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Circadian Rhythm of the Plasma Cortisol* Level in Cases of Prolonged Coma

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OKUYAMA, H., ENDO, M., OHARA, Y., TAKASE, S. and ITAHARA, K. *Circadian Rhythm of the Plasma Cortisol Level in Cases of Prolonged Coma.* Tohoku J. exp. Med., 1977, **123** (1), 33–47 —— Circadian variations of plasma cortisol were studied in four different groups of subjects; 17 patients with prolonged coma, 3 patient with tetraplegia with alert consciousness and without body movement, 4 patients with stabilized chronic infection with alert consciousness and with normal body movement, and control subjects consisting of 7 healthy volunteers and 5 patients with various neuromuscular disease without disturbance of consciousness, motor dysfunction or infection. The maximum level of plasma cortisol attained during the circadian variations was low in prolonged coma, whereas the minimum level was high in prolonged coma, as compared to other three groups. The amplitudes between the maximum and the minimum level were significantly smaller in prolonged coma than in control ($p<0.005$). The tendency that the maximum level appeared at early morning and the minimum at late evening was similarly observed in both prolonged coma and control groups suggesting that there is no phase shift of the circadian rhythm of cortisol in prolonged coma. The responses of plasma TSH to synthetic TSH-releasing hormone or those of plasma cortisol to ACTH were not different between prolonged coma and control, suggesting that the reduced amplitude in prolonged coma is not attributed to the function of the patients' pituitary and/or adrenal cortex. Also, there were no differences in diurnal variations in plasma glucose or non-esterified fatty acid between the prolonged coma and control, nevertheless the former was fed with liquid food via a nasal tube. Therefore, highly significant reduction of the amplitude of circadian variation of cortisol in prolonged coma may not be due to exogenous factors, such as a poverty of body movement, complications due to chronic infection, or tube feeding. The results seem to suggest that the reduced amplitude of the circadian variations in plasma cortisol may have relation to the unconsciousness of prolonged coma due to severe damage to the central nervous system.——
circadian rhythm; cortisol; 17-OHCS; prolonged coma; central nervous system

Circadian rhythm in the hypothalamo-pituitary-adrenal system is well known as one of the most prominent biorhythms. This rhythm is shown to be induced by the hypothalamus (Takebe et al. 1972; Seiden and Brodish 1972). The hypothalamus, however, is not regarded as necessarily being the center which primarily produces this rhythm, although it is apparently controlled by the central nervous system (CNS) (Eik-Nes and Clark 1958; Perkoff et al. 1959).

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* Cortisol: 11β, 17, 21-trihydroxypregn-4-ene-3,20-dione.

Although a number of reports have appeared, the circadian rhythm of hypothalamo-pituitary-adrenal system has been poorly understood in regard to patients suffering from damage to the CNS. For the following reasons, prolonged coma due to severe brain damage can be regarded as a unique model for study of the effects of the damaged CNS on the rhythm. First, prolonged coma may be viewed as a stable or non-stressful state despite the presence of severe brain damage as compared with the acute stage of CNS damage which is more stressful. Second, it is practically impossible to keep normal subjects without body movement for a

TABLE 1. *Summary of*

Case No.	Sex Age	Diagnosis*	Complications	Duration prior to study
1	F, 52	Hemorrhage into blt. cereb. hemispheres		3 yrs & 4 mos
2	M, 64	Occlusion of basilar artery	Diabetes mellitus, Parkinson's disease	3 yrs & 6 mos
3	F, 48	Cereb. anoxia during surgical operation	Neurinoma of XI nerve, surgical operation	3 yrss
4	F, 65	Hemorrhage into blt. cereb. hemispheres		1 yr & 4 mos
5	F, 70	Hemorrhage into blt. cereb. hemispheres		1 yr & 6 mos
6	F, 57	Hemorrhage into pons	Arterial hypertension	2 yrs & 8 mos
7	M, 69	Multiple cereb. embolism	Mitral stenoinsufficiency, auricular fibrillation	4 mos
8	F, 76	Occlusion of basilar artery		1 yr & 6 mos
9	M, 38	Contusion of rt. cereb. hemisphere	Skull fracture, surgical operation	4 yrs & 4 mos
10	F, 38	Contusion of brain stem	Fracture of skull base	2 yrs & 10 mos
11	F, 65	Occlusion of basilar artery	Arterial hypertension	2 mos
12	F, 63	Occlusion rt. A. cereb. media		2 yrs & 10 mos
13	F, 61	Hemorrhage into blt. cereb. hemispheres	Arterial hypertension	1 yr & 5 mos
14	M, 79	Occlusion of rt. A. carot. interna & lt. A. cereb. media		1 yr
15	M, 46	Contusion of blt. cereb. hemispheres	Skull fracture, surgical operation	7 yrs & 4 mos
16	F, 28	Contusion of lt. cereb. hemisphere & brain stem		2 yrs & 6 mos
17	M, 50	Contusion of brain stem, Extradural hematoma	Fratcure of skull base	4 yrs & 5 mos

* rt.=right lt.=left blt.=bilateral cereb.=cerebral
† Decort.=decorticate rigidity Decereb.=decerebrate rigidity

long period of time, while patients of prolonged coma exhibit no spontaneous body movements. Third, it is extremely difficult to isolate normal subjects from "synchronizers" (Halberg et al. 1954). As the result of severe unconsciousness in prolonged coma, however, these patients probably have some difficulty in receiving synchronizers.

The purpose of the present paper is to study circadian variation of the plasma cortisol levels in prolonged coma, and to discuss the effects of damaged CNS on circadian variation of cortisol.

patients with prolonged coma

Chief neurological signs†	Decubitus	Tracheotomy	ESR‡
		Urethral catheter	
Decort., forced crying, teeth grinding	Healed	−/−	25 45
Decereb., sucking reflex, tremor, Myerson's sign	+	+/−	33 66
Decereb., teeth grinding, forced crying		+/+	28 46
Decort., sucking reflex, forced crying	Healed	+/−	50 76
Decort.	+	−/−	25 34
lt. spastic hemiplegia, rt. hand tremor	Exudative	+/+	58 80
Decort., teeth grinding, muscle twiching of lt. face & lt. arm	+	+/+	23 37
Decort., rt. hand tremor	+	−/−	25 55
Decereb., grand mal	Healed	+/−	52 79
Decereb., teeth grinding, nystagmus	Healed	−/−	45 70
Decereb., forced crying, tremor of rt. arm	Exudative	−/−	16 39
Decort., forced crying, teeth grinding	−	+/+	52 125
Decort., teeth grinding, sucking reflex	+	−/+	33 68
Decort., teeth grinding, sucking reflex	+	+/+	38 62
Decereb., sucking reflex	Healed	+/−	70 105
Decereb., teeth grinding, nystagmus	Healed	+/+	31 43
Decort., grand mal, sucking reflex	Exudative	+/+	78 115

‡ ESR=erythrocyte sedimentation rate (1 hr mm / 2 hr mm)

Subjects and Methods

Group of patients in prolonged coma: This group consisted of 17 patients who were in the state of "persistent vegetative state" as termed by Jennett and Plum (1972). Levels of their consciousness were evaluated according to the following 12 items; response to calling, voluntary speech, recognition of the presence of another person, emotional expression in the face, movement of the eyeballs, responses to flashing light and to clicking noises, voluntary movements of the extremities, responses to pin pricks, coughing and swallowing reflexes, and modes of urination. Twelve of the patients had suffered cerebrovascular accidents, the other 5 cases had head trauma (Table 1). Age ranged from 28 to 78 years (mean age: 56.4 years); there were 11 women and 6 men. Duration of prolonged coma prior to the study ranged from 2 months to 7 years 4 months. With regard to limb postures decorticate rigidity was detected in 9 cases of this group, decerebrate rigidity in 7, and the Mann-Wernicke type in one. Decubituses were found in 9, healed in 6, and not detected in 2. Tracheotomies had been performed on 11 cases, but none were set on an artifical respirator.

All cases were maintained at adequate nutrition with liquid foods via nasal tubes. Diapers or ureter tubes were placed on all cases because of urinary disturbances. None received the drugs which might have effect on the hypothalamo-pituitary-adrenal system such as glucocorticoid, ACTH, atropine (Hedge and Smelik 1968), reserpine (Smelik 1967), barbiturate (Krieger 1970), and L-DOPA. Cases which exhibited obvious signs of acute infection, e.g. fever, leucocytosis in peripheral blood, shadows in chest radiographs, abnormal sediments in the urine, were excluded from this group. Nevertheless, many cases of prolonged coma were suspected to have had chronic infections which were stabilized by antibiotics, since they showed accelerated erythrocyte sedimentation rates (Table 1). This was probably due to their chronic infections around decubituses or infections in the respiratory and/or urinary systems.

Control group: The control group (Table 2) consisted of 7 healthy volunteers (daytime workers) and 5 patients suffering from various neuromuscular diseases such as early-stage of amyotrophic lateral sclerosis and the ocular type of myasthenia gravis without medication. The mean age of this group was 35.1 years.

Tetraplegic group: Three cases of tetraplegia (with alert consciousness without body movement) were examined. They were one case resulting from pontine vascular lesion and 2 cases resulting from high cervical spinal lesion (Table 2).

Stabilized chronic infectious group: This group (with alert consciousness and normal body movement) was also examined for the purpose of determining whether chronic infection itself might have effect on the circadian rhythm or not (Table 2).

Chemical determinations: Plasma cortisol, glucose, and nonesterified fatty acid (NEFA) were determined in these 4 groups during their most stable and non-stressful periods. The group of prolonged coma was examined at least two months after the onset when the acute phases of the disorders resulting in prolonged coma had probably elapsed, and thus the disorders themselves were not considered to effect on the hypothalamo-pituitary-adrenal system as stresses. The tetraplegic and chronic infectious groups, also, were studied at least one month after serious complications and stress-producing diagnostic examinations.

Heparinized blood samples were withdrawn from the subjects by venipunctures at 04:00, 08:00, 12:00, 16:00, 20:00 and 00:00, centrifuged immediately, and the plasma was stored at −80°C. Plasma cortisol was measured by competitive protein-binding assay ("Cortipac" R.C.C. kit.). Plasma glucose and NEFA were determined with the ortho-toluidine boric acid method and the modified Dumcombe method (NEFA-Test, Wako, Japan), respectively.

After the fast of 14 hr, synthethic ACTH (Tetracosactide 0.25 mg) was administered intravenously to the patients with prolonged coma and the controls at 9:00. Response to the synthetic ACTH was studied in these two groups by measuring plasma cortisol levels prior to, 30 and 60 min after the administration of ACTH.

TABLE 2. *Summary of control, tetraplegic and stabilized chronic infection cases*

Control cases				Tetraplegic cases			
Case No.	Sex Age	Diagnosis	ESR*	Case No.	Sex Age	Diagnosis	ESR*
1	M26	Healthy volunteer		1	M, 50	Locked-in syndrome	39 75
2	M, 25	Healthy volunteer		2	M, 63	Thickening of posterior longitudinal ligament (chronic pyelonephritis, exudative decubitus)	100 115
3	M, 33	Healthy volunteer					
4	F, 27	Healthy volunteer					
5	F, 28	Healthy volunteer					
6	F, 23	Healthy volunteer		3	M, 25	Cervical spine dislocation	45 65
7	M, 42	Healthy volunteer					
8	M, 43	Amyotrophic lateral sclerosis	10 25	Stabilized chronic infection cases			
9	F, 24	Myasthenia gravis	5 10	1	F, 67	Chronic pyelonephritis	38 75
10	M, 56	Healed viral encephalitis	10 23	2	F, 58	Chronic pyelonephritis	45 95
11	F, 30	Myasthenia gravis	7 12	3	F, 64	Infected bronchiectasis	71 105
12	F, 63	Marie's ataxia	15 32	4	F, 64	Chronic bronchitis	40 75

* ESR=erythrocyte sedimentation rate (1 hr mm / 2 hr mm)

Urinary excretion of 17-hydroxy-corticosteroids (17-OHCS) during a 24-hr period was measured according to the Porter-Silber method in these two groups.

The response to synthetic TSH-releasing hormone (TRH, Protirelin 0.5 mg), administered intravenously at 09:00 after the fast of 14 hr, was compared between prolonged coma and control groups. TSH levels of the plasma, which were collected shortly before, 15, 30, 45, 60 and 120 min after the administration of TRH, were determined by radioimmunoassay (TSH kit "Daiichi", Japan).

RESULTS

The level of consciousness in the prolonged coma of the present paper was not real "coma", for its wakefulness was easily distinguished from its sleep according to clues such as blinking of eyelids and movements of eyeballs as reported by Jennett and Plum (1972). Among the 17 cases of prolonged coma, grinding of teeth during wakefulness was observed in 8, forced crying was observed in 4. It was of special interest that periods of sleep and wakefulness of prolonged coma alternated with one another for short durations lasting from only about 30 min to about 2 hr in length, and that the durations of sleep and wakefulness of prolonged coma in the daytime were not different from those in the night-time.

There were not significant differences in variations in levels of plasma glucose and NEFA during a 24 hr period between the group of prolonged coma, which was fed via tubes, and control group (Table 3, Fig. 1). Also, no differences were discernible in responses to synthetic TRH and ACTH between the two groups

TABLE 3. *Measured data from cases of prolonged coma (P), control (C),*

Case No.		Variations in levels of plasma cortisol (a), 04:00			08:00			12:00		
		a	b	c	a	b	c	a	b	c
P	1	18.5	87	0.865	14.2	86	0.495	17.0	88	0.654
	2	11.6	91	0.576	15.0	90	0.468	14.0	77	0.216
	3	18.3	116	0.366	15.7	122	0.275	14.1	136	0.428
	4	17.3	89	0.633	17.5	93	0.522	11.3	104	0.286
	5	9.0	98	0.267	16.7	92	0.592	10.9	126	0.306
	6	16.9	71	0.312	22.0	109	0.521	13.6	100	0.239
	7	19.5			17.0			14.5		
	8	18.5			15.0			13.5		
	9	20.0			9.2			9.5		
	10	15.1			12.9			15.7		
	11	13.2	86	0.364	16.3	107	0.952	10.7	137	0.273
	12	11.2	80	0.606	12.2	107	0.406	13.6	83	0.282
	13	8.8	92	0.376	12.6	92	0.479	12.2	104	0.212
	14	17.3	85	0.291	11.1	86	0.424	13.3	109	0.248
	15	13.3			13.7			15.8		
	16	10.3	95	0.634	14.4	121	0.550	11.7	112	0.538
	17	22.8	85	0.492	21.0	97	0.628	12.7	95	0.454
Mean±S.D.		15.4 ±4.2	88.8 ±11	0.482 ±0.18	15.1 ±3.3	98.7 ±13	0.526 ±0.16	13.2 ±2.0	104 ±19	0.345 ±0.14
C	1	18.0	99	0.931	23.6	90	0.524	9.5	116	0.400
	2	20.0	111	0.409	19.1	96	0.255	6.1	85	0.400
	3	8.8			25.8			9.1		
	4	12.4	100	0.255	17.6	90	0.264	7.7	89	0.333
	5	4.0	86	0.436	14.8	96	0.388	8.7	91	0.542
	6	7.8	100	0.288	23.8	96	0.415	10.3	86	0.573
	7	10.7	91	0.386	13.9	95	0.316	9.4	100	0.184
	8	11.5			17.0			10.5		
	9	13.5	83	0.202	10.7	96	0.311	10.9	95	0.321
	10	12.3	87	0.247	13.1	115	0.340	8.6	100	0.250
	11	22.5			14.4			8.2		
	12	15.2			18.0			19.8		
Mean±S.D.		13.1 ±5.3	94.6 ±9.7	0.394 ±0.23	17.7 ±4.7	96.8 ±7.8	0.352 ±0.09	9.9 ±3.4	95.3 ±11.0	0.383 ±0.14
T	1	14.6			18.0			14.7		
	2	22.8			18.6			11.1		
	3	19.3			17.8			9.9		
I	1	14.5			20.6			10.0		
	2	8.8			15.8			11.2		
	3	12.4			15.5			11.2		
	4	14.4			20.1			11.2		

* a: cortisol (μg/100 ml), b: glucose (mg/100 ml), c: non-esterified fatty acid (mEq/liter).
during one day (μg/100 ml), significantly smaller in prolonged coma (P) than in control
‡ Mean of plasma cortisol values measured 6 times (μg/100 ml), larger in prolonged coma

(Table 4, Fig. 2).

Circadian variations of plasma cortisol levels in the prolonged coma and control are shown in Fig. 3. From this chart, it seems apparent that the maximum

tetraplegia (T) and stabilized chronic infection (I)

glucose (b), and NEFA (c) during one day*

16:00			20:00			00:00			Amplitude†	Mean‡ value
a	b	c	a	b	c	a	b	c		
14.8	96	0.243	7.6	83	0.619	8.1	90	1.027	10.9	13.4
10.2	98	0.446	5.2	128	0.326	9.1	91	0.541	9.8	10.9
8.7	126	0.197	6.8	118	0.194	5.3	100	0.344	13.0	11.5
8.6	98	0.283	9.1	100	0.197	7.1	94	0.453	10.4	11.8
8.3	104	0.275	7.0	98	0.267	13.2	110	0.344	9.7	10.9
14.2	158	0.248	17.0	132	0.233	17.0	61	0.476	8.4	16.8
15.7			15.7			15.2			5.0	16.3
13.8			10.8			13.8			7.7	14.2
14.7			6.6			6.5			13.5	11.1
12.9			12.7			15.0			3.0	14.1
9.3	105	0.761	10.8	112	0.248	8.5	105	0.382	7.8	11.5
15.1	100	0.539	8.6	85	0.242	8.9	84	0.273	6.5	11.6
7.5	116	0.255	6.9	123	0.230	7.9	105	0.282	5.7	9.3
11.2	86	0.261	9.7	104	0.170	16.1	94	0.373	7.6	13.1
7.1			7.7			9.0			8.7	11.1
11.3	100	0.454	4.8	130	0.292	4.0	102	0.724	10.4	9.4
16.3	97	0.382	11.2	99	0.330	12.0	97	0.472	11.6	16.0
11.8	107	0.362	9.3	110	0.278	10.4	93	0.474	8.8	12.5
±3.1	±20	±0.16	±3.4	±18	±0.12	±4.0	±13	±0.21	±2.7	±2.2
12.2	110	0.397	14.0	153	0.852	6.3	100	0.458	17.3	13.9
11.2	109	0.370	14.2	110	0.706	4.7	100	0.433	15.3	12.6
13.5			6.3			8.5			19.5	12.6
5.3	119	0.421	4.7	119	0.394	3.1	124	0.318	14.5	8.5
10.3	88	0.406	3.7	105	0.936	6.4	89	0.318	11.1	8.0
8.4	84	0.973	9.5	82	0.497	5.0	108	0.321	18.8	10.8
5.5	97	0.357	3.8	110	0.211	2.6	99	0.451	11.3	7.7
8.7			7.0			3.0			14.0	9.6
6.9	112	0.356	4.6	106	0.298	5.3	106	0.298	8.8	8.7
4.0	111	0.253	3.7	114	0.187	10.2	108	0.256	9.4	8.7
7.9			7.5			4.6			17.9	10.9
6.6			9.0			11.3			13.2	13.3
8.4	103	0.442	7.3	112	0.498	6.0	104	0.357	14.3	10.4
±2.9	±13	±0.22	±3.7	±20	±0.30	±2.8	±10	±0.08	±3.5	±2.1
7.4			8.2			8.6			10.6	11.9
9.9			5.0			4.5			18.3	12.0
11.4			7.3			4.9			14.4	11.8
8.2			4.2			5.0			16.4	10.4
10.3			5.4			5.6			10.4	9.5
10.2			4.6			3.5			12.0	9.6
8.4			9.4			7.9			12.1	11.9

† Difference of plasma cortisol level between the maximum and the minimum attained

(C) ($p<0.005$).

(P) than in control (C) ($p<0.05$).

and minimum levels of circadian variation of plasma cortisol in these two groups appear at approximately the same clock time.

The distribution of the number of the cases in these groups were virtually

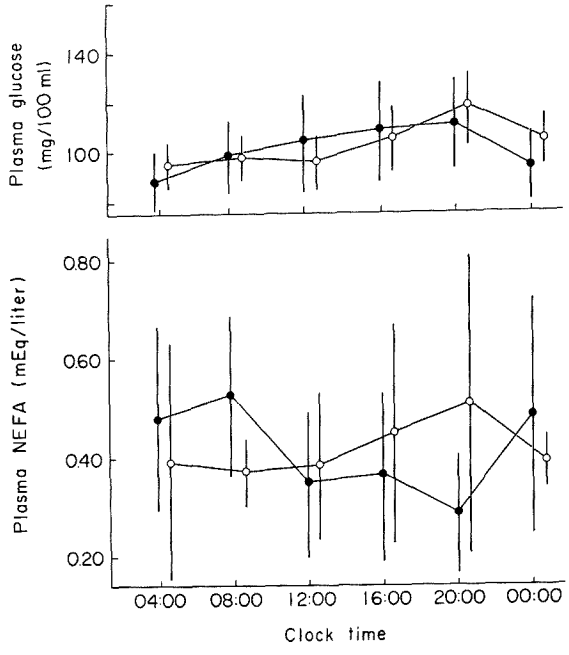

Fig. 1. Variations of plasma glucose and plasma non-esterified faitty acid (NEFA) during one day in cases of prolonged coma and control cases. Mean±S.D. ●——●, cases of prolonged coma (n=12); ○——○, control cases (n=8).

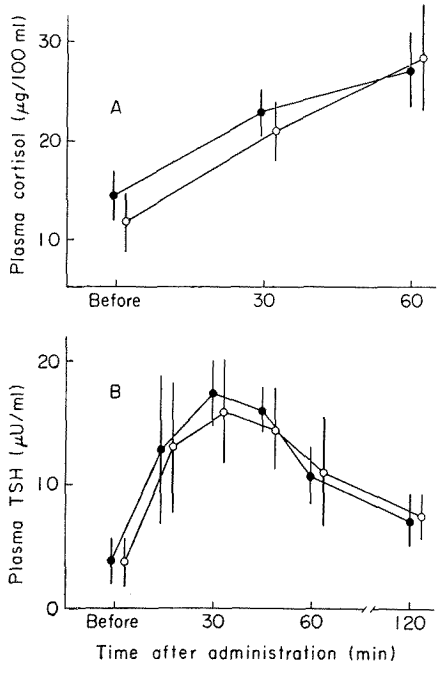

Fig. 2. Effects of intravenous administration of ACTH and TRH on plasma contents of cortisol and TSH in the cases of prolonged coma and control cases. Mean ±S.D. A: Effects of intravenous administration of ACTH (Tetracosactide 0.25 mg) at 9:00 a.m. ●——●, cases of prolonged coma (n=12); ○——○, control cases (n=10). B: Effects of intravenous administration of TRH (Protirelin 0.5 mg) at 9:00 a.m. ●——●, cases of prolonged coma (n=7); ○——○, control cases (n=6).

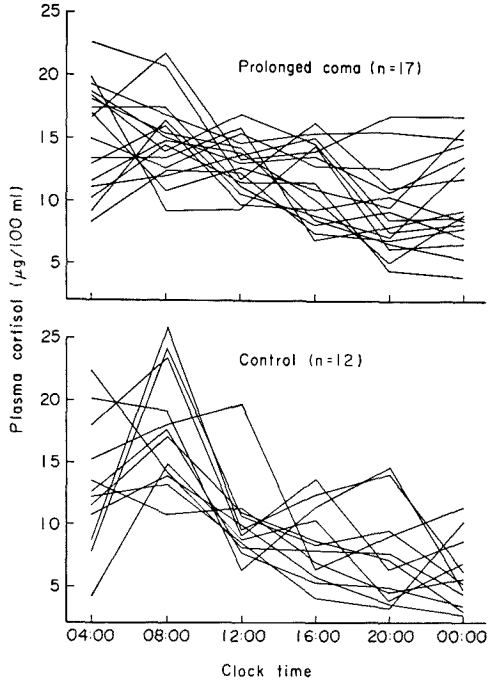

Fig. 3. Circadian variations of plasma cortisol contents in prolonged coma and control cases.

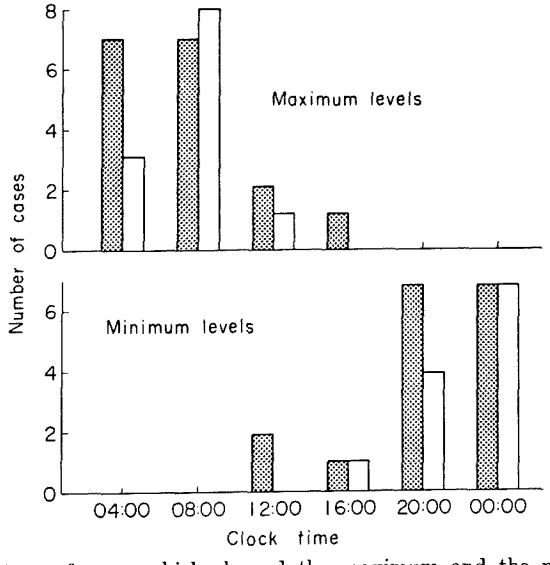

Fig. 4. Distributions of cases which showed the maximum and the minimum levels in circadian plasma cortisol variations at each clock time. ▨, cases of prolonged coma ($n=17$); □, control cases ($n=12$).

identical, when the number of cases exhibited the maximum and minimum in circadian rhythm were plotted at each sampling time (Fig. 4). These results seem to indicate that there is no phase shift in the rhythm in prolonged coma.

On the contrary, the maximum level of the circadian variations in prolonged coma was lower, the minimum level was higher in the group of prolonged coma as compared with the control group (Fig. 3). The amplitudes of circadian variation between the maximum and the minimum levels of plasma cortisol were compared among the 4 groups. As indicated in Table 3, amplitudes in the group of

TABLE 4. *Urinary excretions of 17-OHCS, ACTH test and TRH test of prolonged coma(P) and controls (C).*

Case No.		Urinary 17-OHCS*	ACTH test†			TRH test‡					
			0′	30′	60′	0′	15′	30′	45′	60′	120′
P	1	14.9	13.0	23.6	31.5						
	2	12.3	15.0	21.8	30.0	2.5	10.5	16.5	16.5	10.5	6.6
	3	24.2	17.4	25.8	31.3						
	4		18.5	23.7	26.6	4.0	17.5	19.0	15.0	13.0	10.5
	5		16.7	22.0	26.0						
	6	30.9	17.0	20.5	26.6	2.0	4.3	17.8	17.8	8.2	7.6
	7	30.2	14.5	21.5	22.5						
	8										
	9		10.8	26.5	30.5	3.0	9.0	12.5	13.5	11.0	4.0
	10										
	11	10.1	12.3	19.5	20.5	5.5	9.0	17.0	17.0	9.0	7.0
	12										
	13	3.1	14.6	21.4	23.7	7.5	21.0	19.5	15.5	15.0	10.0
	14	5.1	11.1	20.4	24.3	4.0	19.5	20.5	19.0	9.5	5.0
	15										
	16	26.1									
	17	18.5	12.0	26.7	31.0						
Mean±S.D.		17.5 ±10.1	14.4 ±2.5	22.8 ±2.3	27.0 ±3.6	4.0 ±1.8	13.0 ±5.9	17.5 ±2.4	16.3 ±1.7	10.9 ±2.2	7.2 ±2.2
C	1	11.2									
	2	18.2	13.3	18.5	25.1						
	3	29.3	12.0	22.5	35.2	2.0	7.2	9.4	9.2	6.4	4.5
	4	10.0	15.0	19.5	23.5	4.4	19.0	16.5	14.5	14.0	10.5
	5	9.9	12.0	24.3	29.2	6.0	16.0	18.0	16.0	10.0	4.0
	6	4.0	14.0	18.0	37.3	2.5	12.5	14.5	13.5	12.0	11.0
	7		3.8	16.2	21.8	6.6	19.0	23.0	20.0	18.5	12.5
	8	10.0	12.2	22.0	31.2	2.0	5.0	15.0	14.5	6.5	4.5
	9	14.7	11.7	26.5	32.2						
	10	7.6	11.4	21.5	25.5						
	11	7.0	12.4	20.5	22.4						
	12	6.3									
Mean±S.D.		11.7 ±7.1	11.8 ±2.9	21.0 ±2.9	28.3 ±5.2	3.9 ±1.9	13.1 ±5.5	16.1 ±4.1	14.6 ±3.2	11.2 ±4.3	7.8 ±3.6

* Urinary excretion of 17-hydroxy-corticoids during one day (mg/100 ml).

† Effects of intravenous administration of Tetracosactide 0.25 mg (plasma cortisol μg/100 ml).

‡ Effects of intranveous administration of Protirelin 0.5 mg (plasma thyroid stimulating hormone μU/ml).

prolonged coma were reduced significantly in comparison to those of the control group ($p<0.005$). However, in the group of prolonged coma, no correlation was seen in each case between the amplitude and its conscious level. Also, the two subgroups in prolonged coma which showed different neurological signs, i.e. cases of decortication and those of decerebration were not significantly different in their amplitude. In contrast, the amplitudes of the tetraplegic and the stabilized chronic infection groups were not different from those of the control group (Table 3). As shown in Table 3, the mean plasma cortisol values measured every 4 hr during one day were compared in each case of prolonged coma and of control. The mean values of the group of prolonged coma were larger than those of the control group ($p<0.05$).

Urinary excretion of 17-OHCS during one day in the prolonged coma and control was also examined (Table 4). The mean value of 17-OHCS excretion in the former was larger than that in the latter, but the difference between them was not so significant ($0.01>p>0.05$).

Discussion

In summing up, the amplitudes of circadian rhythms of plasma cortisol were significantly reduced in prolonged coma as compared with control ($p<0.005$). In contrast, there was no phase shift in the rhythm in the prolonged coma from that of the control. Moreover, the data suggest, if not conclusive, that the output of the hypothalamo-pituitary-adrenal system during one day was probably accelerated in prolonged coma.

Previously, Eik-Nes and Clark (1958) have examined 4 cases probably identical to those in prolonged coma of the present paper, and they reported that circadian rhythm of plasma 17-OHCS was erratic, namely it was often observed that its plasma level varied without showing no consistent relation to day time and the pattern of the variation was variable in successive occasions.

Perkoff et al. (1959) also found significant "loss" of circadian rhythm of plasma 17-OHCS in patients with alternating consciousness. However, they did not report specifically on the reduction of the amplitude of the circadian rhythm of plasma 17-OHCS. The results of the present study were interestingly in accordance with the experiments of Moberg et al. (1971), in which they showed sectioning of the fornix of rats abolished the circadian rhythm in plasma corticosterone by raising the lowest and decreasing the highest value in one day.

Body movement, chronic infection, and tube feeding. Besides the disturbances of consciousness and other neurological signs of prolonged coma as the result of CNS damage, the chief differences in states of patients between the prolonged coma and the control may be as follows: First, as mentioned above, the patients with prolonged coma rarely showed body movements. Second, there was chronic infection in most of them. Third, all patients in prolonged coma were fed with nasal tubes. However, three patients in the tetraplegic group examined in this paper showed amplitudes similar to the control group (Table 3).

Perkoff et al. (1959) also found a virtually normal circadian rhythm of 17-OHCS in 10 patients with poliomyelitis, rheumatic arthritis, etc., who had been bed-ridden or chair-ridden for one or more years. Cases of chronic infection without CNS damage in this study also showed amplitudes similar to the control group (Table 3). Perkoff et al.'s patients with various chronic infections were reported to show normal patterns of circadian variation of 17-OHCS (1959).

In addition, in spite of tube feeding in the group of prolonged coma, variations of plasma glucose and NEFA during a 24-hr period were not different from the control group (Fig. 1, Table 3). Moreover, circadian rhythm of 17-OHCS has been reported to be affected neither by 24-hr period of starvation nor by meals given repeatedly at 4-hr intervals (Laidlow et al. 1954). Consequently, the significant reduction of amplitude of circadian variations of plasma cortisol in this group is probably not due to the above-mentioned three external differences, but it is apparently due to unconsciousness of prolonged coma resulting from severe damage in the CNS.

Sampling time and age. Plasma glucocorticoid does not change so smoothly as previously supposed, but does periodically, with 5–10 peaks (Krieger et al. 1971). Accordingly, the problem arose as to when sampling should be made in order to record correct circadian variation of plasma cortisol. Krieger et al. (1971) studied 92 normal subjects using the same sampling times as utilized in the present paper and found good reproducibility in circadian rhythm of 11-OHCS, and concluded that either age or sex had no effect on it. Also, it is reported that the circadian rhythm of 17-OHCS of normal subjects was constantly reproduced with the same sampling clock-times as in this study (Perkoff et al. 1959).

Therefore, according to the sampling times chosen in the present paper, the rhythm of plasma cortisol is supposed to have been recorded correctly. Although the ages of the group of prolonged coma were older than those of the control group, significant reduction of amplitude in the former was probably not due to this factor, since age has been reported to have no effect on circadian rhythm of 11-OHCS (Krieger et al. 1971).

Venipuncture and interruption of sleep. Plasma samples were obtained from subjects of 4 groups by venipunctures every 4 hr, and thus their sleep was interrupted on some occasions. Krieger et al. (1971) reported that obtaining samples by venipunctures, rather than through an indwelling catheter, did not appear to alter significantly plasma corticosteroid levels at any given sampling time, thereby eliminating the stress of venipuncture as a variable. Moreover, Orth et al. (1967) have reported that alteration of the sleep-wake schedule during a single day did not alter the plasma 17-OHCS cycle. Therefore, venipuncture and interruption of sleep does not appear to have any effect on the cortisol circadian rhythm of subjects in this paper.

Responses to exogenous TRH and ACTH. Responses to TRH and ACTH were studied in the group of prolonged coma and control group by measuring plasma

TSH and cortisol, respectively. There were no apparent differences between these two groups (Table 4, Fig. 2). Although TRH was not concerned with the hypothalamo-pituitary-adrenal system directly, TRH was used to examine the functions of pituitaries in two groups in stead of ACTH-releasing factor, which up to now has not been put to clinical use. Therefore the reduced amplitude of the rhythm of plasma cortisol in prolonged coma may be due to their unconsciousness because their functions of pituitary and adrenal cortex were not different from those of control.

Mean plasma cortisol values and urinary excretion of 17-OHCS during one day. The mean plasma cortisol values of 6 samples measured every 4 hr were probably one of the indicators of the output from the adrenals during one day. These values were higher in the group of prolonged coma than those of the control group ($p<0.05$) (Table 3). These data suggest that the function of the adrenal (probably of the hypothalamo-pituitary-adrenal system) in prolonged coma was not reduced, it seems but to have been accelerated. As a matter of fact, the value of urinary excretion of 17-OHCS in this group during one day was also higher than that of the control group (Table 4). The higher mean plasma cortisol level in prolonged coma than that of control results from possible removal of the inhibitory influences of CNS, because the following similar results were presented in patients suffering from organic and functional disorders of the CNS. The mean plasma levels of 17-OHCS, measured successively during the day, were higher in 4 subjects suffering from cerebral damage than those of control (Eik-Nes and Clark 1958). Elevated plasma and urinary corticosterone levels were seen in 3 patients with severe depression (Butler and Besser 1968). Isolation of the hypothalamus in rat was reported to produce elevated plasma corticosterone levels (Halász et al. 1967). At any rate, the function of the hypothalamo-pituitary-adrenal system in prolonged coma was not reduced, since the mean values of plasma cortisol and urinary excretion of 17-OHCS in one day in the this state were also normal or somewhat higher than the control.

Effect of the CNS on circadian rhythm of plasma cortisol. There have been reports suggesting that circadian rhythm in hypothalamo-pituitary-adrenal system is affected by the CNS. Normal circadian rhythm of plasma corticosterone was reported to be abolished in rats after preparation of acute diencephalic islands, implying that neural inputs apparently necessary for the rhythm were lacking in the isolated diencephalon (Ondo and Kitay 1972). Moreover, the limbic system (Ganong 1963), the reticular formation (Endrőczi and Lissák 1960), and the fornix (Moberg et al. 1971) were reported to have effects on this rhythm. Although alteration of the sleep-wake schedule for a single day did not appear to alter the rhythm of 17-OHCS, it can be formed within at least a week synchronized with a new schedule (Orth et al. 1967). Phase shift in circadian rhythm of 17-OHCS was detected after long air travel east to west, but the rhythm adapted gradually to the local time (Flink and Doe 1959). According to these facts, the circadian rhythm of

hypothalamo-pituitary-adrenal system may be understood to be adaptable to synchronizers in the external environment. Moreover, if the CNS was indispensable for receiving synchronizers, these reports also suggest that the CNS has an effect on circadian rhythm. More specifically, abnormal circadian rhythms were found in disturbances of consciousness such as coma arising from various causes (Eik-Nes and Clark 1958; Perkoff et al. 1959), delirium, depression (Butler and Besser 1968). Also, these data seem to indicate that the CNS has effect on this circadian rhythm.

Possible mechanism of reduced amplitude. The state of prolonged coma has been reported to originate from various lesions in CNS, such as those in the cortex itself, the subcortical structures of the hemisphere, the brain stem, or all these sites (Jennett and Plum 1972). Consequently, it is difficult to determine a single location in the CNS which gives rise to a significant reduction in amplitude of circadian rhythm of plasma cortisol. Amplitudes in the two subgroups within the group of prolonged coma showing entirely different neurological signs, i.e., decortication and decerebration, were virtually equal. These results seem to suggest that the reduction in amplitude was probably not due to single lesion in CNS of prolonged coma, but rather possibly due to common sign of it, i.e., severe unconsciousness. In other words, as a result of severely affected consciousness, the reception of synchronizers required to produce circadian rhythm is impeded in prolonged coma Meanwhile, it requires further studies to conclude that the peculiar sleep pattern with short duration noted in prolonged coma in this study is one of the factors of reduced amplitude of the rhythm.

According to the present data, the amplitude of circadian variation of plasma cortisol was significantly reduced in prolonged coma; however, the phase shift of it was not observed in prolonged coma. This may point to two possibilities. One, on the assumption that amplitude of circadian rhythm of cortisol is produced by a mechanism entirely different from that of phase, only the amplitude mechanism was obstructed in this state. The other, assuming that the amplitude and phase are controlled by the same mechanism but that the intensities of exogenous stimuli required to produce the amplitude are stronger than those required to produce the phase, perhaps the patients with prolonged coma were able to receive the stimuli strong enough to produce the phase but not those strong enough to produce normal amplitude. Certainly, from the present study, it was impossible to decide which of the two possibilities is most probable.

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