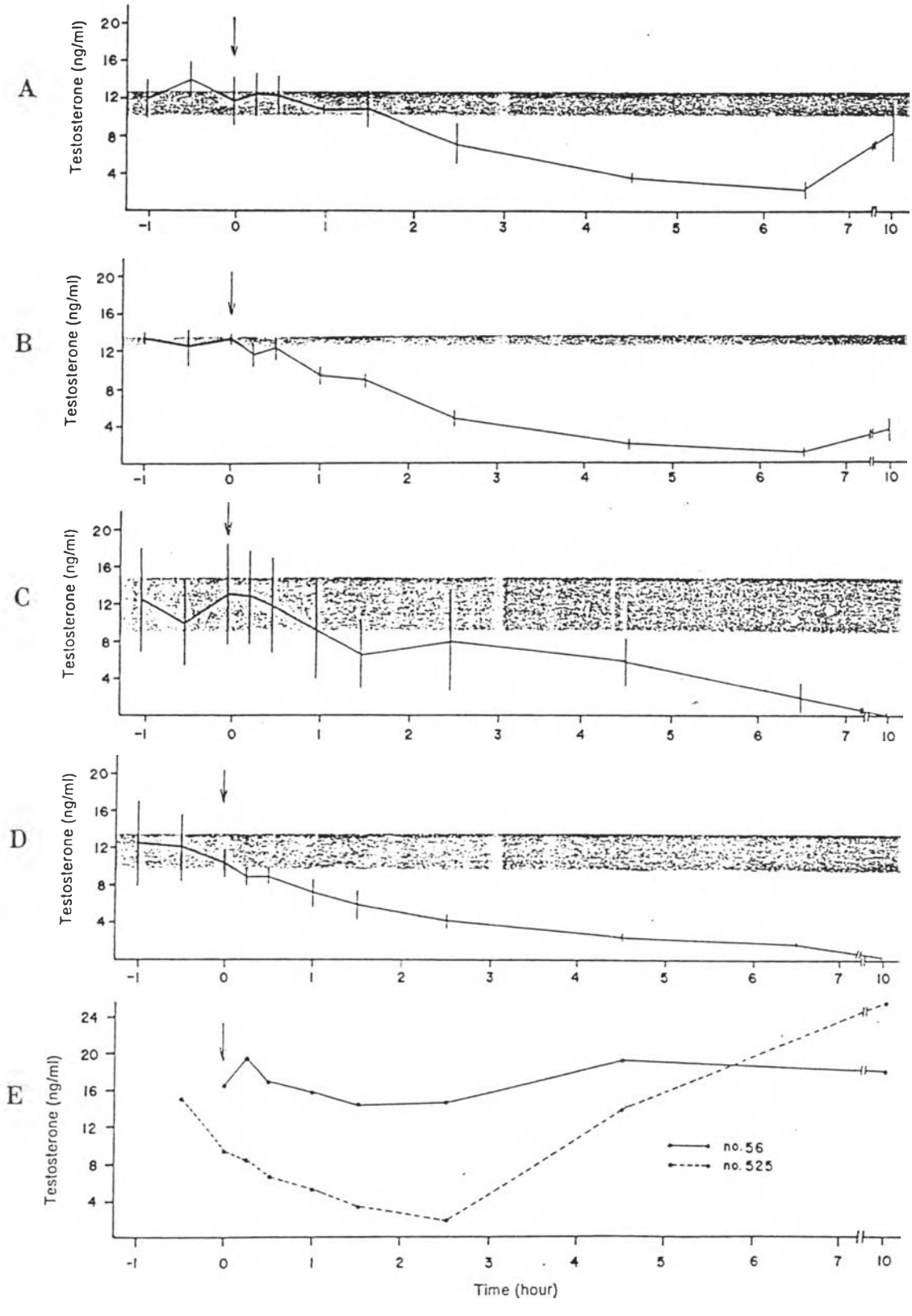

Figure 18 Mean(±SE) serum testosterone levels in each monkey group after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg, respectively at time 0 compared to saline-injected control(E).
A,B and D : adult male monkeys; C : pubertal male monkeys.
■ : $\bar{X}$±SE of pretreatment values.

dose-response manner. The lowered testosterone levels reach a nadir within 6.5-10.0 hours. The lowest dose of morphine injection (1.5 mg/kg) to adult monkeys caused slowly reduction from and early increment to the basal values. The reduction was initiated within 1.5-2.5 hours to a peak level of depression of 2.5 ± 0.8 ng/ml at 6.5 hours from pretreatment level of 11.5 ± 1.2 ng/ml. The testosterone level was gradually increased to, but less than, the basal level at 10.0 hours (8.5 ± 3.0 ng/ml). After acute morphine administration in dose 3.0 mg/kg to adult males, testosterone values began to fall within 0.5-1.0 hour. The lowest concentration happened at 6.5 hours as in monkeys treated 1.5 mg/kg morphine. But the nadir level was lower in this group (1.2 ± 0.3 vs 2.3 ± 0.8 ng/ml). However, the level was also gradually increased at 10.0 hours and lower than monkey treated with 1.5 mg/kg morphine (3.6 ± 1.2 vs 8.5 ± 3.0 ng/ml). Adult males received the highest dose of morphine (6.0 mg/kg), the levels of testosterone initiated to decrease as early as at 0.25 hour and further decline could be seen as last as 10.0 hours after morphine injection.

At the same dose of morphine (3.0 mg/kg) given to puberty and adult males induced a dramatic different response in testosterone levels. In puberty, the testosterone reduction was slower (1.5 hours post-injection) but more sustained decline until the last blood collection was evidenced. In saline-injected control group, adult male exhibited a consistently high testosterone levels, while in pubertal male the testosterone levels were progressively decline during the first 2.5 hours of injection and gradually increase, thereafter.

3. The effect on serum cortisol levels

As in figure 19 and 20, a clear diurnal variation of serum cortisol levels was seen. In all monkey groups, the period of lowest levels was between 1800-1900 h or at 10.0 hours after morphine injection. Levels between 0700-0800 h or 1.0 hour prior morphine injection were significantly higher than levels during 1800-1900 h ($p<0.05$, 564.13 $\pm$ 23.20 vs. 191.22 $\pm$ 18.07 ng/ml). The pretreatment values of serum cortisol varied greatly among monkeys groups. As compared to a saline injected control, the basal values only in adult males treated with 1.5 and 6.0 mg/kg morphine hydrochloride respectively showed a non-significant difference. Whereas, the basal levels in adult (585.38 $\pm$ 42.01 ng/ml) and pubertal (693.46 $\pm$ 54.30 ng/ml) monkeys treated with 3.0 mg/kg morphine were significantly higher than in control monkeys (481.50 $\pm$18.90 ng/ml). Therefore, values obtained during morphine treatment were considered as changes from baseline values as predicted in figure 21. During first hour of morphine treatment, cortisol levels were rather high, then, fell steadily below the basal levels. In adult monkeys treated with 1.5 and 3.0 mg/kg morphine showed a minimum levels within 2.5 hours (294.35 $\pm$ 42.65 ng/ml, $p<0.01$; 446.33 $\pm$ 44.04, $p<0.05$ respectively). Morphine in dose 3.0 mg/kg also reduced a mean cortisol level of pubertal monkeys at 2.5 hours but not significant ($p>0.05$) as compared with the saline-injected control. There was no significant change in any time points in adults treated with the high dose of 6.0 mg/kg morphine when compared to the saline-injected control.

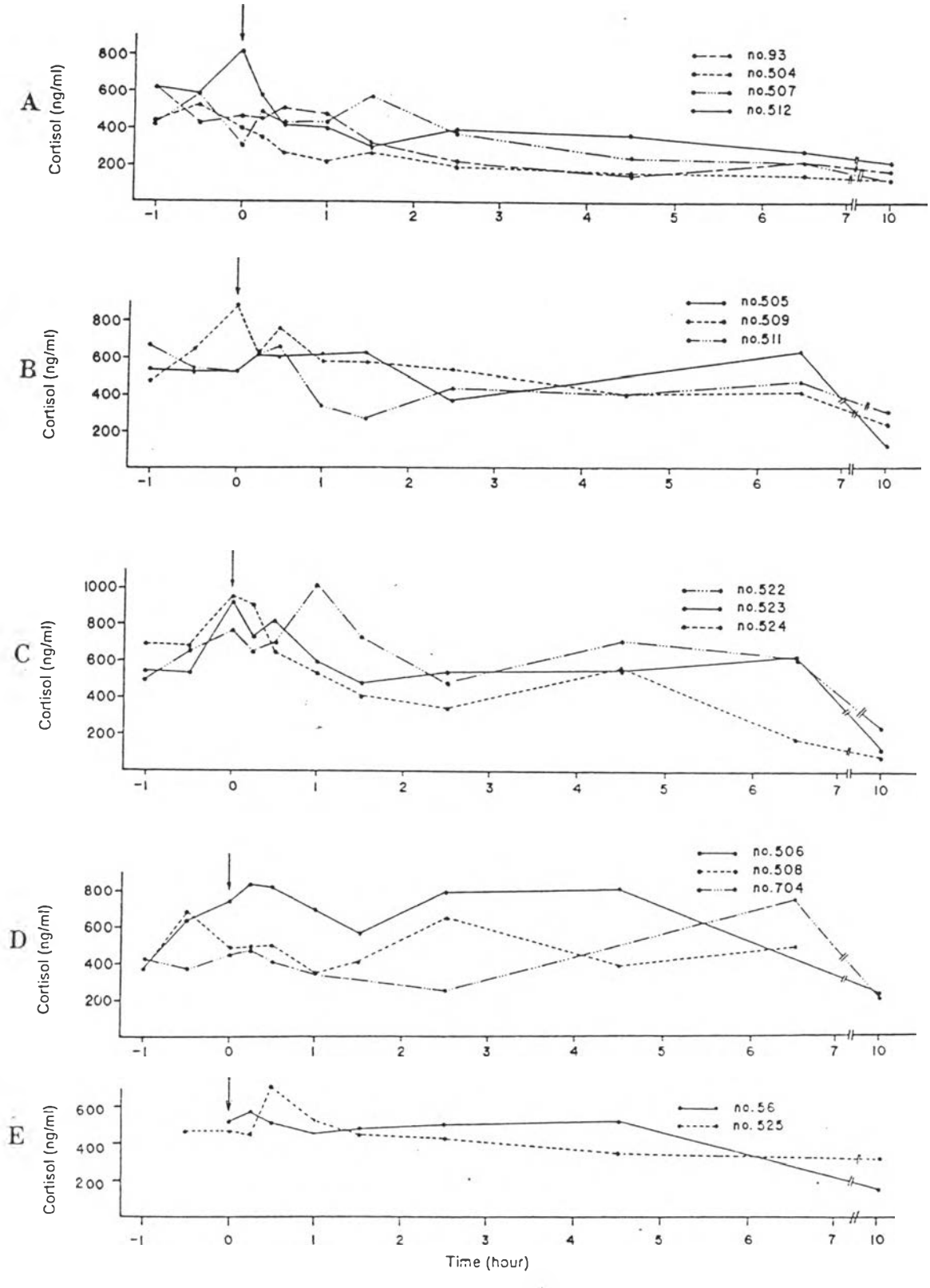

Figure 19 Serum cortisol levels in each monkey after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg, respectively at time 0 compared to saline-injected control(E).
A,B and D : adult male monkeys; C : pubertal male monkeys.

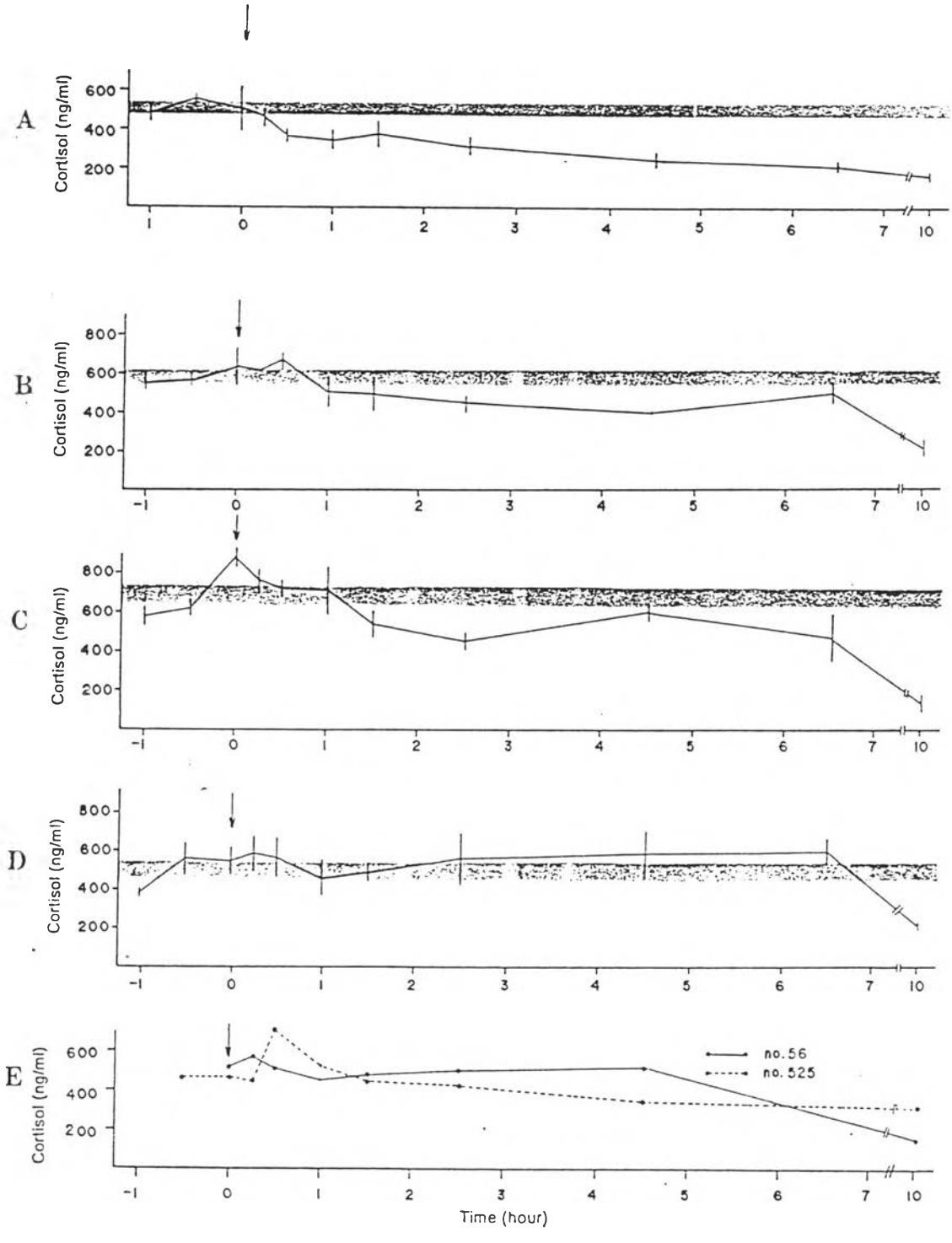

Figure 20 Mean(±SE) serum cortisol levels in each monkey group after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg, respectively at time 0 compared to saline-injected control(E).
A,B and D : adult male monkeys; C : pubertal male monkeys.
■ : $\bar{X}$±SE of pretreatment values.

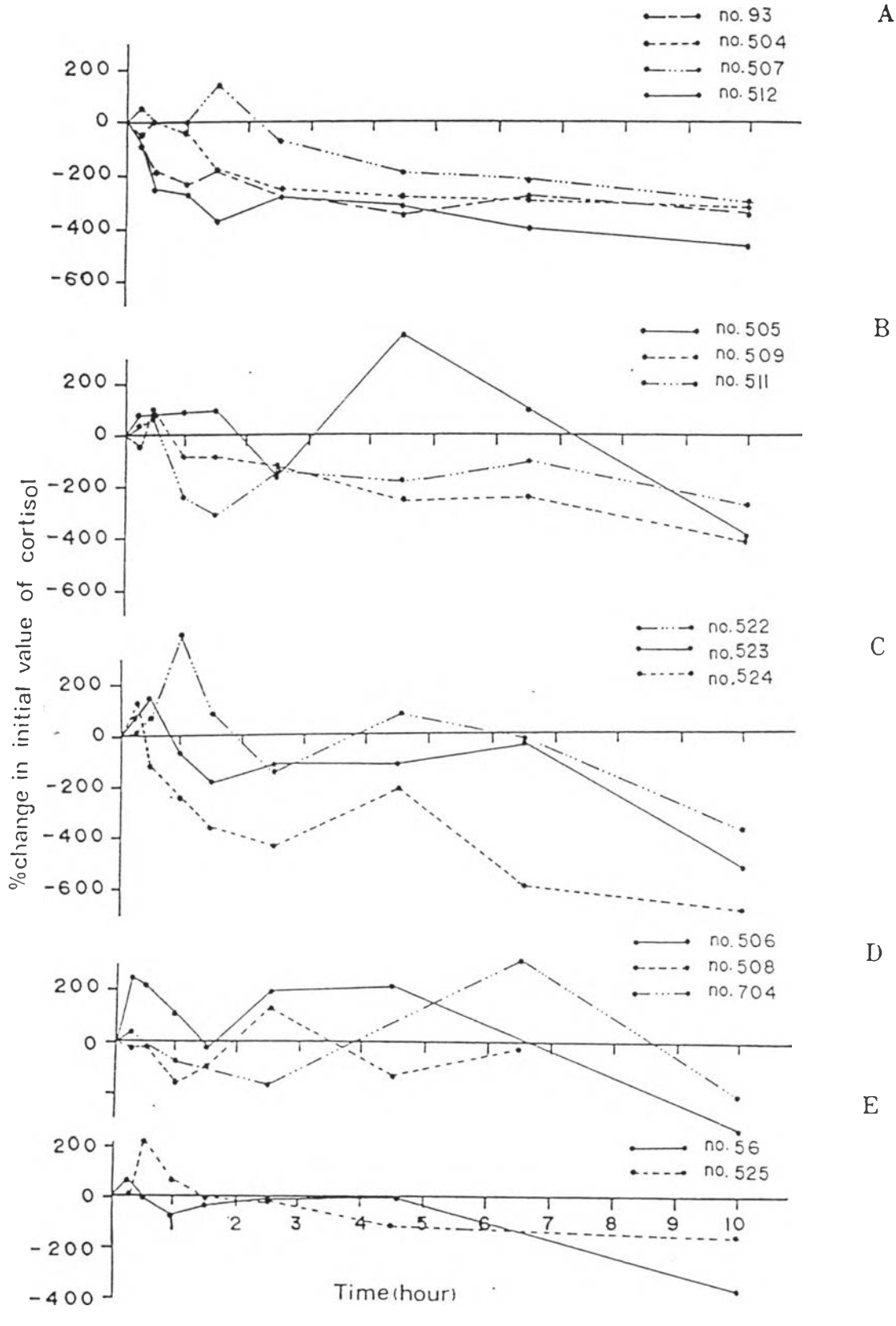

Figure 21 The percentage change in an initial value of cortisol concentration after subcutaneous injection of morphine hydrochloride 1.5 (A), 3.0 (B,C) and 6.0 (D) mg/kg, respectively compared to saline-injected control(E).
A,B and D : adult male monkeys; C : pubertal male monkeys.

4. The effect on serum TSH levels

There were significant differences in pretreatment values among any monkey groups. The mean TSH levels in adult monkey group treated with 1.5 mg/kg morphine were higher than in the other groups, and after the administration of morphine hydrochloride serum TSH levels were much more elevation than pretreatment values ($0.001<p<0.05$; except at 2.5 and 4.5 hours, $p<0.10$) (Figure 22-24) In contrast, higher doses of morphine (3.0 and 6.0 mg/kg) showed no significant effect on serum TSH levels in adult male monkeys. It, however, tended to be higher than the pretreatment values in some time-points; at 10.0 hours of treated dose 3.0 mg/kg and at 1.0 and 2.5 hours of treated dose 6.0 mg/kg ($p<0.10$). In pubertal males, serum TSH levels were also increase significantly at 2.5 and 10.0 hours ($p<0.05$), respectively. When compared to saline-injected control, the significant rise was typically found in pubertal (at 2.5 and 10.0 hours) and in adult (at 0.5 hour) monkeys injected subcutaneously with 1.5 and 3.0 mg/kg morphine hydrochloride respectively. There were no significant differences in the other adult monkey groups at any time points when compared to the saline controls.

5. The effects on serum T_4 levels

In all adult groups, the basal serum T_4 levels were significantly higher than the saline-control (Figure 25 and 26). The values were also tened to be higher but were not statistically significant ($p<0.10$) than the saline-control in pubertal monkeys. Following injection of morphine hydrochloride, all monkeys elicited a significant increase in T_4 levels when compared to the pretreatment

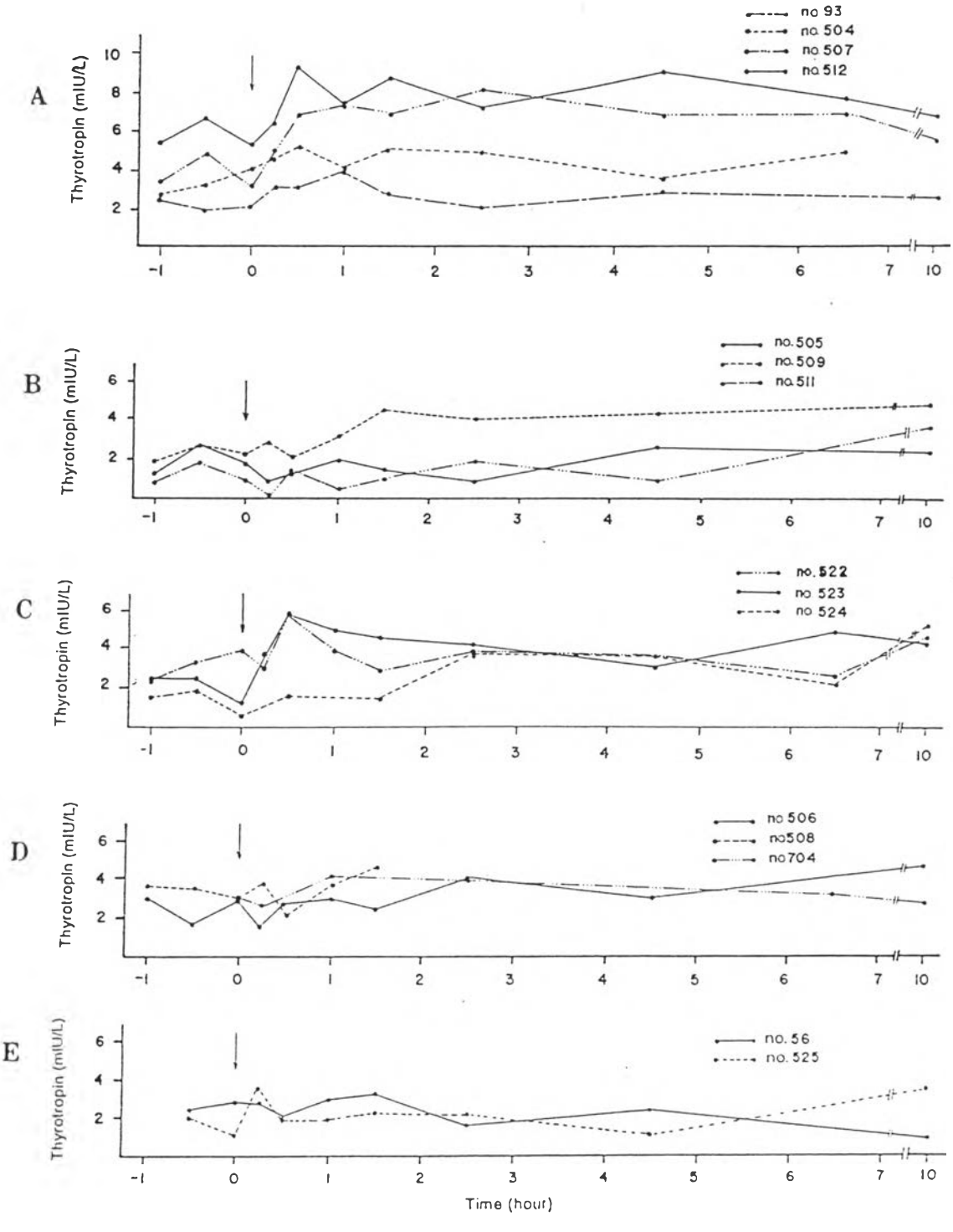

Figure 22 Serum thyrotropin levels in each monkey after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg, respectively at time 0 compared to saline-injected control(E).
A,B and D : adult male monkeys; C : pubertal male monkeys.

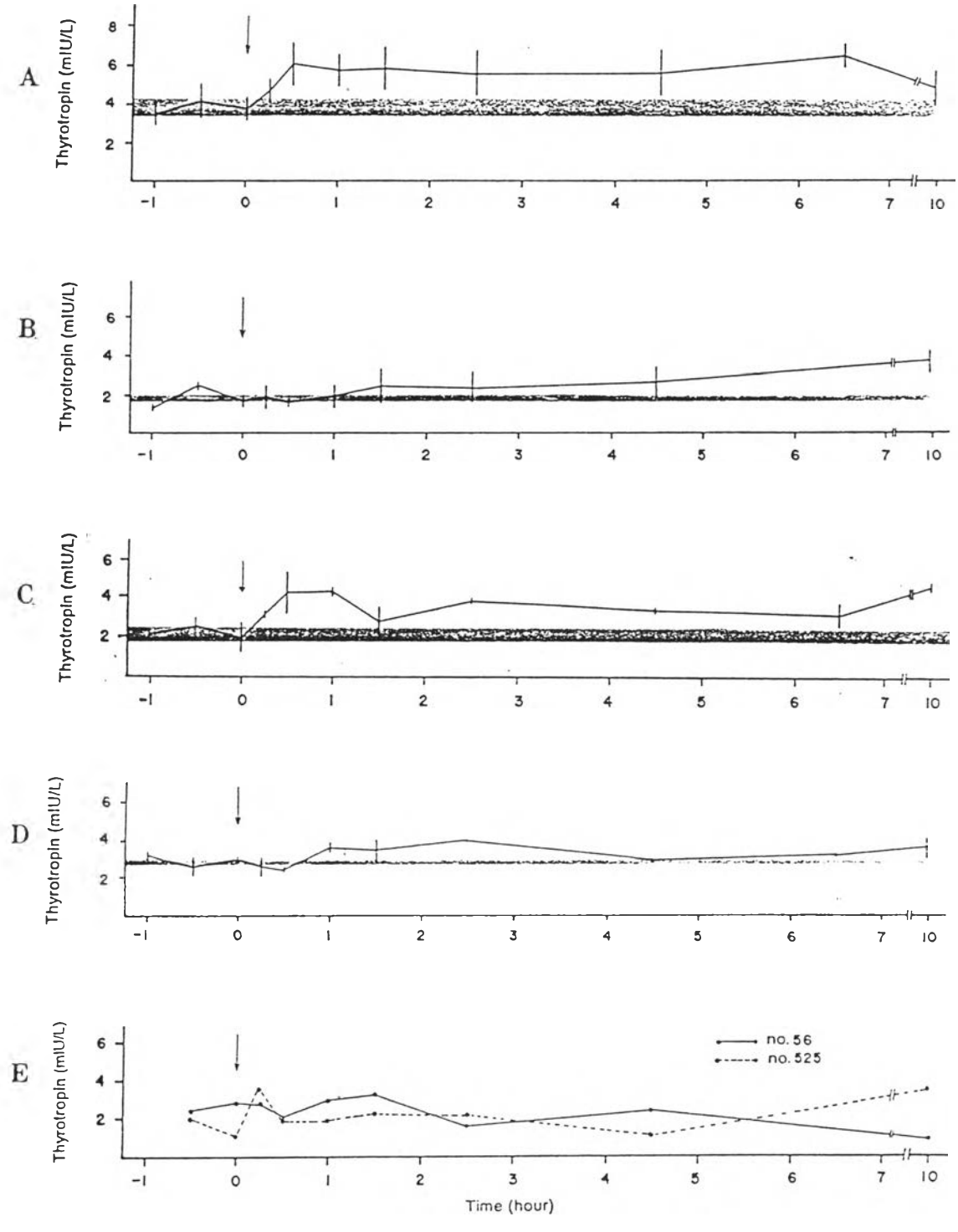

Figure 23 Mean(±SE) serum thyrotropin levels in each monkey group after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg, respectively at time 0 compared to saline-injected control(E)
A,B and D : adult male monkeys; C : pubertal male monkeys.
■ : $\bar{X}$±SE of pretreatment values.

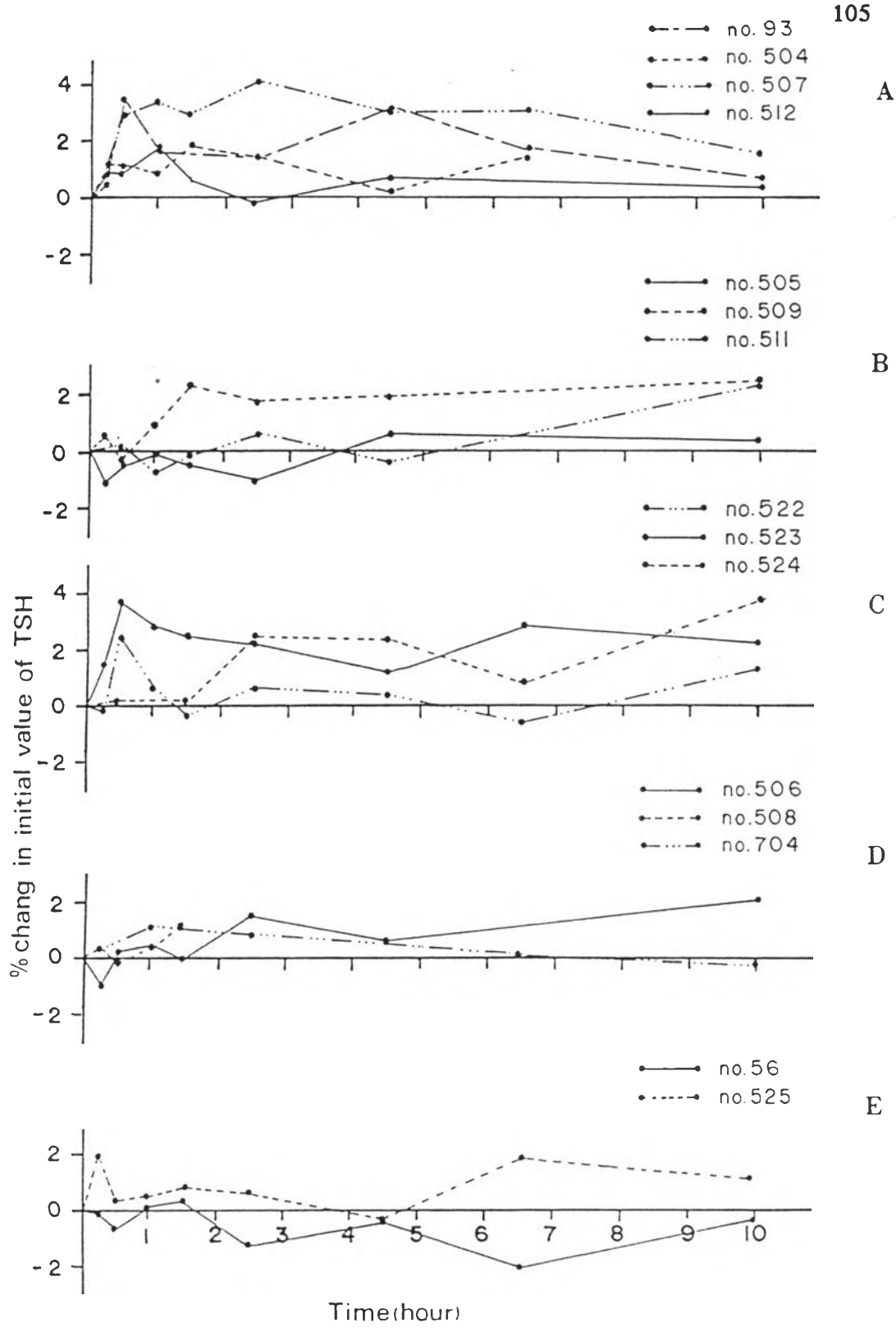

Figure 24 The percentage change in an initial value of thyrotropin concentration after subcutaneous injection of morphine hydrochloride 1.5 (A), 3.0 (B,C) and 6.0 (D) mg/kg , respectively compared to saline-injected control(E). A,B and D : adult male monkeys; C : pubertal male monkeys.

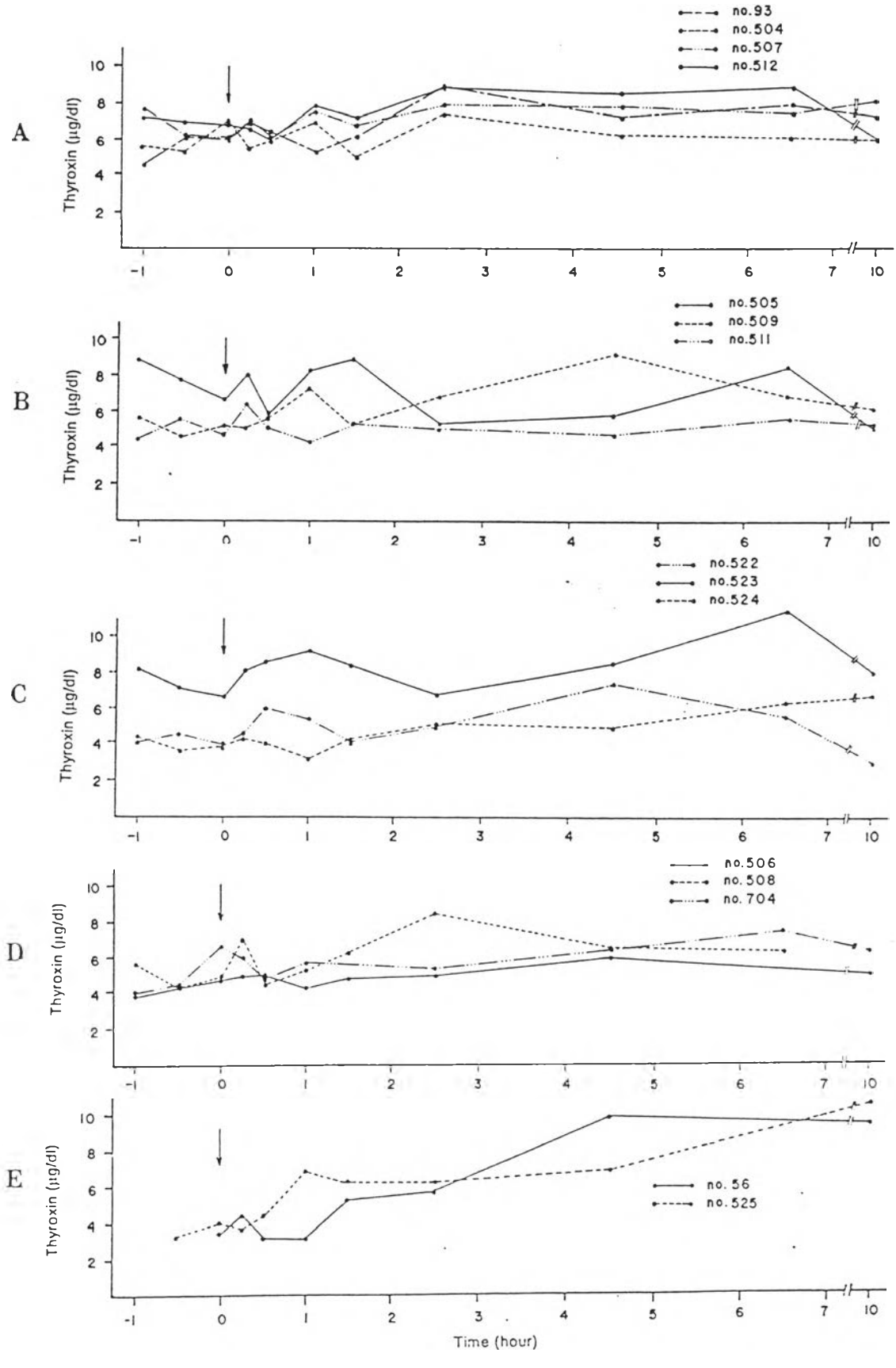

Figure 25 Serum thyroxin levels in each monkey after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B.C) and 6.0(D) mg/kg, respectively at time 0 compared to saline-injected control(E).
A.B and D : adult male monkeys; C : pubertal male monkeys.

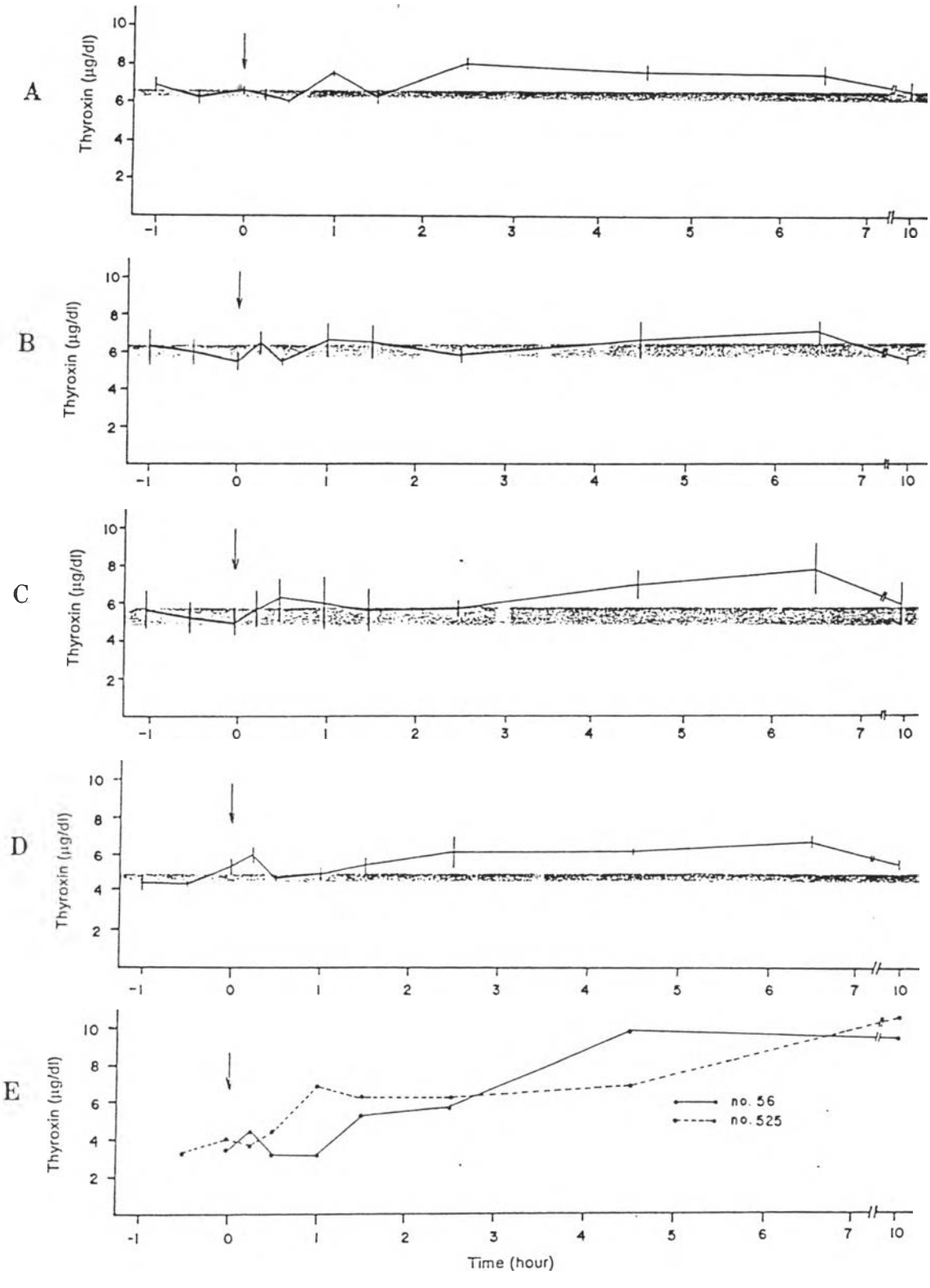

Figure 26 Mean(±SE) serum thyroxin levels in each monkey group after subcutaneous injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg, respectively at time 0 compared to saline-injected control(E).
A,B and D : adult male monkeys; C : pubertal male monkeys.
■ : $\bar{X}$±SE of pretreatment values.

values within 2.5-6.5 hours ; at 2.5-6.5 hours, 6.5 hours, and 4.5 hours ($p<0.05$) in adult groups treated with 1.5, 3.0 and 6.0 mg/kg morphine, respectively, and at 6.5 ($p<0.05$) in pubertal monkeys. Saline injection also led to a significant increase of serum T_4 at 2.5 hours ($p<0.01$) and at 10.0 hours ($p<0.05$). Serum T_4 levels in any monkeys treated with 1.5 mg/kg morphine showed a synchronous pattern for the entire time of observation, but the patterns were quite different in the other monkey groups. Nearly all monkeys showed a slight decline of serum T_4 concentration toward the basal levels at the last point of blood collection (10.0 hours).

6. The acute effect of morphine hydrochloride on hormonal levels in naive monkeys

The study of acute effect of morphine hydrochloride on hormonal levels including TSH, T_4, cortisol, PRL and testosterone in naive monkeys were similar to the recovered morphine-addiction monkeys (Figure 27). There were no significant differences in T_4 levels compared to basal values whereas TSH levels were significantly higher than basal values at 2.5-3.5 hours ($p<0.05$). The cortisol levels showed a progressive rise prior to morphine adminitration, which were significantly higher at third samples (0 hour) than at initial samples (-1 hour). Following morphine injection, cortisol levels were gradually decline with a nadir at 3.5 hours ($p<0.01$) and returned to the basal values afterward. Similarly to the recovered morphine-addiction monkeys, PRL levels exhibited a prompt rise at 15 minutes post-injection and began to decrease at 1.5 hours. But the levels were still higher than basal values at the last points of blood collection (at 5.5 hours) ($p<0.01$). Finally,

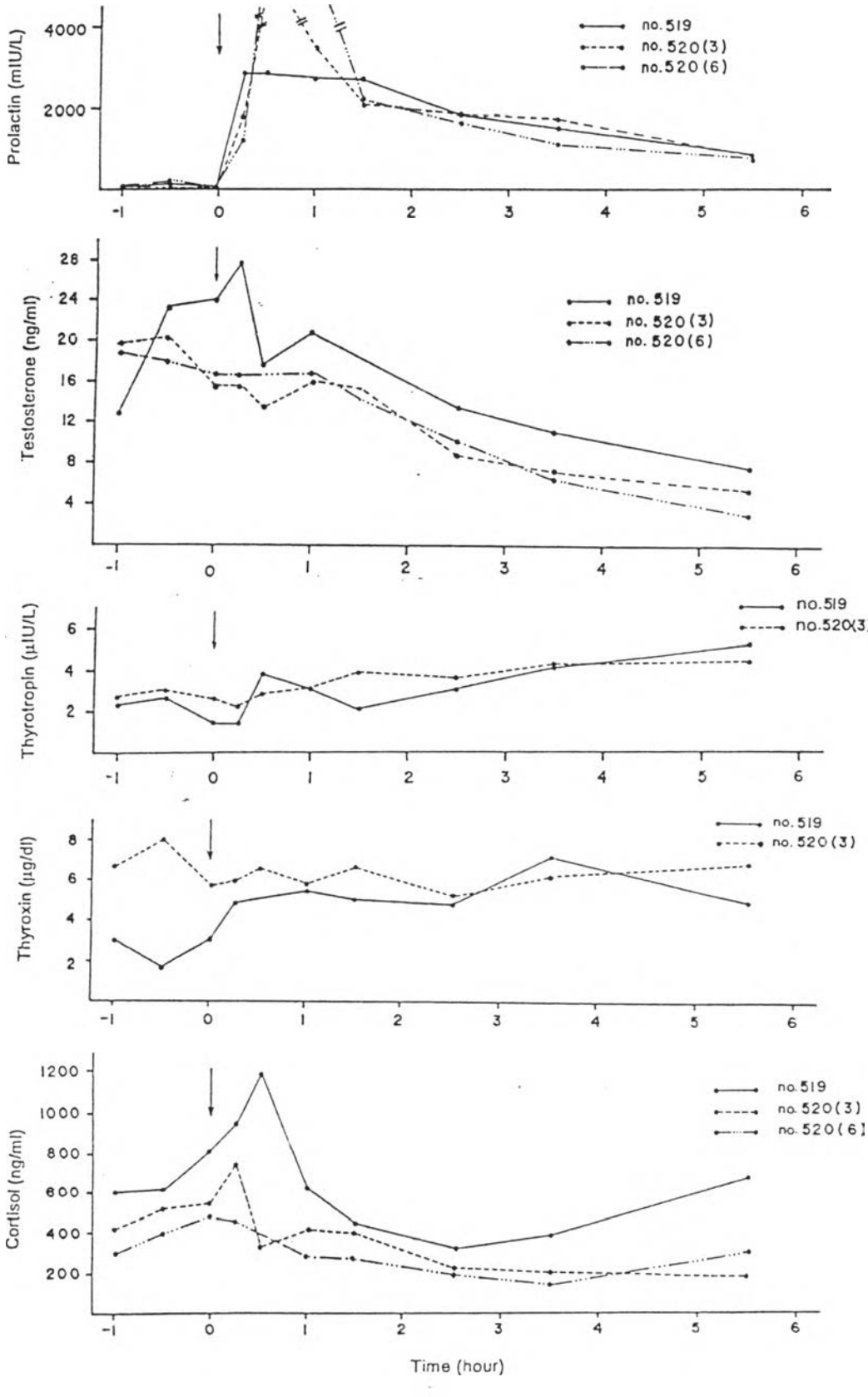

Figure 27 Hormonal profile in naive monkeys after subcutaneous injection of 3.0 mg/kg (no.519 and no.520(3)) or 6.0 mg/kg (no.520(6)) morphine hydrochloride at time 0.

testosterone levels were significantly lowered than basal values at 2.5 hours ($p<0.01$) with a further subsidence until 5.5 hours. Owing to the blood samples collected in these monkeys were lasted at 5.5 hours which was shorter time than the study in recovered morphine-addiction monkeys, however, the patterns of hormonal changes were resemble.

Chronic Effect of Morphine Hydrochloride

For the convenience determination and understanding, the hormonal levels after each dose of morphine administration in any monkeys will be combined and discussed beforehand in the terms of mean hormonal levels, after that, all of any measured hormonal profiles in the onset of study will be integrated and considered for the interrelation of hormonal changes.

1. The effect on serum PRL levels

Mean seurm PRL levels in all monkey groups during pretreatment and treatment periods could not be observed any prominent changes (Figure 28). Whereas the mean values were increased for a short instance (28-36 days) during withdrawal periods, it was initiated at day-115 and day-129 in adult and pubertal monkeys treated with 3.0 mg/kg/day morphine, respectively, and at day-163 in monkeys treated with 6.0 mg/kg/day morphine hydrochloride. It is of interest that the values of standard deviation in adult monkey group treated with 3.0 mg/kg/day morphine hydrochloride are rather high. Since, the basal PRL levels in monkey no.509 were typically higher than the other monkeys (referred to the acute effect study).

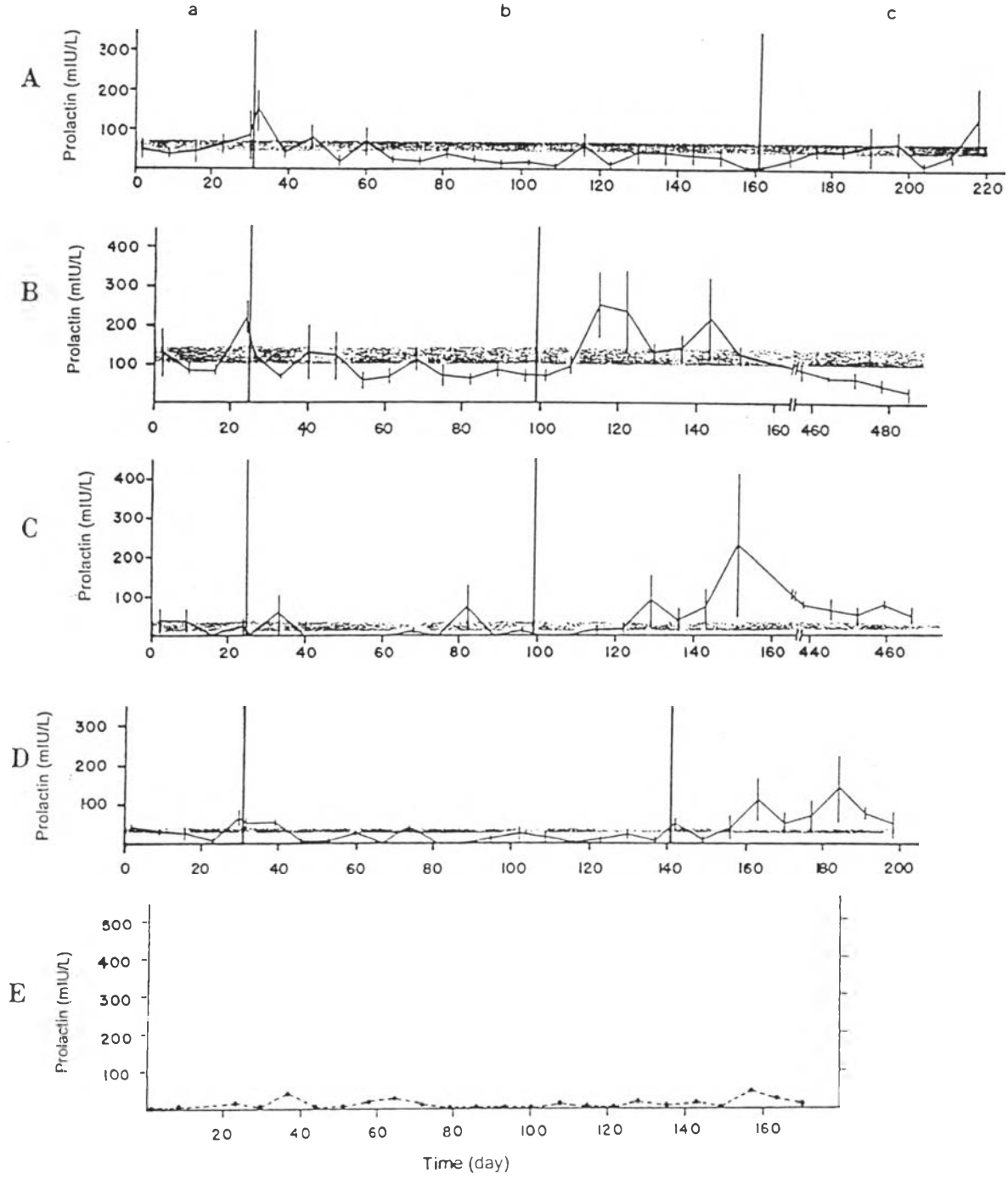

Figure 28 Mean(±SE) serum prolactin levels taken 20 hours after each injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg/day respectively in each monkey group. a,b and c represented pretreatment, treatment and posttreatment periods, respectively. A, B and D : adult male monkeys; C : pubertal male monkeys; E : saline-injected control. ▩ : $\bar{X}$±SE of pretreatment values.

2. The effect on serum testosterone levels

In adult monkeys, mean serum testosterone levels tended to be decline for approximately 3 weeks during abstinence periods in the higher doses of morphine treatment (3.0 and 6.0 mg/kg/day) (Figure 29). Moreover, the monkey group injected with 3.0 mg/kg/day morphine seemed to elicited a protracted drop of testosterone levels (>20 hours) after the first dose of injection. This detected decline level was recovered to the basal values, thereafter. In pubertal monkeys, mean serum testosterone levels were very fluctuated during treatment and early withdrawal periods. The statistical analysis of these values was significantly higher than basal levels ($p<0.05$) only in the early withdrawal period (11.52 $\pm$ 2.42 vs 7.63 $\pm$ 1.78 ng/ml) at day-101 to day-129.

3. The effect on serum TSH levels

There were no statistical changes in mean serum TSH levels between any treatment periods in nearly all adult monkey groups (Figure 30), except in adults treated with 1.5 mg/kg/day morphine hydrochloride, the posttreatment values were significantly higher than treatment values ($p<0.01$; 5.40 $\pm$ 0.46 vs 4.81 $\pm$ 0.56 mIU/L). Alternatively, the mean TSH values in pubertal monkeys during posttreatment period were significantly higher than both pretreatment ($p<0.05$; 4.39 $\pm$ 0.41 vs 3.88 $\pm$ 0.77 mIU/L) and treatment periods ($p<0.005$; 4.39 + 0.41 vs 3.76 $\pm$ 0.52 mIU/L).

4. The effect on serum T_4 levels

Mean serum T_4 levels during treatment periods in any groups were slightly increased (Figure 31). But only in adult

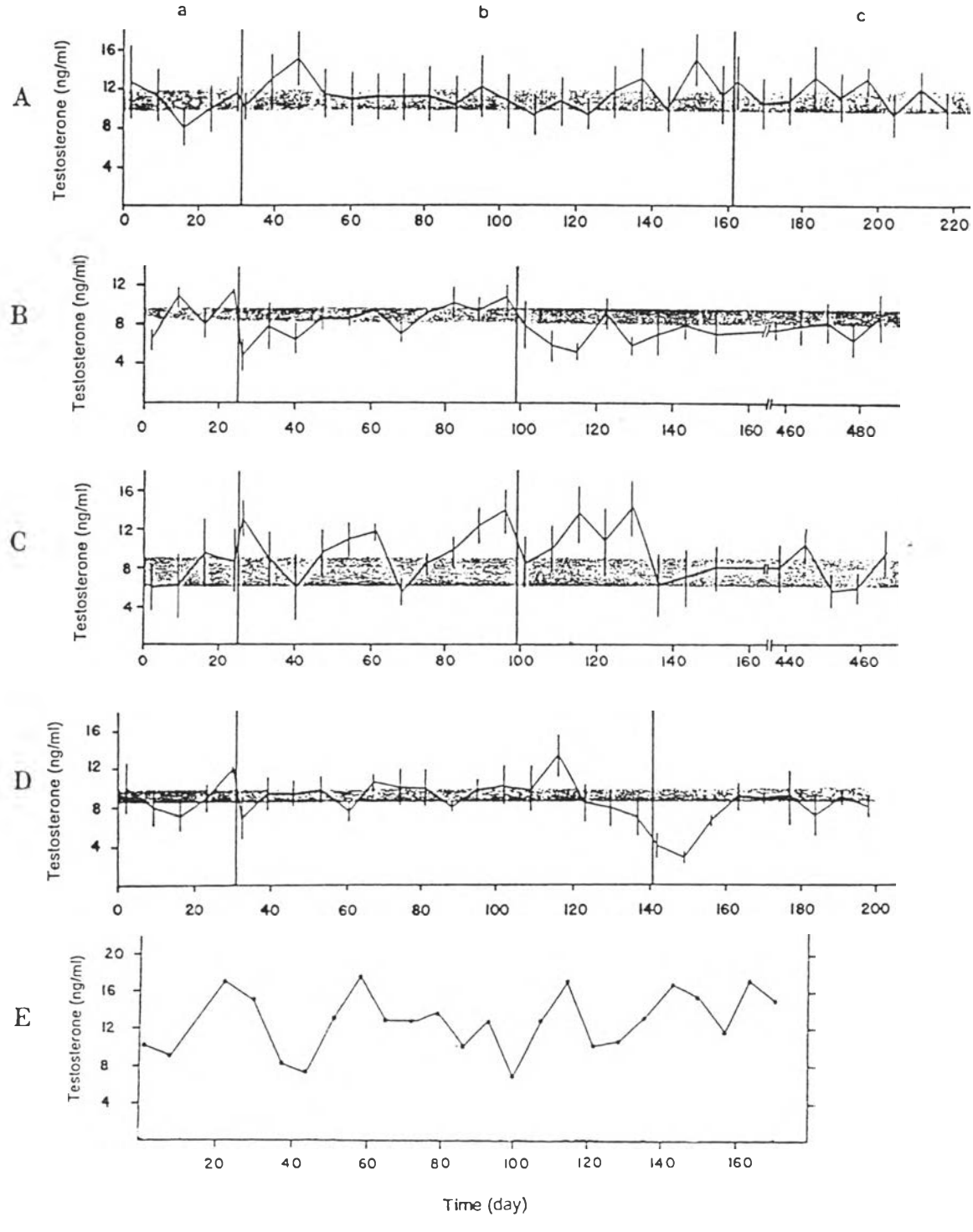

Figure 29 Mean(±SE) serum testosterone levels taken 20 hours after each injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg/day respectively in each monkey group. a,b and c represented pretreatment, treatment and posttreatment periods, respectively. A, B and D : adult male monkeys; C : pubertal male monkeys; E : saline-injected control. ▩ : $\bar{X}$±SE of pretreatment values.

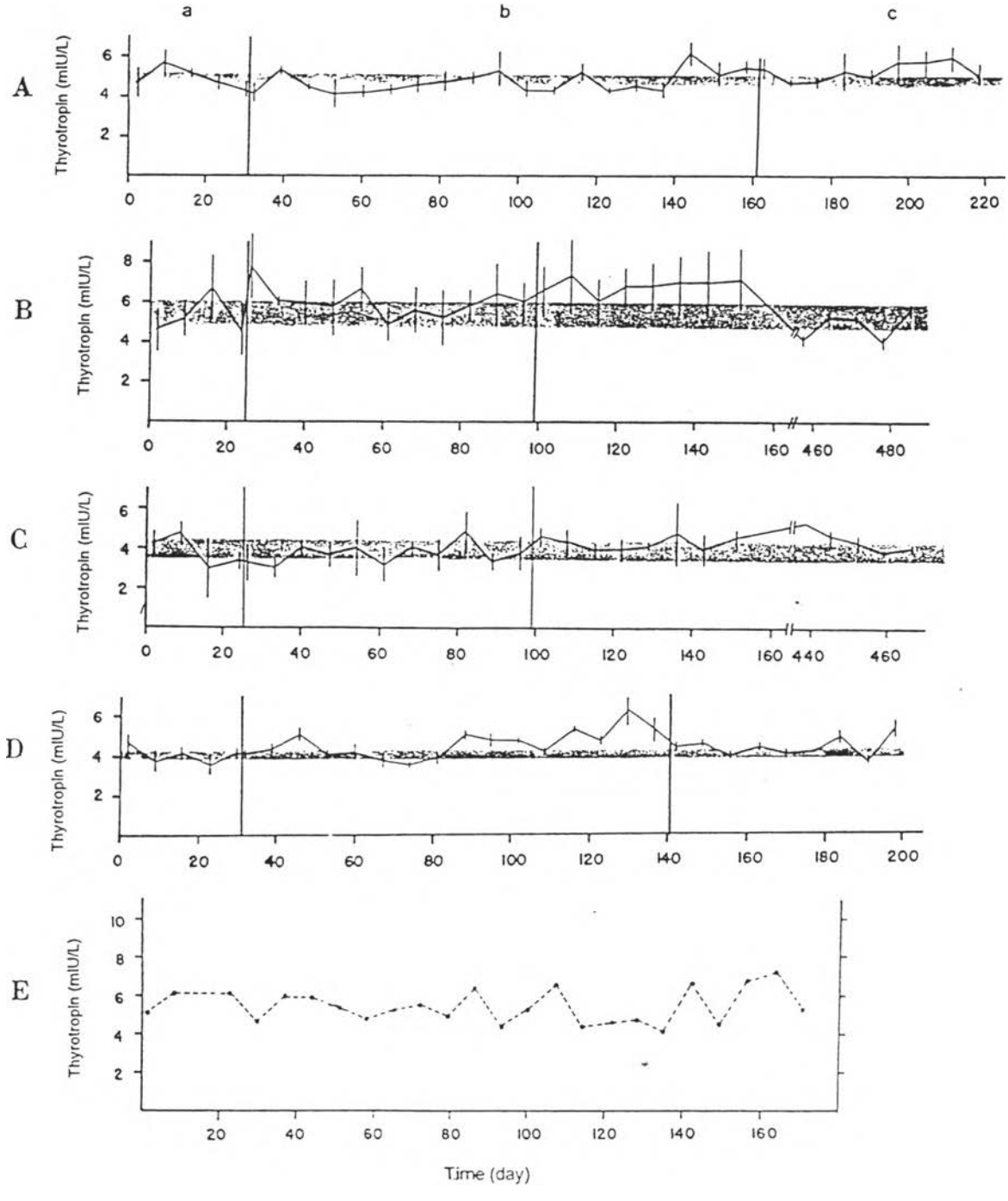

Figure 30 Mean(±SE) serum thyrotropin levels taken 20 hours after each injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg/day respectively in each monkey group. a,b and c represented pretreatment, treatment and posttreatment periods, respectively. A, B and D : adult male monkeys; C : pubertal male monkeys; E : saline-injected control. ▪ : $\bar{X}$±SE of pretreatment values.

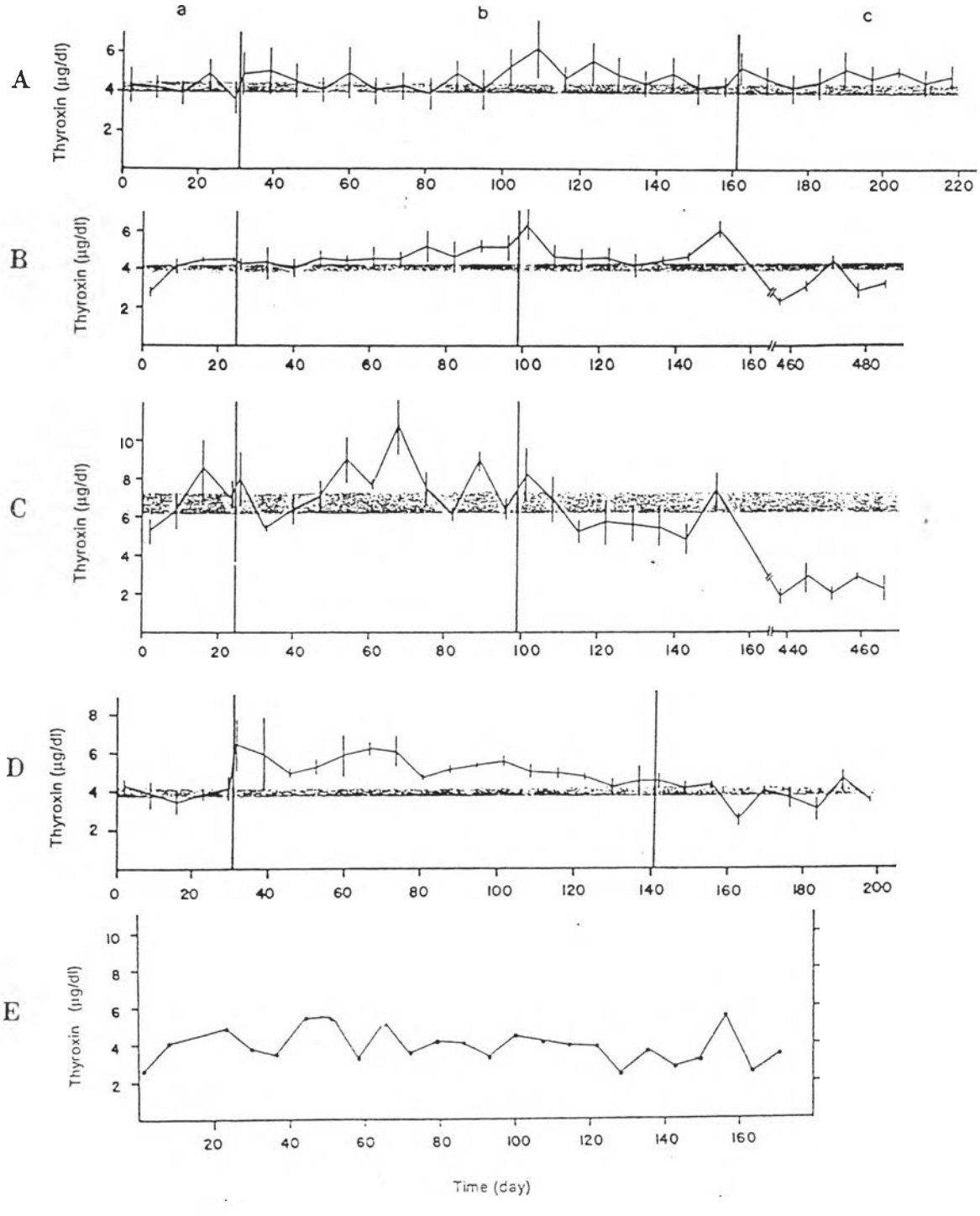

Figure 31 Mean(±SE) serum thyroxin levels taken 20 hours after each injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg/day respectively in each monkey group. a,b and c represented pretreatment, treatment and posttreatment periods, respectively. A, B and D : adult male monkeys; C : pubertal male monkeys; E : saline-injected control. ■ : $\bar{X}$±SE of pretreatment values.

group treated with 6.0 mg/kg/day morphine showed a significantly higher than pretreatment values ($p<0.05$; 5.38 $\pm$ 0.63 vs 3.98 $\pm$ 0.34 ug/dl) which were gradually decline to the basal levels in the posttreatment period (3.83 $\pm$ 0.73 ug/dl). The lowest dose of morphine administration in adult monkeys (1.5 mg/kg/day) continued to increase T_4 levels until the posttreatment period and those values were significantly higher than pretreatment values ($p<0.025$; 4.78 $\pm$ 0.36 vs 4.18 $\pm$ 0.48 ug/dl). Surprisingly, mean serum T_4 levels in adult and pubertal monkeys given 3.0 mg/kg/day morphine exhibited a profound decline during the last posttreatment periods (at day-457 to day-485 and at day-438 to day-466 in adult and pubertal monkeys, respectively) after an experimental withdrawal. The statistical analysis of these values showed a significantly lower than the early posttreatment values ($p<0.005$) at day-101 to day-143, particularly in pubertal monkeys these values were also lower than the basal values ($p<0.005$).

5. The effect on serum cortisol levels

The sudden increase of mean cortisol levels could be found in all monkey groups during withdrawal periods (Figure 32). Of interest, the monkeys injected in lower doses of morphine hydrochloride occupied a longer lag times before hormonal elevation; 1, 44 and 57 days in adult monkey groups given 6.0, 3.0 and 1.5 mg/kg/day morphine, respectively, and 44 days in pubertal monkeys. In contrast, the mean hormonal levels during treatment periods were without any significant alterations compared with pretreatment values.

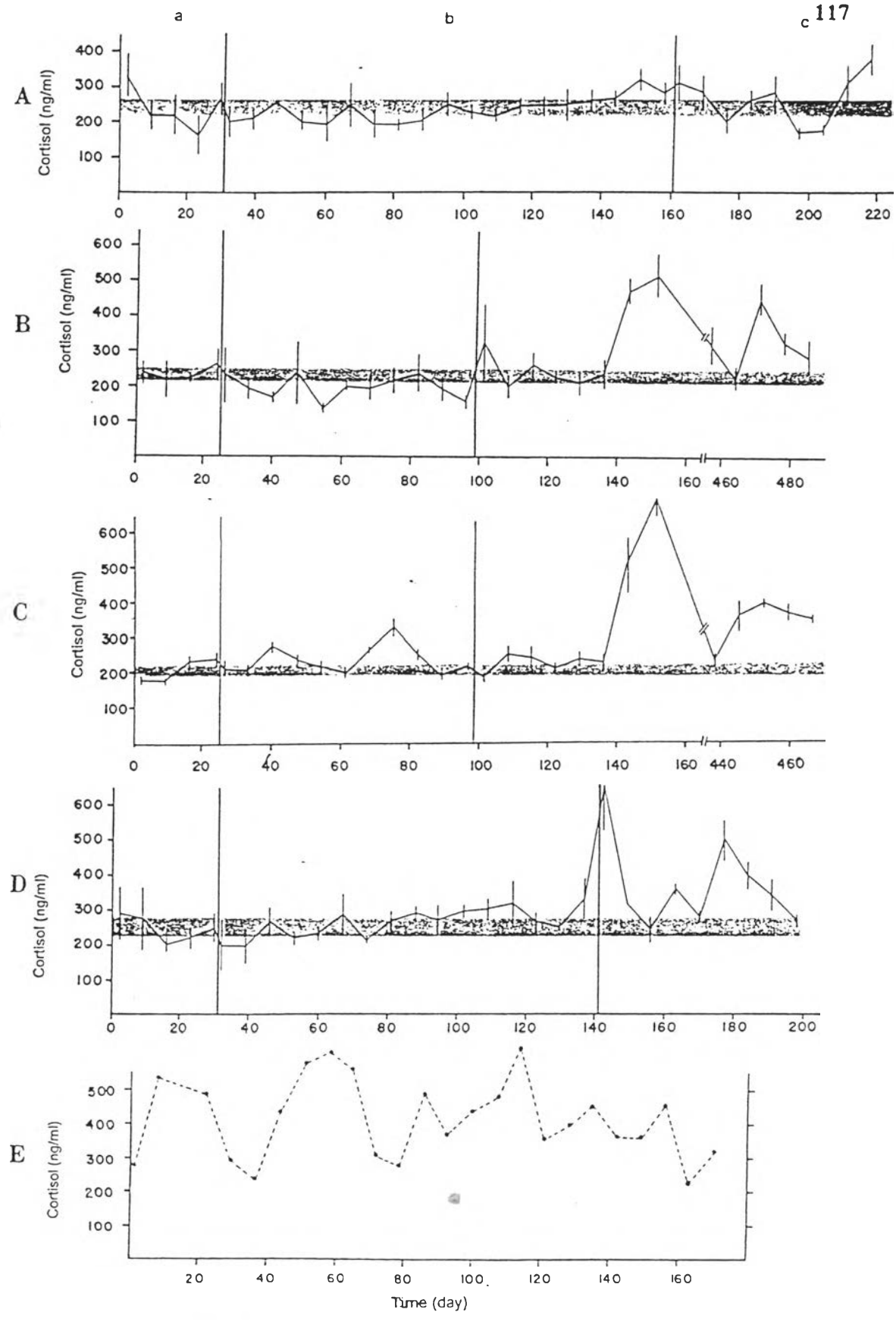

Figure 32 Mean(±SE) serum cortisol levels taken 20 hours after each injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg/day respectively in each monkey group. a,b and c represented pretreatment, treatment and posttreatment periods, respectively. A, B and D : adult male monkeys; C : pubertal male monkeys; E : saline-injected control. ■ : $\bar{X}$±SE of pretreatment values.

6. The effect on serum E_2 levels

Mean basal E_2 levels in pubertal monkeys were notably lowered than in all adult monkeys (Figure 33). During late treatment and early withdrawal periods, mean E_2 levels tended to be lower than the basal values in all adult monkey groups. However, the significant changes could observe only in the monkeys recieved in the longer-time of morphine injection (110 and 130 days in doses 6.0 and 1.5 mg/kg/day) but not in the shorter-time of morphine injection (74 days in dose 3.0 mg/kg/day). Therefore, the pretreatment values of mean E_2 levels were significantly higher than treatment and posttreatmnet values in adult monkeys given 1.5 and 6.0 mg/kg/day morphine hydrochloride, respectively. In adult males injected 6.0 mg/kg/day morphine the treatment values were also significantly higher than posttreatment values (17.77 $\pm$ 4.22 vs 8.86 $\pm$ 3.71 pg/ml).

Integral Alteration in Hormonal Levels During Chronic Morphine Treatment

1. Adult male monkeys treated with 1.5 mg/kg/day morphine

The hormonal levels in these monkeys were rather consistent and there were some statistical differences in some values of hormones (Figure 34-37). Serum T_4 levels tended to increase during treatment period in all monkeys. In monkey no.93 T_4 levels were significantly higher in posttreatment period than in treatment and pretreatment periods ($p<0.05$; 2.74 $\pm$ 1.06, 1.85 $\pm$ 0.51 and 1.78 $\pm$ 0.56 ug/dl), respectively. However, E_2 and PRL levels were lowered during treatment period ($p<0.05$). The lowered

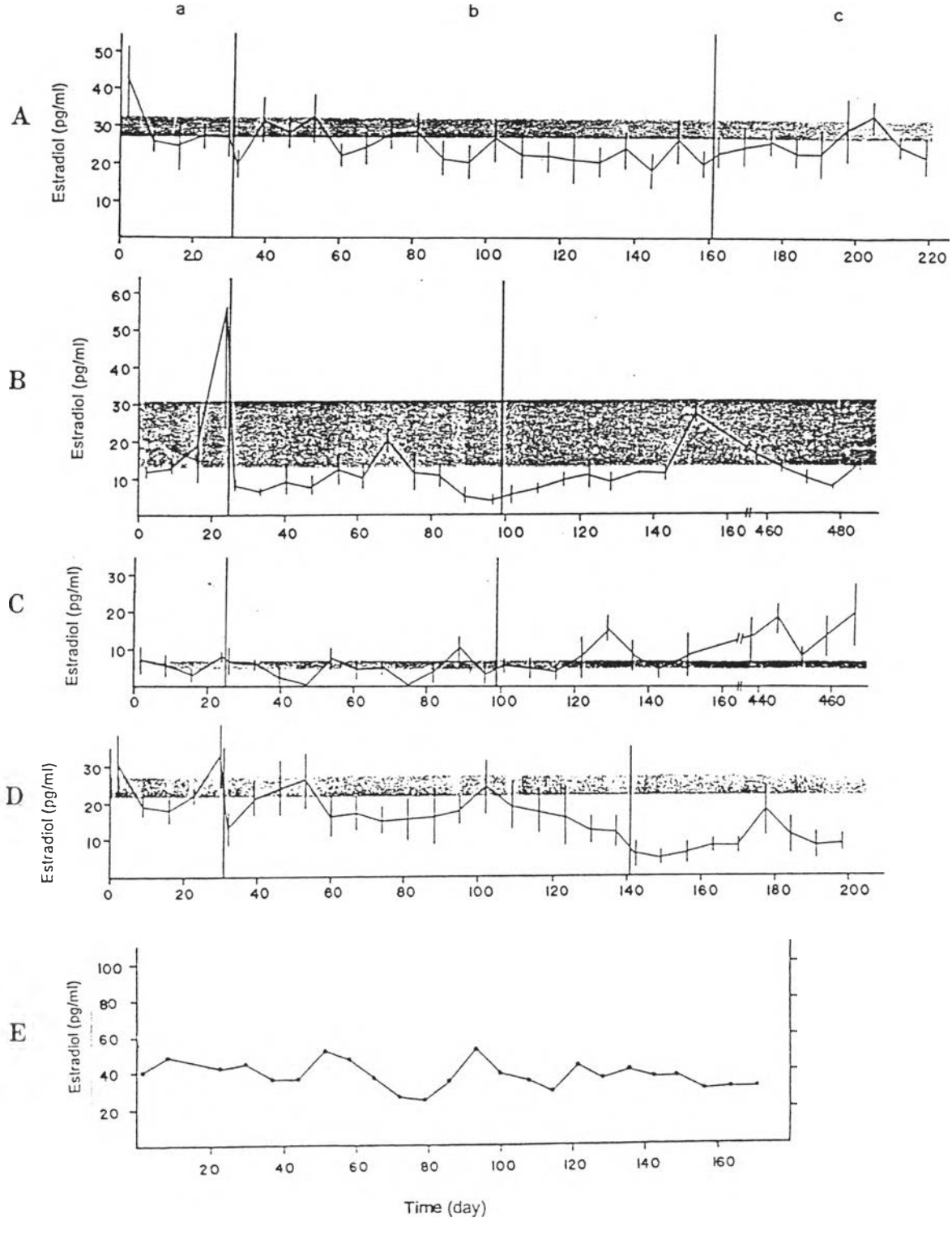

Figure 33 Mean(±SE) serum estradiol-17ß levels taken 20 hours after each injection of morphine hydrochloride 1.5(A), 3.0(B,C) and 6.0(D) mg/kg/day respectively in each monkey group. a,b and c represented pretreatment, treatment and posttreatment periods, respectively. A, B and D : adult male monkeys; C : pubertal male monkeys; E : saline-injected control. ■ : $\bar{X}$±SE of pretreatment values.

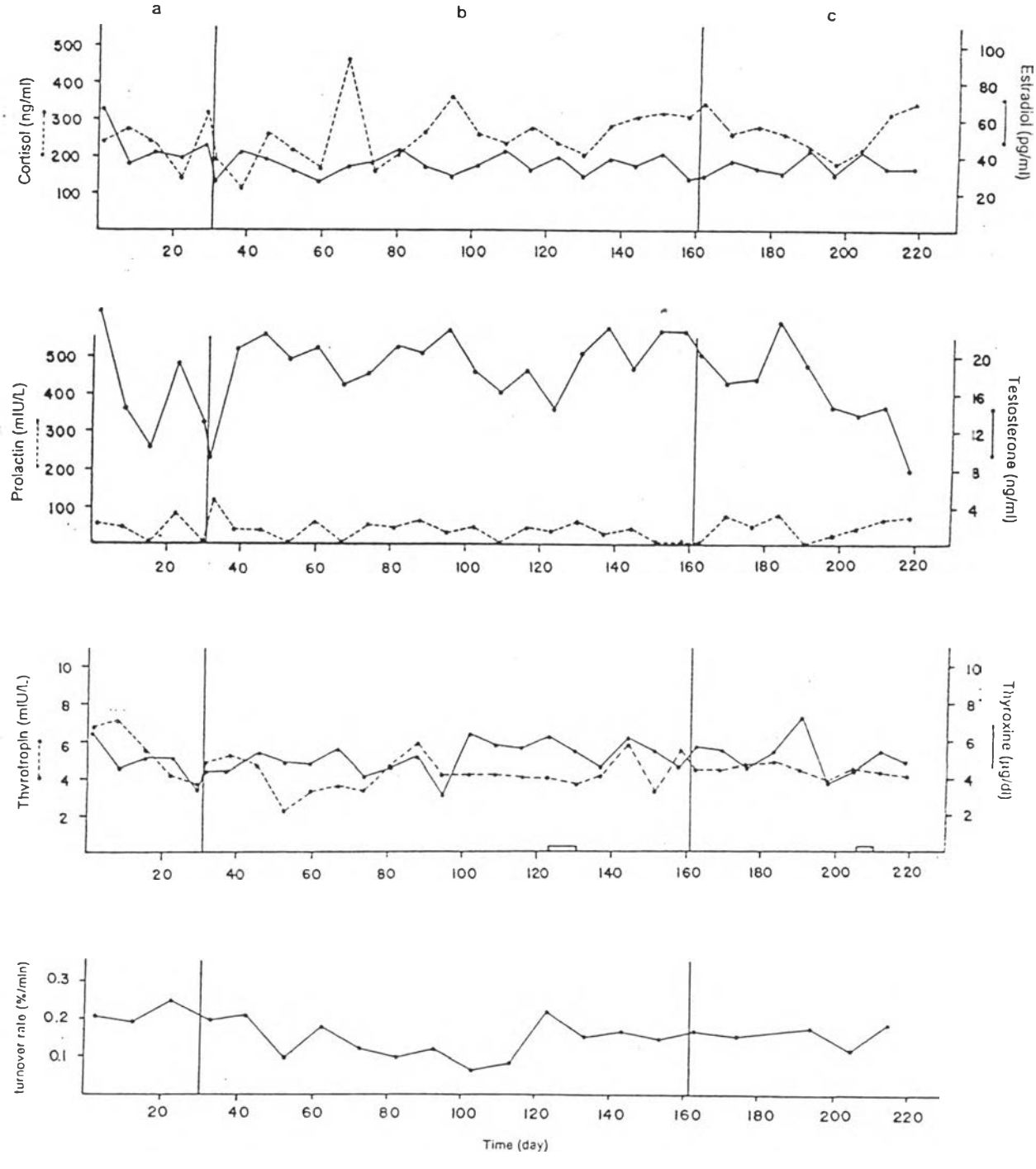

Figure 34 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 1.5 mg/kg/day in adult male monkey no.504. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

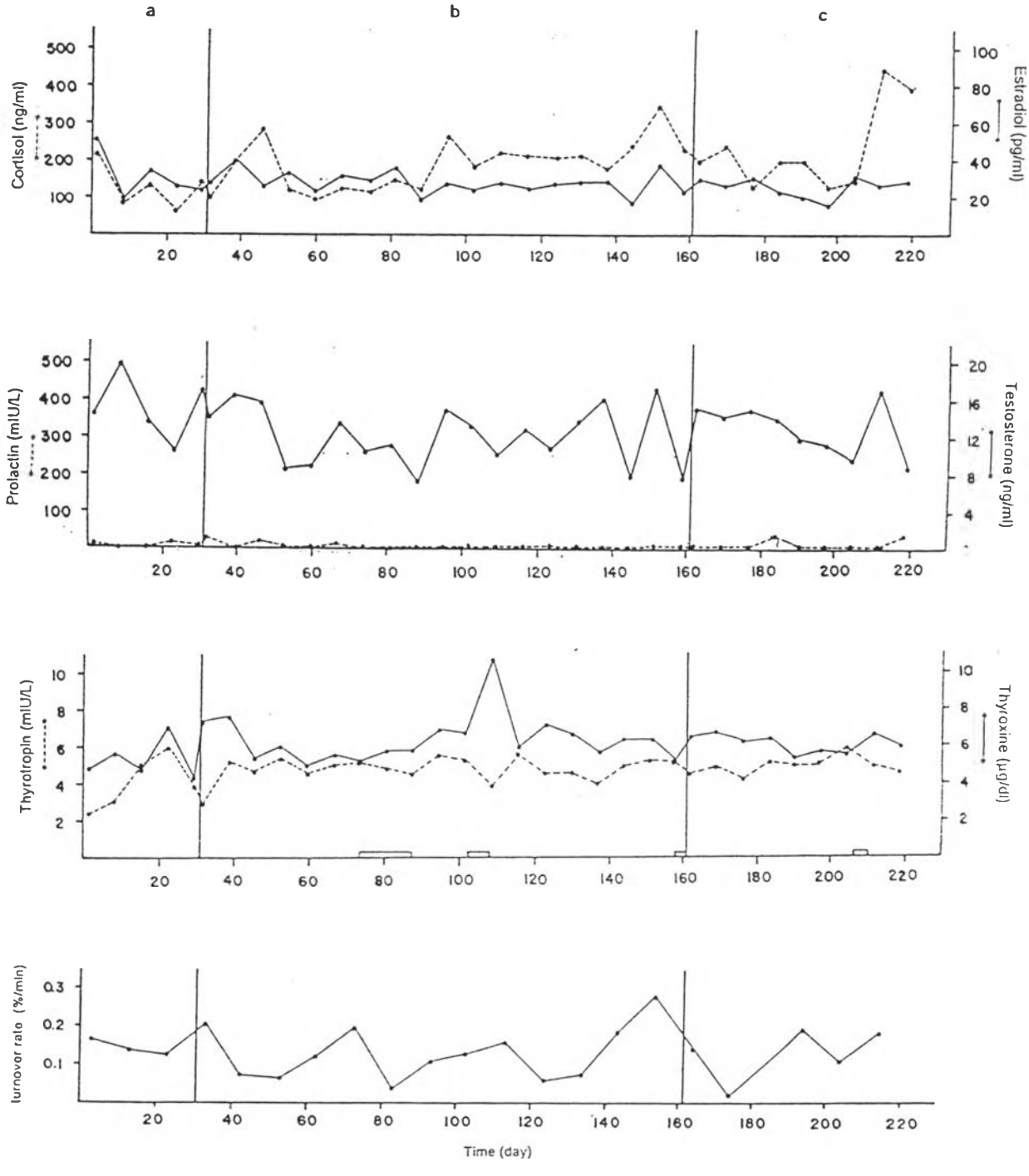

Figure 35 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 1.5 mg/kg/day in adult male monkey no.507. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

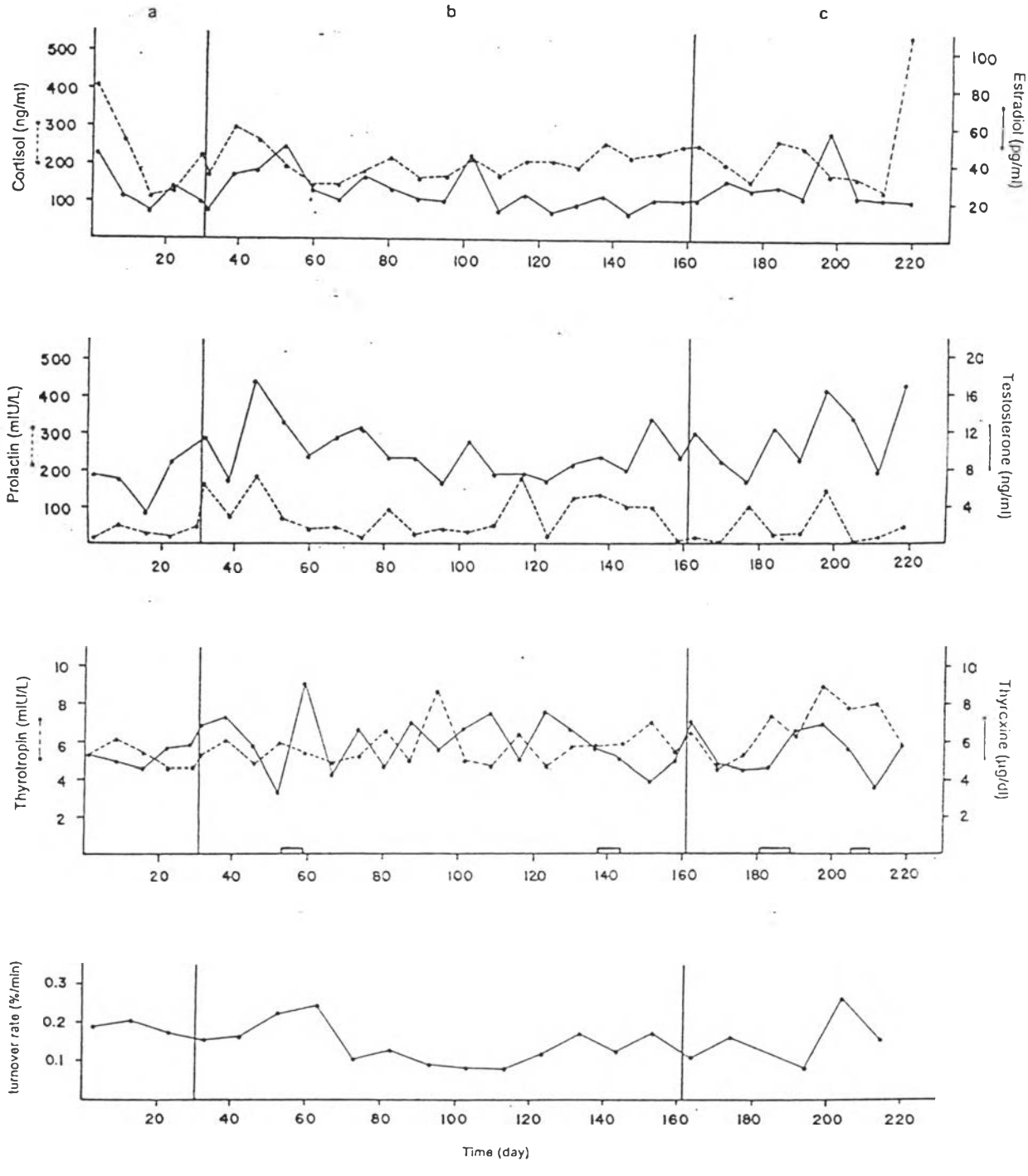

Figure 36 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 1.5 mg/kg/day in adult male monkey no.512. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.
□ : watery excretion from the mammary gland
■ : milky excretion from the mammary gland

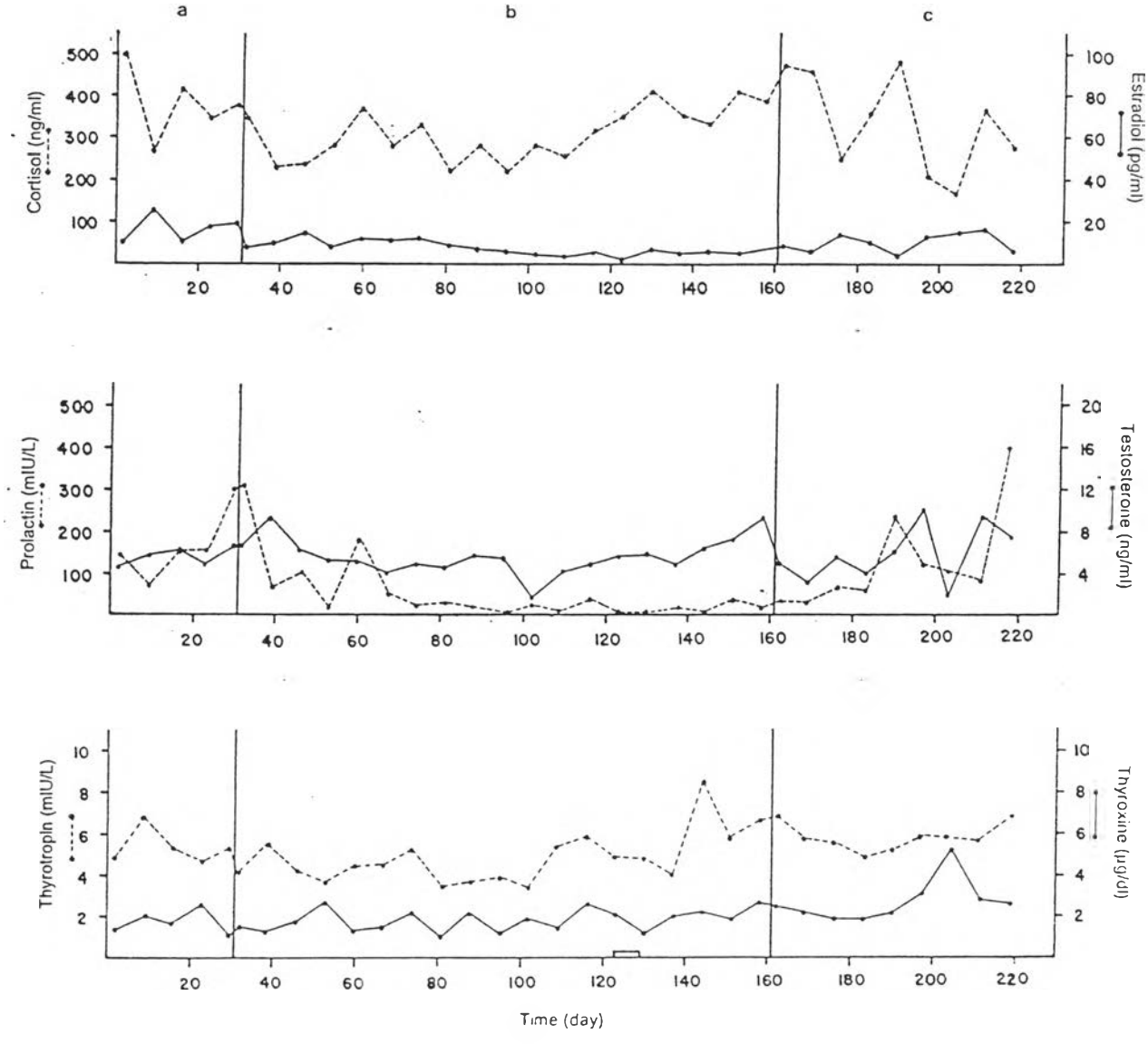

Figure 37 Patterns of hormonal levels taken 20 hours after each injection of morphine hydrochloride 1.5 mg/kg/day in adult male monkey no.93. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

testosterone levels in this monkey during early withdrawal period were particularly concurred with the cortisol rise.

There were lacks of correlation between PRL and cortisol, T_4 and TSH, and E_2 and testosterone levels in all monkeys. Additionally, all monkeys could be extruded watery fluid from the mammary gland.

2. Adult and pubertal monkeys treated with 3.0 mg/kg/day morphine

Two out of three adult monkeys (no.505 and 509) had a significant decrease in TSH and T_4 levels during the last posttreatment period (at day-457 to day-485) (Figure 38 and 39). Then T_4 levels showed a positive correlation with TSH levels in monkey no.505 ($r = 0.55$, $p<0.05$) and no.509 ($r = 0.71$, $p<0.01$). There was no significant change in T_4 levels but had a progressive rise of TSH levels in monkey no.511 (Figure 40). Testosterone concentration was decrease during early treatment period and return to normal level thereafter in monkey no.505 and 511 whereas it was subsided until the early posttreatment period in monkey no.509. All monkeys showed a PRL rise during early posttreatment period. The maximal cortisol levels during posttreatment period were mostly synchronous with the testosterone drop. Surprisingly, there was a long continuous duration of milky excretion in monkey no.509, and his basal PRL levels were higher than the other monkeys. While it was without any excretion from the mammary gland of monkey no.505.

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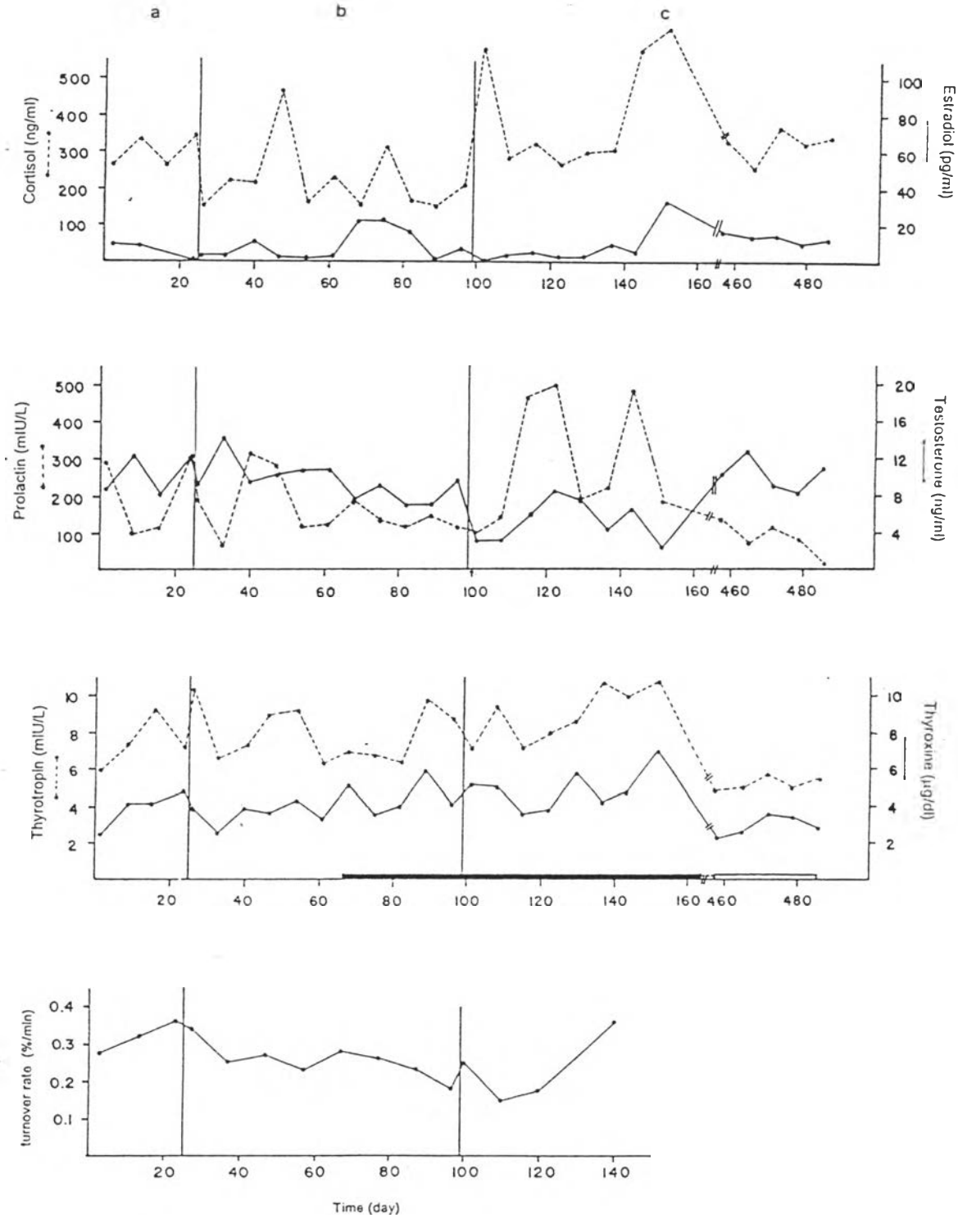

Figure 39 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 3.0 mg/kg/day in adult male monkey no.509. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.
□ : watery excretion from the mammary gland
■ : milky excretion from the mammary gland

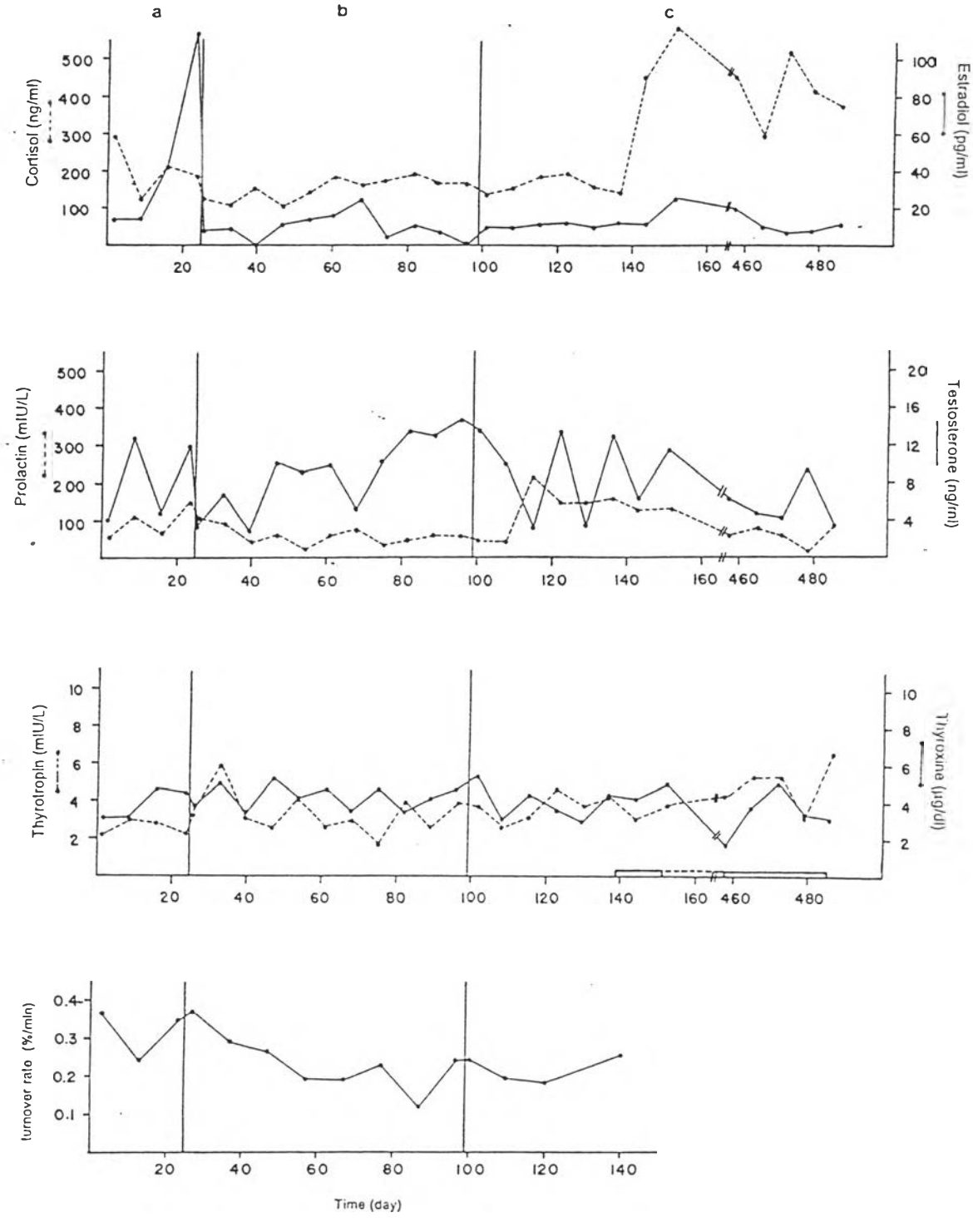

Figure 40 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 3.0 mg/kg/day in adult male monkey no.511. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

As in adult monkeys, there were lowered serum T_4 levels in all pubertal monkeys during the last posttreatment period (at day-438 to day-466), while the cortisol levels were increase. (Figure 41-43). Thereafter, T_4 levels showed a negative correlation with TSH levels ($r = -0.41$, 0.125 and -0.44 in monkey no.522, 523 and 524, respectively). Serum T_4 levels tended to be high during treatment period in all pubertal monkeys. The cortisol rise was compatible with the testosterone drop.

All pubertal monkeys treated with morphine exhibited watery excretion from the mammary gland. Mankeys no.522 and 523 showed watery excretion interrupted with a short duration of milky secretion during the transition period of morphine treatment and morphine withdrawal.

3. Adult monkeys treated with 6.0 mg/kg/day morphine

A transient drop of cortisol, testosterone and E_2 levels were found in monkey no.506 and 508 during early treatment period. In contrast, there were a decline in testosterone and E_2 levels with a cortisol peak during early withdrawal period in all monkeys (Figure 44-46). Serum T_4 levels were increased significantly during treatment period in all monkeys, however, there was no alteration in TSH levels. Then, it resulted in a lack of correlation between T_4 and TSH levels. Alternatively, there was a high positive correlation between E_2 and testosterone levels ($r = 0.54$, 0.66 and 0.55 in monkey no.506, 508 and 704, respectively). Of interest, monkey no.508 did not show any excretion from the mammary gland during the entire period of study.

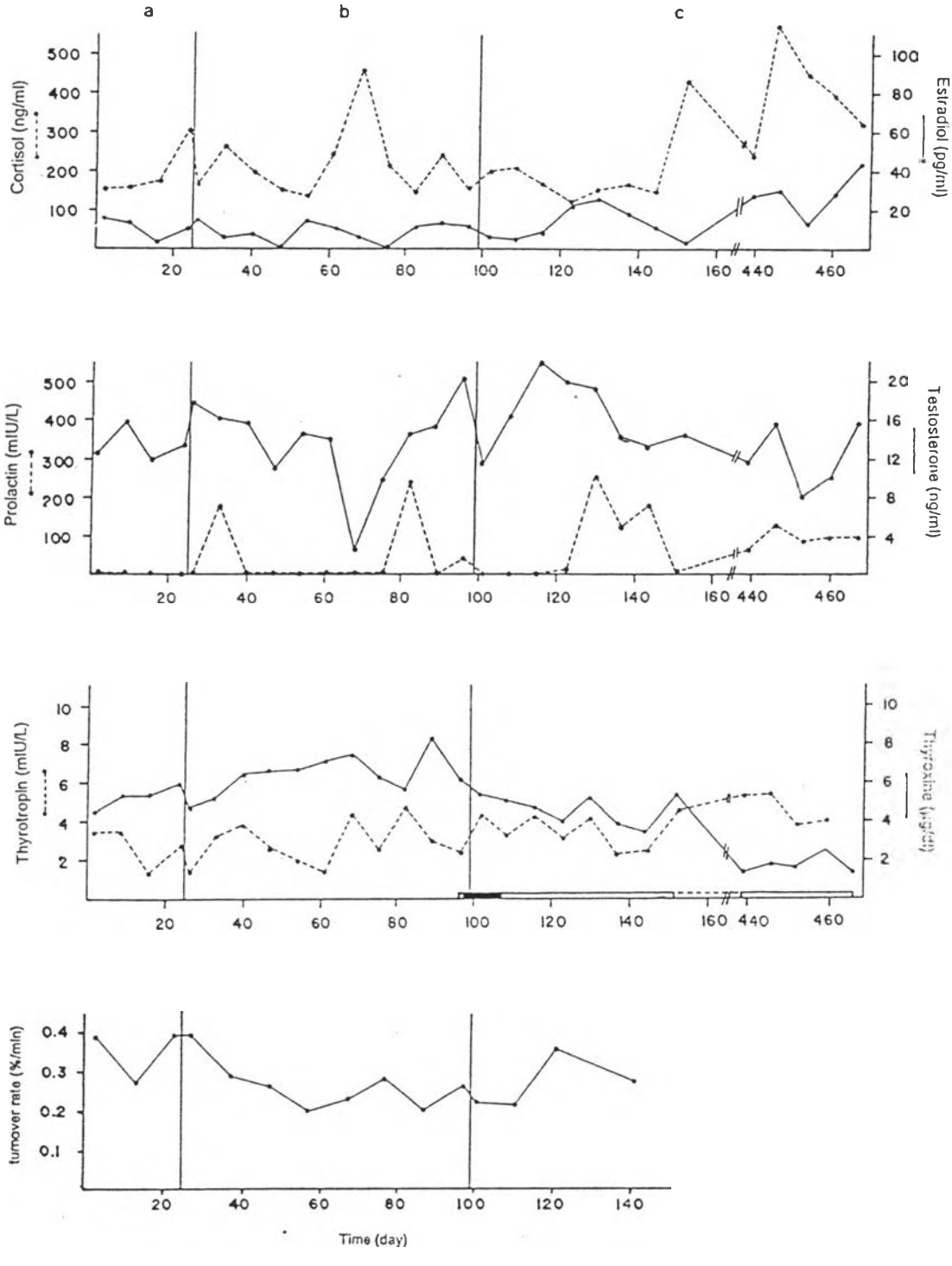

Figure 41 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 3.0 mg/kg/day in pubertal male monkey no.522. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.
□ : watery excretion from the mammary gland
■ : milky excretion from the mammary gland

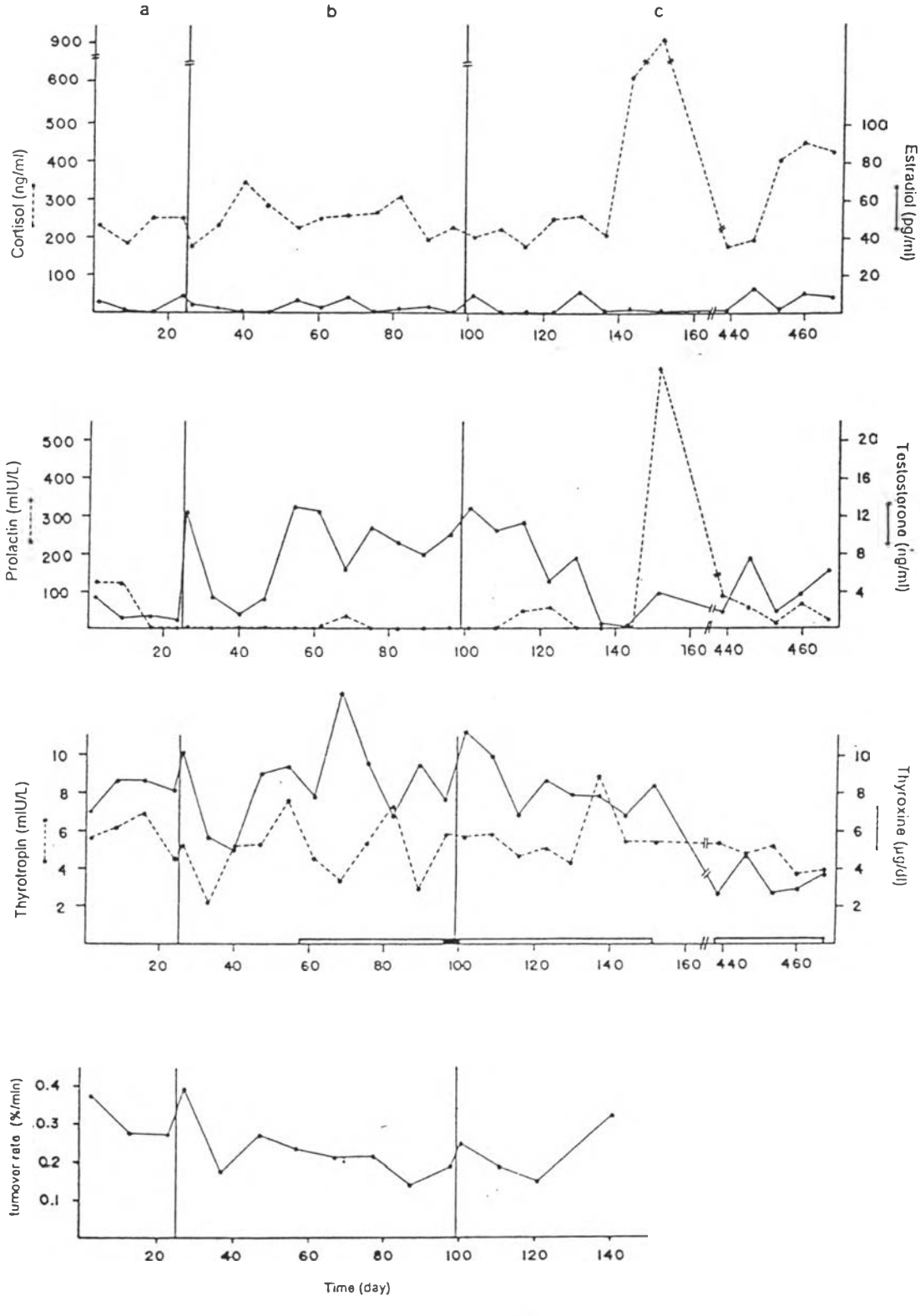

Figure 42 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 3.0 mg/kg/day in pubertal male monkey no.523. a. b and c : represented pretreatment. treatment and posttreatment periods. respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

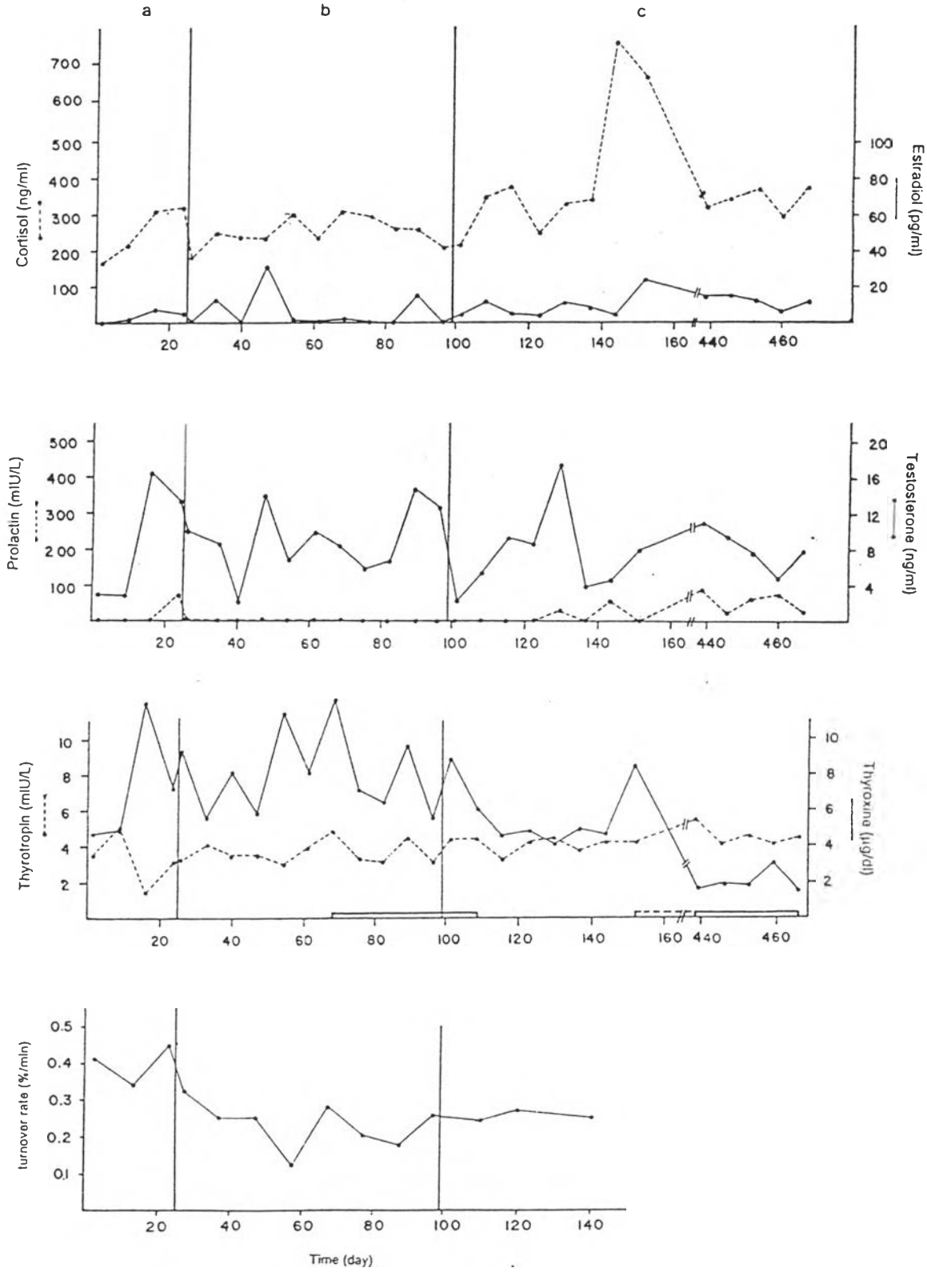

Figure 43 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 3.0 mg/kg/day in pubertal male monkey no.524. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

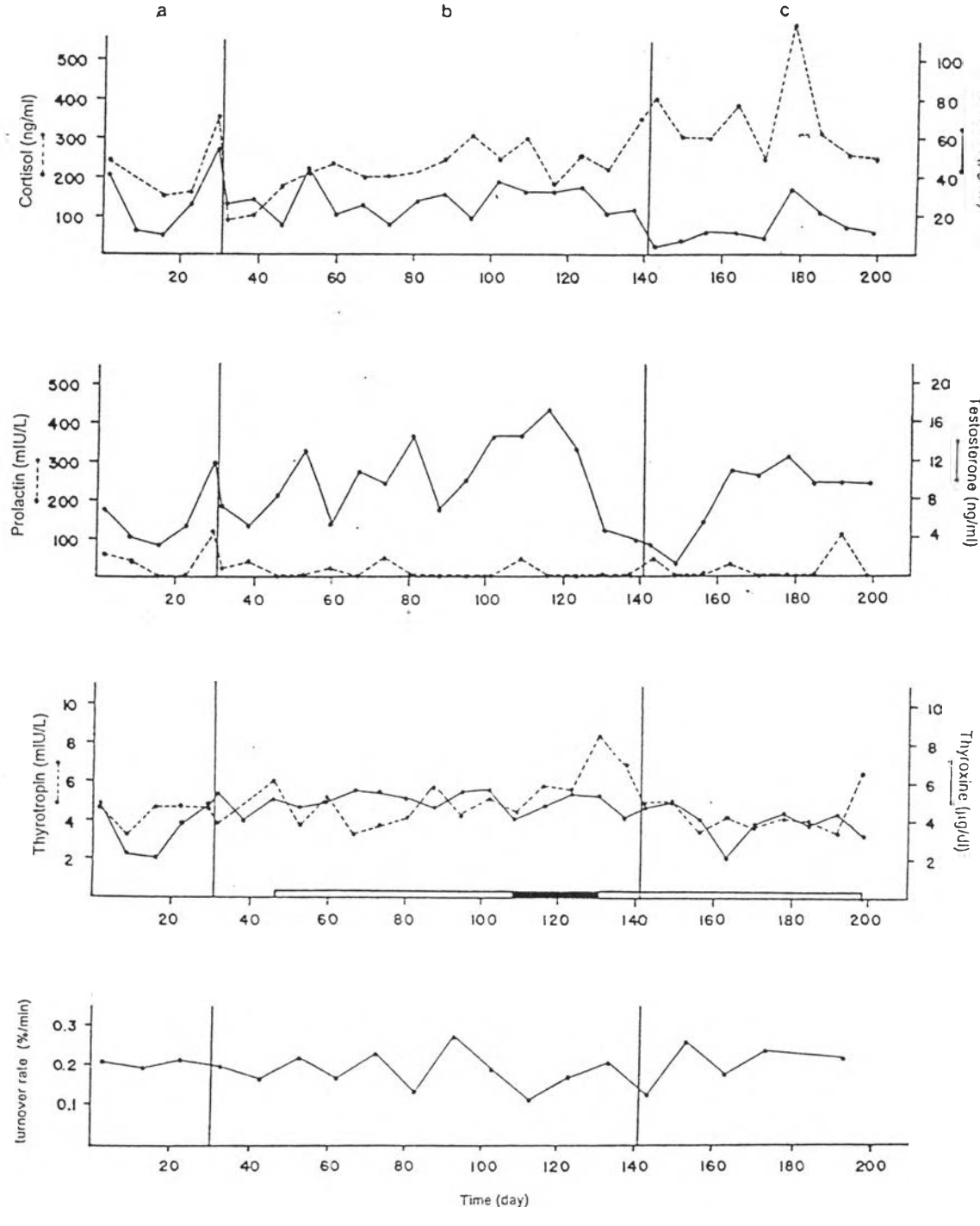

Figure 44 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 6.0 mg/kg/day in adult male monkey no.506. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

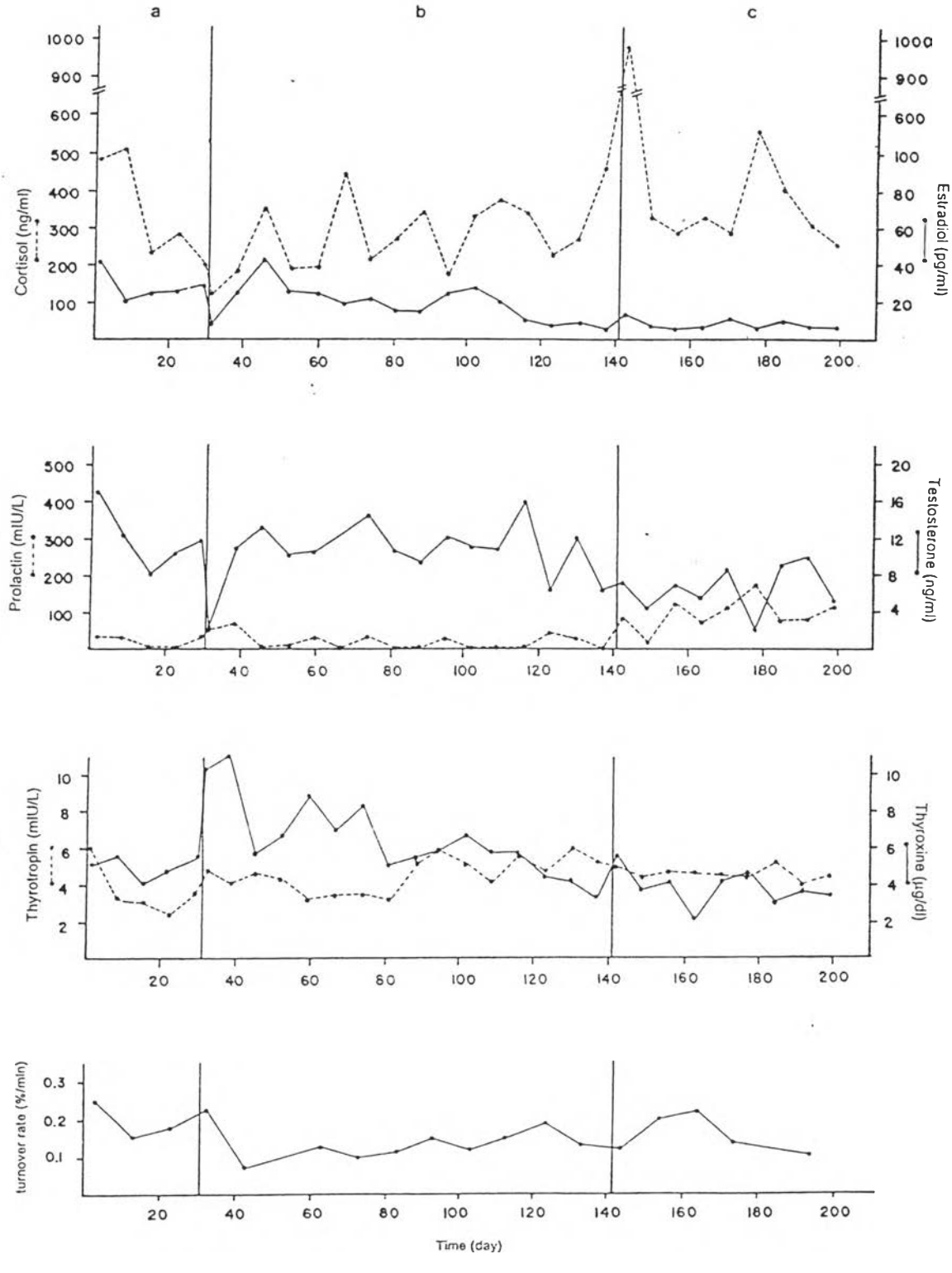

Figure 45 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 6.0 mg/kg/day in adult male monkey no.508. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

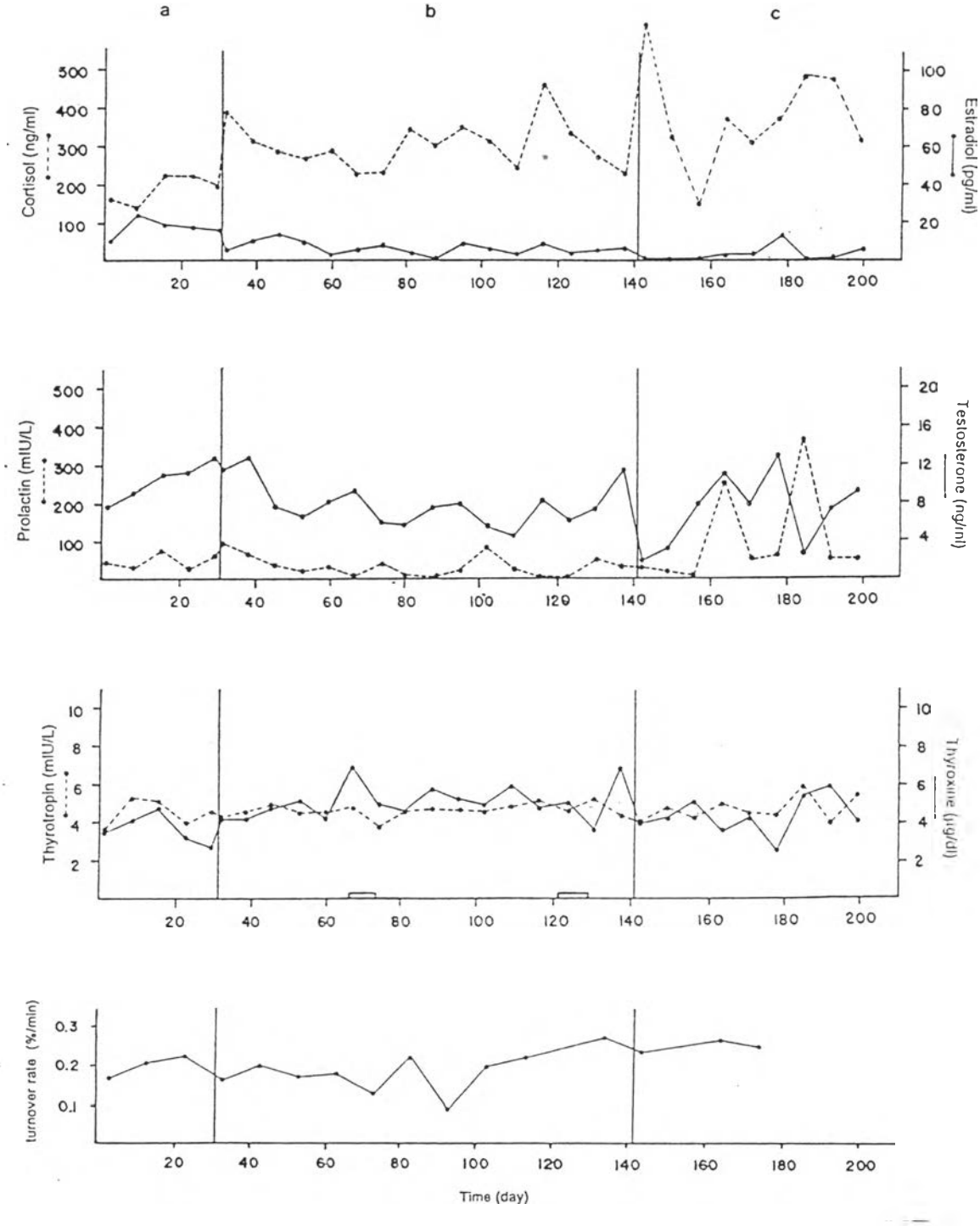

Figure 46 Patterns of metabolic turnover rate and hormonal levels taken 20 hours after each injection of morphine hydrochloride 6.0 mg/kg/day in adult male monkey no.704. a, b and c : represented pretreatment, treatment and posttreatment periods, respectively.

□ : watery excretion from the mammary gland

■ : milky excretion from the mammary gland

Metabolic Turnover of Morphine

When ^{3}H-morphine was given intravenously, the elimination half-lives were 154-523 min^{-1} (308.21 $\pm$ 20.30 min^{-1}) and the turnover rates were 0.13-0.45 $\%min^{-1}$ (0.26 $\pm$ 0.08 $\%min^{-1}$) during pretreatment period. After chronic morphine treatment, the turnover rate were altered in nearly all monkeys, except adult monkey no.506 injected 6.0 mg/kg/day with morphine for 110 days. He showed a consistent levels throughout the study period. Monkey no.508 showed a sudden drop of turnover rate after morphine administration for 13 days with a gradual increase to pretreatment values afterward. Result in monkey no.704 conflicted with the monkey no.508, since the value was higher in posttreatment period (0.26 $\pm$ 0.0 $\%min^{-1}$) than in pretreatment (0.20 $\pm$ 0.02 $\%min^{-1}$) and treatment periods (0.19 $\pm$ 0.05 $\%min^{-1}$), respectively. Turnover rates were decline in all adult monkeys treated with 1.5 mg/kg/day morphine for 13-43 days. The turnover rate in monkey that showed a preceded reduction preferentially recovered to normal values earlier. For example, monkey no.504 exhibited the definite decline on day-53 and returned to pretreatment value on day-123 whereas monkey no.512 the notably lowered value was observed on day-73 with a recover found on day-203, respectively. Turnover rates in adult and pubertal monkeys treated with 3.0 mg/kg/day morphine were also decreased during treatment period, then, returned to normal levels in some monkeys after morphine cessation. In monkeys no.511 and 524 the values were unable to increase to pretreatment levels until the end of study onset. Similarly, if the monkey showed an earlier decline in turnover rate value they were also easier returned to pretreatment value. It is of

interest that turnover rates in adult and pubertal monkeys treated with 3.0 mg/kg/day morphine were higher than the values in adult monkeys treated with 1.5 and 6.0 mg/kg/day morphine. In turn, the biological half-lives were shorter in the first ones.

Testicular Measurements

A mean testicular size and body weight elicited a nonsignificant alteration in all study periods in adult males which were given 1.5 mg/kg/day morphine hydrochloride for 130 days (Figure 47). In adult monkeys treated with 3.0 mg/kg/day morphine for 74 days showed a slightly decrease ($p<0.05$) in a mean testicular size and body weight during treatment period and concomitantly increase to the pretreatment values at the end of treatment and posttreatment periods (Figure 48). Further decline in testicular size and body weight were evidenced in monkeys treated 6.0 mg/kg/day morphine for 110 days. The decline in testicular size were started on the third week of morphine treatment and lasted throughtout entired treatment period (Figure 49). Withdrawal of morphine treatment failed to restored the testicular size promptly. It required as long as 57 days for reaching the pretreatment value. On the other hand, the testicular size and body weight in pubertal group treated with 3.0 mg/kg/day morphine were not decrease but trended to be increase toward the end of treatment (Figure 50). When compared with the adults, the puberties displayed smaller testicular size and initiated to reach an adult value at about 4.74 ± 0.65 years old. Averaged serum testosterone concentration in pubertal males was also less than in all adult males.

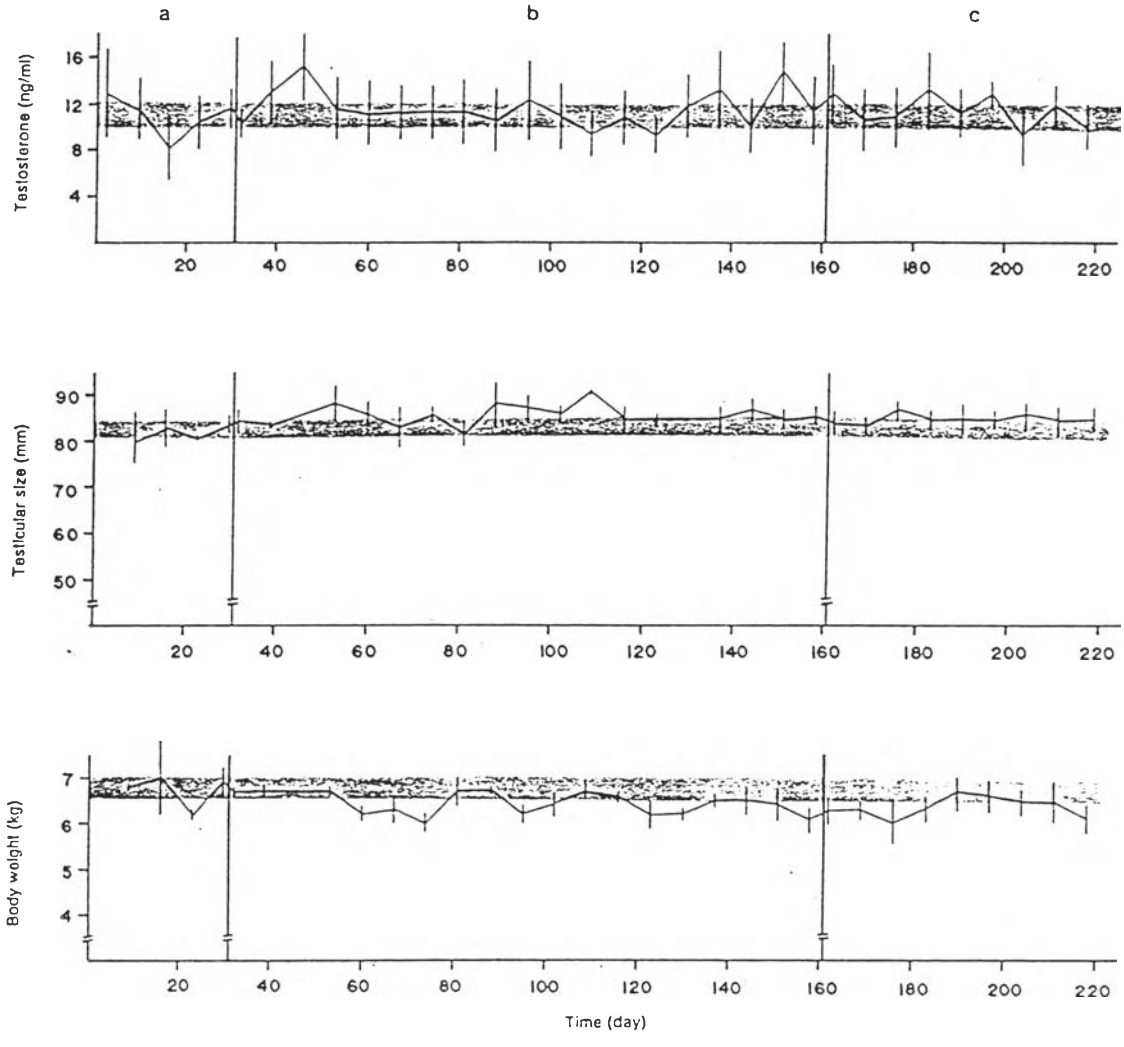

Figure 47 Mean(±SE) serum testosterone, testicular size and body weight profiles in adult male monkeys long-term treated with 1.5 mg/kg/day morphine hydrochloride. a,b and c represented pretreatment, treatment and posttreatment periods, respectively.
■ : $\bar{X}$±SE of pretreatment values.

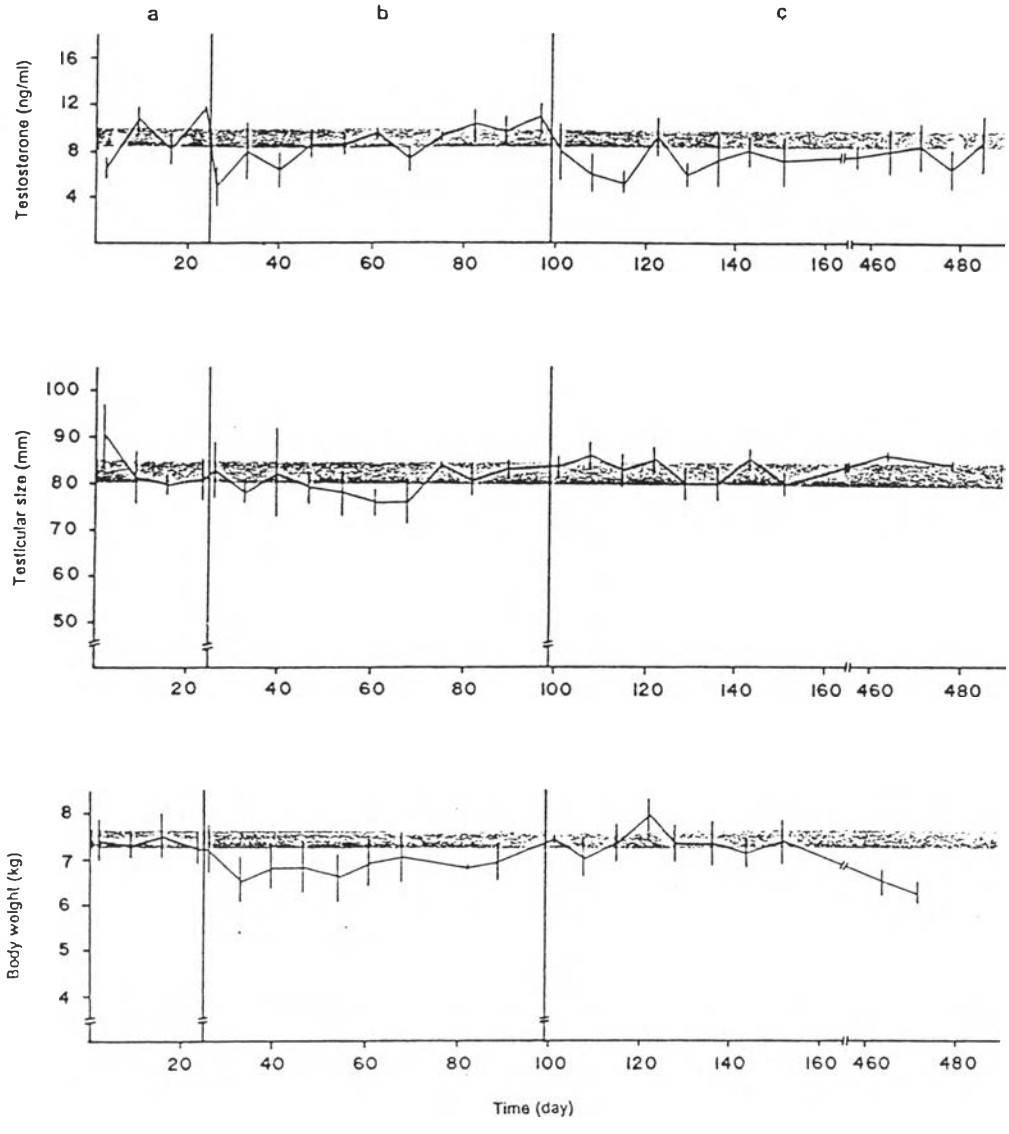

Figure 48 Mean(±SE) serum testosterone, testicular size and body weight profiles in adult male monkeys long-term treated with 3.0 mg/kg/day morphine hydrochloride. a,b and c represented pretreatment, treatment and posttreatment periods, respectively.
▮ : $\bar{X}$±SE of pretreatment values.

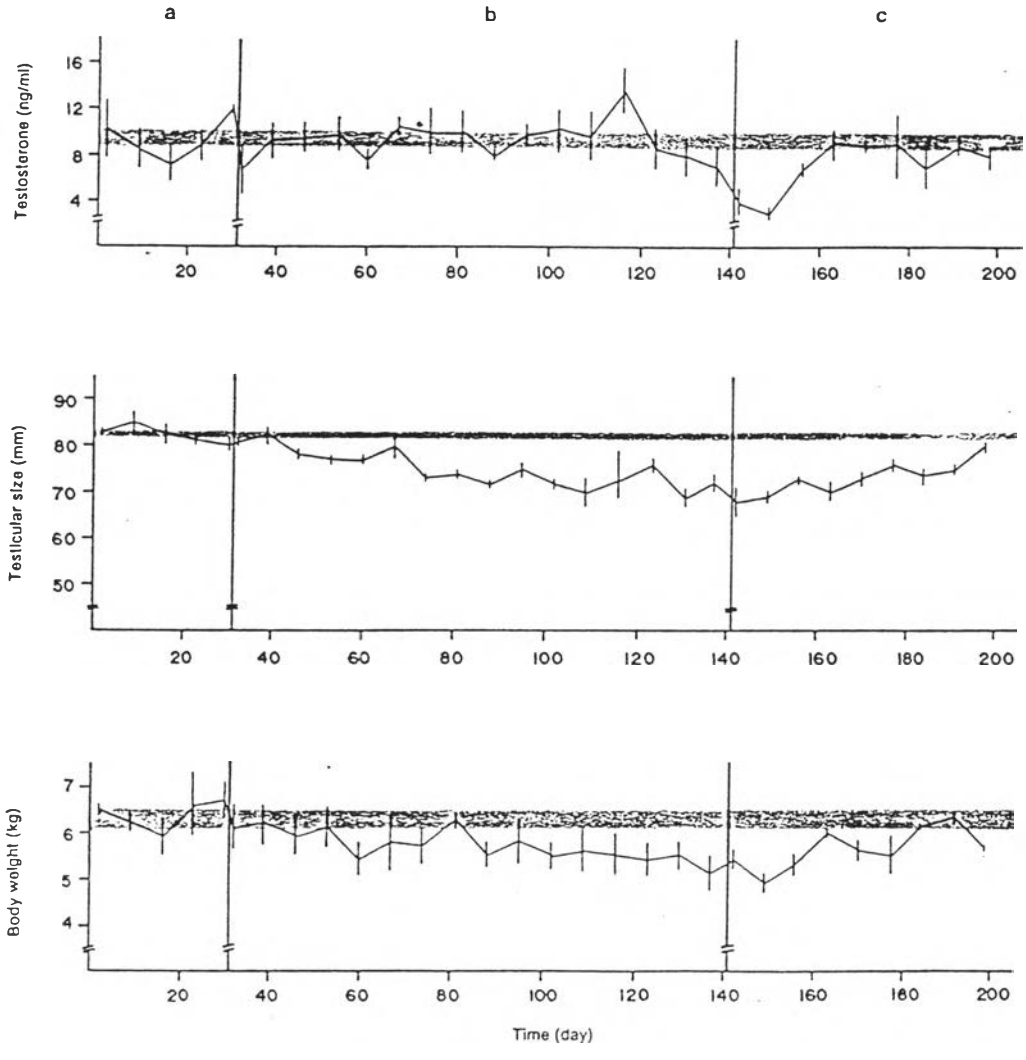

Figure 49 Mean(±SE) serum testosterone, testicular size and body weight profiles in adult male monkeys long-term treated with 6.0 mg/kg/day morphine hydrochloride. a,b and c represented pretreatment, treatment and posttreatment periods, respectively.
■ : $\bar{X}$±SE of pretreatment values.

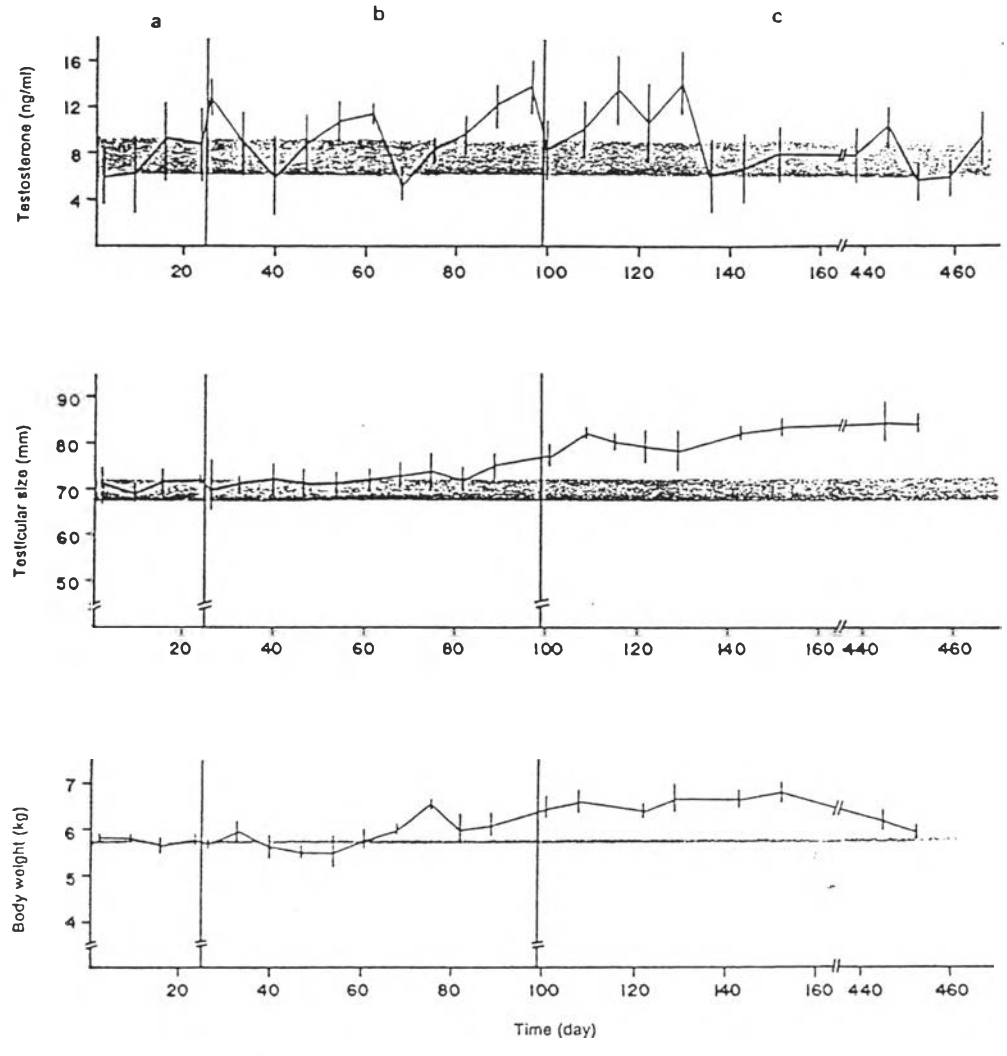

Figure 50 Mean(±SE) serum testosterone, testicular size and body weight profiles in pubertal male monkeys long-term treated with 3.0 mg/kg/day morphine hydrochloride. a,b and c represented pretreatment, treatment and posttreatment periods, respectively.
■ : $\bar{X}$±SE of pretreatment values.

In monkeys treated with 6.0 mg/kg/day morphine showed a correlated alteration between testicular size and body weight ($r=0.65$, $p<0.01$), however, the testosterone levels did not change along with this evident. Monthly mean levels of testicular size in each monkey were integrated and determined the correlation with age and body weight (Figure 51 and 52). Testicular size exhibited a greater correlation with body weight ($r=0.63$, $p<0.01$) than age ($r=0.45$, $p<0.01$) during an onset of study.

Galactorrhea Symptom

A number of male monkeys showed a spontaneous galactorrhea after morphine injected for a moment. The earliest symptom initiated at day-16 after morphine injection in monkey no.506. Only monkey no.509 and 506 had a continued milk secretion throughout the end of study onset while the other monkeys showed a transient secretion. Most of them represented a watery excretion from a mammary gland, except monkey no.522, 523, 509 and 506 were also exhibited a milky excretion for some duration. It is of interest that monkey no.509 was the only treated monkey whom excreted milky fluid continually (Figure 53). He showed the highest basal PRL levels and detected of galactorrhea when testosterone levels tended to be decline. As referred to an acute effect of morphine, the monkeys whom showed a greatest degree of PRL elevation after morphine injection tended to occur galactorrhea. For example, monkey no.509 and 511 treated with 3.0 mg/kg/day, and no.506 and 704 treated with 6.0 mg/kg/day morphine respectively showed a high peak in PRL response with a galactorrhea whereas monkey no.505 and 508 showed a

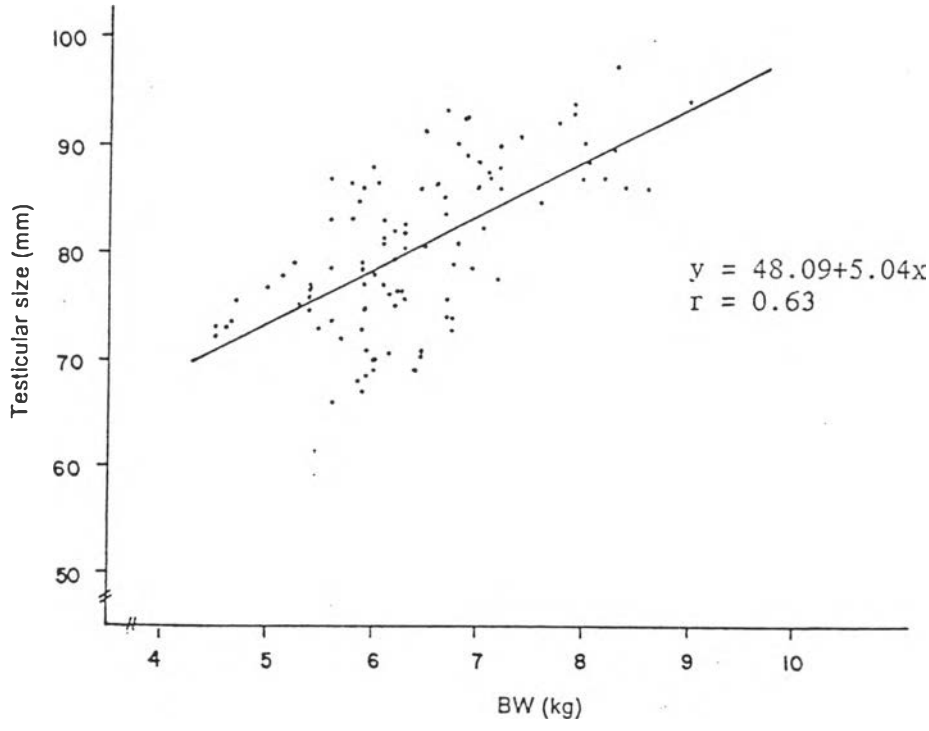

Figure 51 The relationship between testicular size and body weight in cynomolgus monkeys during long-term treatment of morphine hydrochloride.

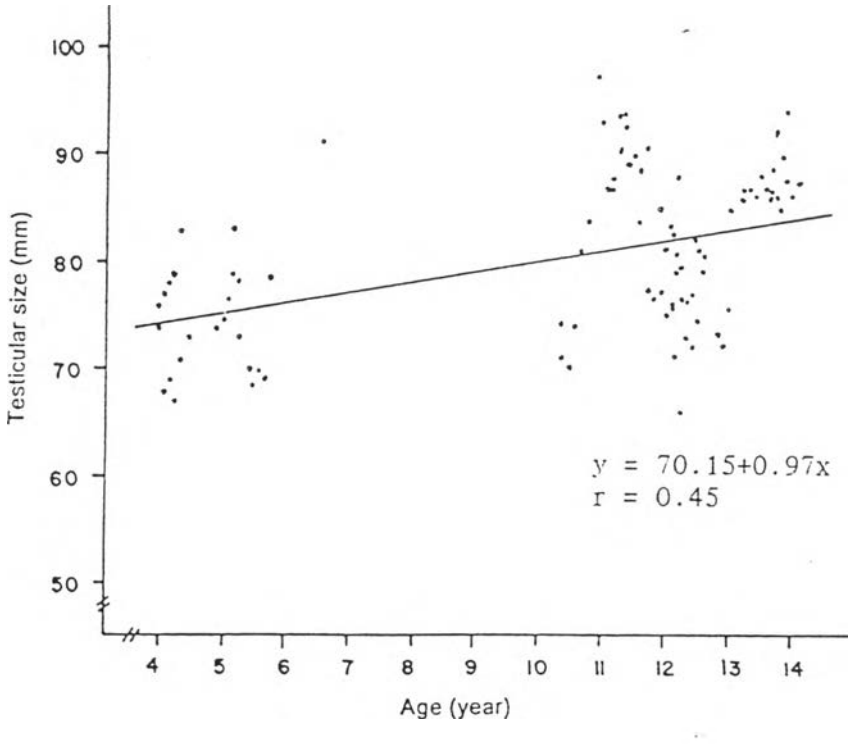

Figure 52 The relationship between testicular size and age in cynomolgus monkeys during long-term treatment of morphine hydrochloride.

low peak after morphine injection and without galactorrhea afterward. An interesting finding was that milk secretion has been continued to occur in spite of morphine cessation. Indeed, the quantity of milk is in less amount and milk could not obtain without pressure expulsion on nipples. After the milk was readily extruded for a few times, nipple manipulation could not serve any excretion again.

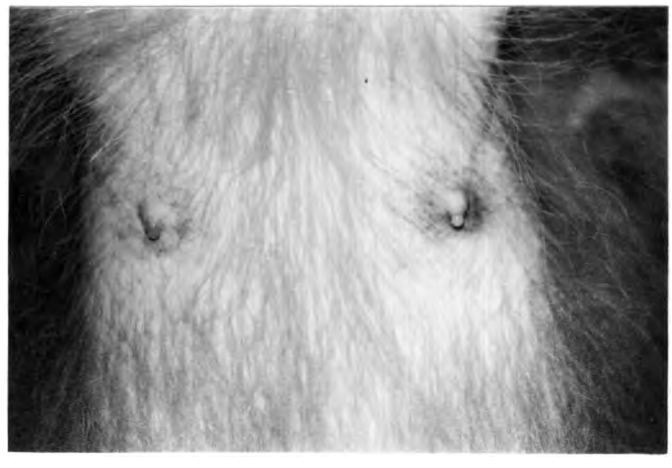

Figure 53 Milky excretion from the mammary gland in monkey no.509 as observed on day-40 of morphine treatment.

Stress and Hormonal Alterations

The monkey no.525 in figure 54 was a pubertal male monkey born in the colony. His age at the begining of study was 4.83 year-old and started of hair loss at approximately 4.0 year-old. He exhibited an excessive hair loss around his trunk, legs, arms and head until the end of study onset. At first, he was selected to be a pubertal control for comparing to morphine-injected pubertal monkeys. But his pattern of hormonal oscillations were seemingly very unexceptional, and more surprisingly he was also extruded watery

excretion from the mammary gland for any transient periods. He was postulated exposure to stress. Usually, he always calls for food when seeing monkey-care man by clapping his hands.

The consecutive blood sampling showed the progressive decline in E_2 and testosterone levels and compatible with the progressive rise in PRL and cortisol levels (Figure 55). Whereas, T_4 and TSH values seem to be a consistancy. The highly positive correlation was seen between levels of E_2 and testosterone ($r = 0.545$; $p<0.01$) and a negative correlation between testosterone and PRL ($r = 0.497$; $p<0.05$). Whereas less correlations were seen between the levels of testosterone and PRL to cortisol hormone (testosterone and cortisol, $r = -0.222$; PRL and cortisol, $r = 0.241$, respectively). The correlation between T_4 and TSH was non-significant ($r = 0.270$).

Figure 54 Excessive hair loss in pubertal monkey no.525 during the study period. He was subcutaneously injected with 0.5 ml saline once a day.

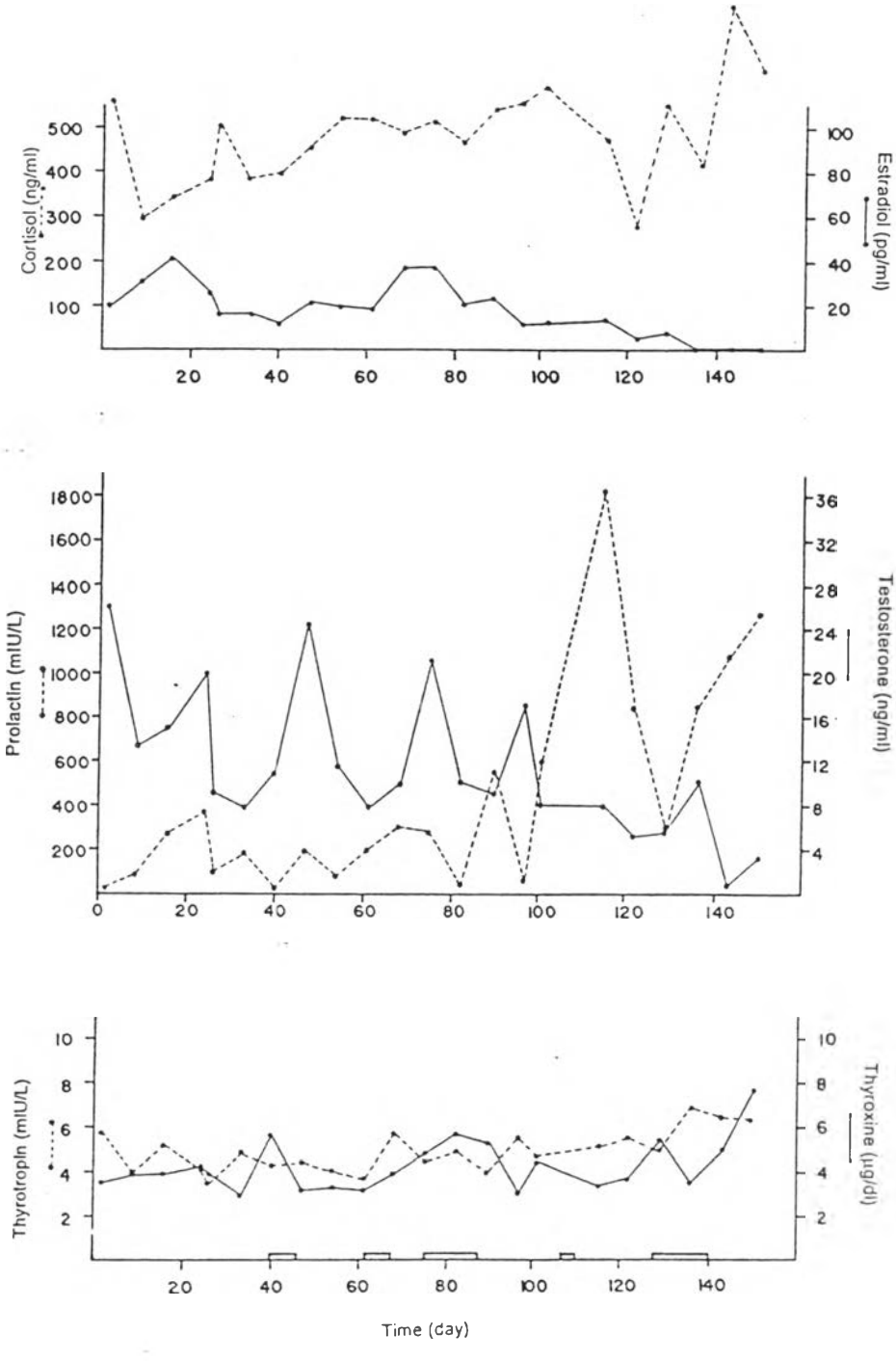

Figure 55 Patterns of hormonal levels taken 20 hours after each injection of saline in pubertal male monkey no.525.
□ : watery excretion from the mammary gland
■ : milky excretion from the mammary gland